



HAL
open science

True ileal amino acid digestibility and digestible amino acid scores (DIAASs) of the cruciferin and napin fractions of rapeseed: Impact of processing

Romain Kapel, Celine Niquet-Léridon, Frédéric Tessier, Jean-Luc Mession, Caroline Buffière, Nouredine Hafnaoui, Carole Migné, Bérénice Houinsou-Houssou, Alain Riaublanc, Véronique Solé-Jamault, et al.

► To cite this version:

Romain Kapel, Celine Niquet-Léridon, Frédéric Tessier, Jean-Luc Mession, Caroline Buffière, et al.. True ileal amino acid digestibility and digestible amino acid scores (DIAASs) of the cruciferin and napin fractions of rapeseed: Impact of processing. *Food Chemistry*, 2025, 474, pp.143161 (art. number). <10.1016/j.foodchem.2025.143161>. <hal-04990785>

HAL Id: hal-04990785

<https://normandie-univ.hal.science/hal-04990785v1>

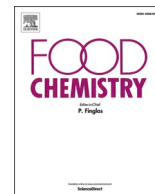
Submitted on 14 Mar 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License



True ileal amino acid digestibility and digestible amino acid scores (DIAAS) of the cruciferin and napin fractions of rapeseed: Impact of processing

Romain Kapel^a, Celine Niquet-Léridon^b, Frédéric Tessier^b, Jean-Luc Mession^a,
Caroline Buffière^c, Nouredine Hafnaoui^c, Carole Migné^c, Bérénice Houinsou-Houssou^d,
Alain Riaublanc^d, Véronique Solé^d, Didier Rémond^{c,*}

^a CNRS, Université de Lorraine, LRGP, Laboratoire Réactions et Génie des Procédés, F-54500 Vandoeuvre-les-Nancy, France

^b Institut Polytechnique UniLaSalle, Université d'Artois, ULR, 7519, Beauvais, France

^c Université Clermont Auvergne, INRAE, UNH, Unité de Nutrition Humaine, F-63000 Clermont-Ferrand, France

^d INRAE, BIA, Biopolymères Interactions Assemblages, F-44300, Nantes, France

ARTICLE INFO

Keywords:
Rapeseed
Protein
Digestibility
DIAAS

ABSTRACT

Processes are being developed to exploit proteins from the residue of rapeseed oil extraction. Two main fractions of proteins can be purified: cruciferin and napin. The present work was carried out to characterize the nutritional quality of these fractions, while exploring the effect of the extraction conditions (neutral or acid), and that of the post extraction process (heating 1 h, 120 °C). The true ileal digestibility (TID) of their amino acids (AA) was measured in minipigs, to calculate the digestible indispensable amino acid score (DIAAS). The TID of cruciferin was high (95 %) compared to that of napin (87 %). The extraction process and the heat treatment had no effect on the TID of napin. The DIAAS for adults was 117 % for cruciferin, and 82–89 % for napin (branched-chain AA being the limiting AA). Improving the TID of napin would enable this interesting source of cysteine and lysine to be better exploited in human nutrition.

1. Introduction

The edible oil industry produces large amounts of proteins that are currently mainly used in animal nutrition. A non-negligible part of these proteins could probably be directly used in human nutrition with better efficiency. The authorization by the European Commission of the commercialization of rapeseed proteins as a novel food ingredient (implementing decision 2014/424/EU) illustrates this trend.

Rapeseed meal, the by-product yielded by the oil extraction procedure, contains around 35–40 % by weight of crude protein. Rapeseed meal proteins are composed of cruciferin 7S–11S (globulins) and napin 2S (albumins). Cruciferins are hexameric proteins with a molecular weight (Mw) of around 300 kDa consisting of subunits composed of acidic α (30 kDa) and basic β polypeptides (20 kDa) bound with a single disulfide bridge. Napin (14.5 kDa) is a heterodimeric protein constituted of heavy (10 kDa) and light (4.5 kDa) polypeptides bound by two interchain disulfide bridges (Berot, Compoin, Larre, Malabat, &

Gueguen, 2005; Dong et al., 2011). Cruciferin and napin have different amino acid compositions and technological properties, and their fractionation could make the use of rapeseed proteins in food formulations more versatile (Chmielewska et al., 2021).

The most ready-to-do process to obtain napin and cruciferin protein enriched-products consists of: (i) total protein extraction from the meal suspended at either neutral or alkaline pH (7–12.5), followed by (ii) cruciferin precipitation by acidification (pH \approx 3–6), whereas (iii) soluble proteins (napin) are recovered by ultrafiltration (Berot et al., 2005; Dong et al., 2011; Ghodsvali, Haddad Khodaparast, Vosoughi, & Diosady, 2005; Pedroche et al., 2004; Yoshie-Stark, Wada, & Wäsche, 2008). Concerning step (i), increasing the pH of the extraction media led to higher total nitrogen extractability from the defatted meal, in the range of \approx 40–55 % and 60–80 % at pH values of 10 and 12, respectively. The authors noted that the harshness of the oil extraction procedure had a strong impact on protein extraction yield (Ghodsvali et al., 2005). In addition, Yoshie-Stark et al. (Yoshie-Stark et al., 2008) reached 78 %

* Corresponding author at: Unité de Nutrition Humaine, INRAE de Clermont-Ferrand/Theix, 63122 St Genès-Champanelle, France.
E-mail address: didier.remond@inrae.fr (D. Rémond).

<https://doi.org/10.1016/j.foodchem.2025.143161>

Received 13 March 2024; Received in revised form 28 January 2025; Accepted 29 January 2025

Available online 1 February 2025

0308-8146/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

protein extractability at pH 7.4, to avoid polyphenol oxidation and complexation with soluble proteins at alkaline pH (Xu & Diosady, 2000). For (ii), acidification at pH values of 5.5–5.8 resulted in the highest level of cruciferin precipitation, while (iii) the recovery yield of soluble protein by ultrafiltration was similar (napin, 30–40 %) regardless of the pH applied (Pedroche et al., 2004; Yoshie-Stark et al., 2008). The protein content of the dried cruciferin-enriched fraction is usually lower (70–85 % of the dry matter) than that for napin (>90 % of the dry matter). Alternatively, another possibility of obtaining a napin-enriched product consisted of the selective extraction at pH 2 and purification by ultrafiltration (Nioi, Kapel, Rondags, & Marc, 2012). Data are lacking concerning the performances of the above-mentioned processes in terms of overall protein recovery, composition and purity.

Evaluating cruciferin and napin in human nutrition implies good knowledge of their nutritional quality. The latter can be evaluated not only on the basis of their amino acid composition but also on that of their digestibility in the small intestine. The true ileal digestibility (TID) of rapeseed protein isolate in humans has been previously reported to be rather low 84–87 % (Bos et al., 2007; Deglaire, Bos, Tome, & Moughan, 2009) compare to that of other protein sources: 96 % for milk proteins (Bos et al., 1999), 92 % for meat proteins (Oberli et al., 2015), 94 % for pea protein isolate (Guillin et al., 2022) and 95 % for wheat gluten (Le Bourgot et al., 2023), for instance. Nothing is known regarding the specific TID of the cruciferin and napin fractions of rapeseed. Knowledge of this parameter is however essential to calculate relevant nutritional quality scores, such as the digestible indispensable amino acid score (DIAAS) (FAO, 2013).

In the present study, different production routes of napin and cruciferin protein-enriched products were implemented. The protein true ileal digestibility of the fractions obtained was determined *in vivo* in minipigs, and the DIAAS was calculated.

2. Materials and methods

2.1. Preparation of protein extracts

The rapeseed meal was obtained from dehulled seeds cold-pressed and defatted by hexane (Olead, Pessac, France). Solvent removal was achieved at low pressure and temperature (max. 60 °C). It was ground and sieved into a fine powder ($d_m = 66 \mu\text{m}$, specific area = $0.3 \text{ m}^2 \cdot \text{g}^{-1}$) according to the supplier's instructions. The meal was composed of $29.3 \pm 0.8 \%$ proteins, $61.1 \pm 1.7 \%$ total dietary fibre (TDF), $7.3 \pm 0.3 \%$ ash and $9.4 \pm 1.4 \%$ phytate on a dry matter basis. The relative amount of cruciferin and napin proteins was $61.2 \pm 1.8 \%$ and $37.4 \pm 4.2 \%$, respectively.

In general, protein concentrates are obtained in two steps. The first one is an extraction from the meal using an aqueous solvent. The second one is a purification aiming at removing non protein compounds extracted alongside with proteins at the first step.

Cruciferin and Napin I were obtained using a 0.1 M NaCl and 0.5 g $\cdot \text{l}^{-1}$ ascorbic acid aqueous solution at a solid/liquid extraction of 1:10 wt. $\cdot \text{v}^{-1}$ according to Defaix, Kapel, and Galet (2019). The extraction was carried out under stirring for 45 min at 25 °C after adjustment of the pH at pH 7.0 using HCl 1 M. Insoluble material was removed by centrifugation (10,000 g, 30 min, 20 °C). Then, the supernatant containing both cruciferin and napin was adjusted at pH 3.5 for cruciferin selective precipitation. Acidified samples were stirred for 45 min at 25 °C and centrifuged (10,000 g, 30 min, 20 °C). Cruciferin pellets were washed twice with deionized water and freeze dried to give Cruciferin concentrate. Napin in the remaining supernatant was purified by a tangential filtration process using a 5 kDa-Mw membrane cut-off (hollow fibers UFP-5-C-5 A membrane, GE Healthcare). This cut-off was chosen in order to retain napin and let low molar weight molecules (under 5 kDa) pass through. In brief, supernatant was concentrated four times and diafiltered against deionized water in order to flush low molar weight non protein compounds. Napin enriched fraction was then

freeze dried.

Napin II was obtained after extraction in deionized water after pH adjustment at 2, (2 M HCl) according to Nioi et al. (Nioi et al., 2012). In this condition, napin is extracted selectively (without cruciferin). The solid-to-liquid ratio of the acidic suspension was $1:10 \text{ wt} \cdot \text{v}^{-1}$. Stirring duration, temperature and centrifugation were the same as described above for neutral extraction. Napin proteins were subsequently purified by tangential filtration (like that for Napin I), yielding the Napin II fraction. These processes are displayed in Fig. S1 (supplementary information 1). The SDS-Page technique was used to verify the efficiency of the process in selecting targeted proteins (Fig. 2S, supplementary information 2).

In order to improve their digestibility, part of the Napin I produced was denatured by heating in solution ($20 \text{ g} \cdot \text{l}^{-1}$), for 1 h at 120 °C (Napin I-TT).

2.2. *In vivo* digestibility

All the procedures were performed in accordance with the guidelines formulated by the European Community for the use of experimental animals (2010/63/EU). The study was approved by the Local Committee for Ethics in Animal Experimentation (CREEA d'Auvergne, Aubière, France), and authorized by the French government (APAFIS# 14622-2,018,041,116,527,391 V2).

Six female Yucatan minipigs ($20.1 \pm 1.6 \text{ kg}$ body weight) (bred at INRAE, St Gilles, France) were surgically fitted with permanent catheters in the portal, the hepatic vein and the aorta, and a T-shaped cannula was inserted in the distal ileum (Reynaud et al., 2021). Surgery was performed at least 3 weeks before the beginning of the experiments. The animals were housed in individual pens under ventilation and controlled temperature (21 °C). They had free access to water, and, except on sampling days, they were fed once a day with 500 g of a commercial feed (Porcypima; Sanders Centre Auvergne, Aigueperse, France).

For each protein fraction, the equivalent of 30 g of protein (%N \times 6.25) was incorporated in a meal containing wheat starch (100 g), maltodextrin (116 g), sucrose (30 g), rapeseed oil (10 ml), cellulose (15 g) and water (70 ml), as well as chrome oxide (Cr_2O_3 , 0.3 % of the meal dry matter content) used as a non-hydrolysable, non-absorbable marker of digestive transit. In addition to the four sources of proteins tested, a free amino acid (AA) mixture (in proportion close to the amino acid composition of meat) was used for monitoring the endogenous amino acid composition of ileal effluents (Reynaud et al., 2021). Each of the five test-meals was tested randomly in each animal. Sampling days for a given animal were separated by at least two days. At the end of the experiment, the minipigs were euthanized by injection of $125 \text{ mg} \cdot \text{kg}^{-1}$ body weight of sodium pentobarbital (Doléthol®; Vetoquinol, Lure, France) via the hepatic vein catheter.

2.3. Experimental procedures

To differentiate endogenous and dietary proteins, the pigs were given a continuous perfusion of ^{13}C -leucine solution through the upper hepatic vein catheter (Reynaud et al., 2021) for five days before the experiment. They received a loading dose of $1 \text{ mg} \cdot \text{kg}^{-1}$ and then a continuous dose of $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{j}^{-1}$ at a rate of $1.5 \text{ ml} \cdot \text{h}^{-1}$. It was subsequently maintained throughout the experiment. On the days of sampling, minipigs did not receive the commercial feed and were offered only one of the test-meals. Ileal effluents were collected continuously from 1 h to 9 h after test meal delivery in plastic bags (Sachet Whirl-Pak bags; volume: 120 ml, size: $7.5 \times 18.5 \text{ cm}$) attached to the ileal cannula. The bags were regularly renewed based on bursts of digestion. The effluents collected (accumulated over 1 h intervals) were weighed and immediately frozen at -20 °C . Blood samples (3 ml) from the portal vein were collected in cold syringes with lithium-heparin as an anticoagulant (S-Monovettes, Starstedt), at -15 , 60 and 360 min. Blood samples were immediately centrifuged at 1500 g for 10 min at 4 °C. The resulting

supernatant was frozen in liquid nitrogen and stored at -80°C until further analysis.

2.4. Analytical methods.

2.4.1. Composition of protein fractions

Fractions produced at the laboratory scale were analysed according to AACC Approved Methods of Analysis for dry matter (AACC 44–15.02, n.d.), ash (AACC 08–01.01, n.d.), total fibre content (AACC 32–05.01, n.d.) and crude protein (AACC 46–16.01, n.d.). Proteins were quantified with a nitrogen-to-protein conversion factor 6.25. Phytate content and trypsin inhibitor activity were determined according to Garcia-Esteva et al. (Garcia-Esteva, Guerra-Hernandez, & Garcia-Villanova, 1999) and Bacon et al. (Bacon, Wanigatunga, & Fenwick, 1995), respectively. Furosine, an indicator of the early phase of the Maillard reaction, was quantified by UV-HPLC (Nogueira Silva Lima et al., 2023), and carboxymethyllysine (CML) was determined by LC-MS/MS, after acid hydrolysis of the samples) (Niquet-Léridon & Tessier, 2011).

2.4.2. Purity of protein extracts

SDS-PAGE were carried out using a MiniProtean Tetra cell device (Biorad, Hercules, CA). Samples were prepared between 1 and 5 mg/ml protein concentration with the sample buffer, containing 62.5 mM tris-HCl, pH 6.8, 10 % (w/v) glycerol, 0.005 % (v/v) bromophenol blue, 2 % (w/v) SDS, in presence (reducing condition, R) or not (NR) of 2.5 % (v/v) β -mercaptoethanol (β -ME) 14. Precipitated protein pellets were suspended in a denaturing buffer containing 6 M urea, 0.1 M tris-HCl, pH 8.5 and 1 % (wt/v) SDS at ratio pellet-to-buffer of 1:40 (wt/v) prior mixing with the sample buffer at volume ratio of 1:1. The Mw standards (Precision Plus ProteinTM standards, BioRad) were applied in a separated lane. Fixed proteins were stained with Coomassie Blue R-250 (0.5 % (wt/v) in 20 % (v/v) ethanol. Destaining was achieved using 5 % (v/v) acetic acid and 25 % (v/v) ethanol.

The purity of the fractions was evaluated by SE-HPLC consisting in an LC-20 CE pump and an SPD-M20 A PDA (Shimadzu, Kyoto, Japan), an in-line degasser DGU-20 A3R mounted with a Biosep-SEC-s2000 column (7.8 mm internal diameter x 30 cm length, Phenomenex[®], Torrance, CA) maintained at 35°C in a CTO-20 A oven. The system was equilibrated with an acetonitrile – ultrapure water – trifluoroacetic acid running buffer (volume ratio of 55: 44.9: 0.1), degassed beforehand. The flow rate was set at $0.6\text{ mL}\cdot\text{min}^{-1}$. 10 μL sample volume filtered through 0.22 μm filters made of nitrate/acetate cellulose esters (Millex[®], Merck Millipore, Billerica, Ma.) was injected. Absorbance was monitored at 280 nm and 325 nm. Spectra were recorded and processed with the LC LabSolutions software (v. 5.51). In these conditions, cruciferin and napin eluted between 5 and 6.4 ml and 6.5 and 8 ml respectively (Fig. 2S, supplementary information 2). Cruciferin and napin peak area ratio were used to assess their composition of the total protein fraction (Table 1S supplementary data).

2.4.3. Digestive contents analysis

Ileal effluents were lyophilised and finely ground. The chromium content of ileal digesta was determined using atomic absorption spectrometry (Reynaud et al., 2021). Total nitrogen content in the ileal effluents and the test-meals were determined using an elemental analyser (Vario Isotope cube, Elementar). For each individual kinetics of the effluent sampling, a representative sample of the entire postprandial period was constituted with a fixed percentage of each effluent collected hourly, counting from the first detection of chromium appearance. This pooled sample was used for amino acid analysis and ^{13}C -leucine enrichment determination. Amino acid concentrations and ^{13}C -leucine enrichment in test-meals and pooled ileal effluents were determined as described by Reynaud et al. (Reynaud et al., 2021). Briefly, to estimate amino acid levels, we first performed four separate standardised hydrolysis procedures: sample placement in 6 N HCl for 24 h at 110°C (for aspartate, glutamate, threonine, serine, glycine, alanine, tyrosine,

phenylalanine, histidine, lysine, arginine, and proline), sample placement in 6 N HCl for 48 h at 110°C (for valine, isoleucine and leucine), sample placement in 6 N HCl for 24 h at 110°C after peroxidation with H_2O_2 (for methionine and cysteine); and sample placement in 4 N Ba (OH)₂ for 16 h at 110°C (for tryptophan). Amino acid content was then determined by ion exchange chromatography (Amino acid autoanalyser L8900, Hitachi). After the molecules had been separated using cation exchange chromatography and converted to their *N*-acetyl-propyl derivatives, ^{13}C -leucine enrichment was determined using a gas chromatography combustion isotope ratio mass spectrometer (GC-C-IRMS; Isoprime, GV Instruments). Enrichments were expressed as atom percent excess.

2.5. Calculations and statistical analysis

Equations for TID calculation have been detailed previously (Reynaud et al., 2021). The digestible indispensable amino acid score (DIAAS) was calculated according to FAO guidelines (FAO, 2013). For this calculation, the digestible indispensable amino acid content of the protein fractions was expressed on the basis of the sum of the amino acids instead of the protein content (Nx6.25) as is the case in the FAO formula. Data were analysed using an analysis of variance (ANOVA) with protein source and animal as fixed effects (GLM procedure of SAS[®]). Comparisons of the means were performed using Duncan's multiple range test at a 95 % confidence level. Data are presented as means \pm SEM.

3. Results

The extraction yield of cruciferin from rapeseed meal was 22 %. Napin extraction yields were similar for neutral and acidic extraction processes (38 %). All the fractions produced had high protein contents (80–90 % of the dry matter). The presence of a greater amount of residual fibers and phytates in Napin II (three time greater than that observed in Napin I) lowered its content in total protein. However, the purity of the napin fraction obtained after acid extraction was greater than that after neutral extraction, because of less contamination by cruciferin (Table 1S, supplementary information 3).

The amino acid composition of the different protein fractions is presented in Table 1. The cruciferin fraction is characterized by a higher content in branched-chain amino acids (isoleucine, leucine and valine) and aromatic amino acids (phenylalanine and tyrosine), and a lower content in lysine and sulphur amino acids (methionine and cysteine). Whereas the furosine contents of the different fractions were not very different, the carboxymethyllysine content was 5–6 times higher for the Napin I fraction than for cruciferin and Napin II.

The ileal flow of amino acids observed after ingestion of NapinI and Napin I-TT was twice that observed with cruciferin, that of Napin II was 50 % greater than that of Napin I (Table S2, supplementary information 4). The apparent ileal digestibility (AID) of the different protein fractions was significantly different (Table 2). Overall, the cruciferin fraction presented a higher AID than the napin fractions. Among the napin fractions Napin I AID was 60 % higher than Napin II AID, illustrating the importance of the impact of the extraction process on this parameter. Conversely, heating Napin I only slightly affected this parameter. The ileal flux of endogenous amino acids observed with the different protein fractions is presented in Table 3. While this flux was approximately similar for cruciferin, Napin I and Napin I-TT, it was about twice as high with Napin II. The true ileal digestibility (TID) of the sum of amino acids of cruciferin was high (95 %), compared to that of Napin I and II (88 and 87 %, respectively), and the heat treatment of Napin I did not significantly affect its TID (Table 4). The Digestible Indispensable Amino Acids Score (DIAAS, FAO, 2013) was calculated for the different ages of life (Table 6) from the digestible indispensable amino acid content of the different protein fractions (Table 5). It is noteworthy that cruciferin allowed a balanced supply of amino acids (with no limiting amino acid)

Table 1

Dry matter (DM), crude protein (CP), and amino acid (AA) contents in cruciferin, napin I (neutral extraction), napin II (acid extraction), and napin I-TT (neutral extraction + heating).

	Cruciferin	Napin I	Napin II	Napin I-TT
Crude protein (N x 6.25) (% DM)	93.5	95.3	86.6	95.3
Indispensable amino acids (% DM)				
Histidine	2.2	3.1	2.9	3.1
Isoleucine	4.2	3.1	2.7	3.1
Leucine	7.6	5.7	4.9	5.7
Lysine	4.6	7.3	6.8	7.3
Methionine	2.5	2.8	2.3	2.8
Phenylalanine	5.2	3.5	3.4	3.5
Threonine	5.9	5.2	4.5	5.2
Tryptophan	1.0	1.0	0.8	1.0
Valine	5.7	4.6	4.1	4.6
Total indispensable AAs (% DM)	39.0	36.2	32.7	36.2
Dispensable amino acids (% DM)				
Arginine	5.1	4.6	4.2	4.6
Alanine	4.8	4.3	3.2	4.3
Aspartic acid/Asparagine	9.2	3.2	2.7	3.2
Cysteine	1.3	4.3	3.9	4.3
Glutamic acid/Glutamine	17.1	23.8	21.5	23.8
Glycine	4.6	4.0	3.8	4.0
Proline	4.0	7.6	7.2	7.6
Serine	4.7	4.0	3.6	4.0
Tyrosine	2.8	1.7	1.6	1.7
Total dispensable AAs (% DM)	53.7	57.5	51.6	57.5
Total AAs (% DM)	92.7	93.7	84.3	93.7
Lysine (% CP)	4.9	7.6	7.9	7.6
Methionine + cysteine (% CP)	4.1	7.4	7.1	7.4
Branched-chain amino acids (%CP) ^a	18.7	14.1	13.4	14.1
Furosine (mg/100 CP)	16.0 ± 0.2	13.2 ± 0.3	17.3 ± 0.7	15 ± 0.7
Carboxymethyllysine (mg/100 g CP)	4.41 ± 0.05	30.3 ± 1.5	6.81 ± 0.27	45.1 ± 2.5
Trypsin inhibitors activity (mg of trypsin inhibited/g DM)	0.18 ± 0.04	23.08 ± 1.75	13.63 ± 1.17	0.46 ± 0.04

^a branched-chain amino acids = valine + leucine + isoleucine.

as early as the sixth month of life. Conversely, the low level of branched-chain amino acids in napin fractions, and most particularly in leucine, did not allow covering requirements at all ages. Overall, little impact of extraction process and heat treatment was observed for the DIAAS of napin.

4. Discussion

Because they represent an important protein resource, many studies have focused on the use of proteins from rapeseed meal in human nutrition. This raises challenges in the production of protein fractions having high functional and nutritional quality. Firstly, the way in which the meal is obtained is essential. Indeed, it was shown that cold pressing of dehulled seeds, and desolventizing at low temperature lead to the most suitable starting material for obtaining high quality rapeseed protein, together with high protein extractability from the meal (Ostbring, Tullberg, Burri, Malmqvist, & Rayner, 2019). That is why we selected rapeseed meal obtained using these conditions for the present study. Thereafter, because they have different functional properties (solubility, emulsifying, foaming, and gelling properties), the extraction and purification of protein fractions of cruciferin and napin may be of interest for incorporation into food products (Chmielewska et al., 2021). Nevertheless, the TID of napin and cruciferin fractions had never been

Table 2

Apparent ileal digestibility (AID) of crude protein (N x 6.25) and amino acids of cruciferin, napin I (neutral extraction), napin II (acid extraction), and napine I-TT (neutral extraction + heating) in minipigs (n = 5). Values are means ± SEM.

	Cruciferin	Napin I	Napin II	Napin I-TT	P
N x 6.25, %	69.87 ^a ± 6.58	51.50 ^a ± 2.12	16.39 ^b ± 8.02	55.26 ^a ± 3.48	0.0004
Indispensable amino acids, %					
Histidine	76.47 ^a ± 5.58	66.80 ^a ± 0.85	51.08 ^b ± 4.29	76.43 ^a ± 2.46	0.0035
Isoleucine	85.45 ^a ± 3.23	56.57 ^b ± 2.35	24.76 ^c ± 6.93	63.69 ^b ± 3.45	0.0001
Leucine	88.54 ^a ± 2.62	63.44 ^b ± 2.38	32.29 ^c ± 6.43	76.45 ^{ab} ± 2.17	0.0001
Lysine	85.96 ^a ± 3.57	73.86 ^{bc} ± 1.16	70.25 ^c ± 2.71	80.34 ^b ± 1.75	0.0072
Methionine	96.82 ^a ± 1.20	92.29 ^a ± 3.00	84.70 ^b ± 2.65	95.60 ^a ± 1.15	0.0049
Phenylalanine	87.34 ^a ± 3.26	62.62 ^c ± 1.60	41.95 ^d ± 4.56	74.31 ^b ± 2.32	0.0001
Threonine	78.15 ^a ± 5.03	62.69 ^b ± 1.40	28.02 ^c ± 6.58	67.24 ^{ab} ± 2.75	0.0001
Tryptophan	49.86 ^a ± 8.19	32.75 ^a ± 10.45	-13.55 ^b ± 12.09	47.58 ^a ± 6.45	0.0024
Valine	84.87 ^a ± 3.56	61.97 ^b ± 2.67	34.20 ^c ± 5.82	65.98 ^b ± 3.01	0.0001
Dispensable amino acids, %					
Arginine	87.69 ^a ± 3.02	66.57 ^{ab} ± 1.47	50.40 ^c ± 5.24	77.39 ^{ab} ± 2.47	0.0001
Alanine	79.87 ^a ± 4.78	60.52 ^a ± 3.98	18.36 ^b ± 11.80	63.84 ^a ± 3.24	0.0005
Aspartic acid/Asparagine	81.46 ^a ± 4.23	4.98 ^b ± 6.25	-49.46 ^c ± 13.68	-4.46 ^b ± 9.81	0.0001
Cysteine	59.32 ^{ab} ± 10.18	72.5 ^a ± 3.51	45.84 ^b ± 5.04	65.84 ^{ab} ± 4.97	0.0555
Glutamic acid/Glutamine	87.47 ^a ± 3.25	76.12 ^a ± 2.58	57.65 ^b ± 4.21	76.80 ^a ± 2.97	0.0011
Glycine	52.01 ^a ± 14.04	28.09 ^a ± 5.08	-39.77 ^b ± 14.62	36.70 ^a ± 7.61	0.0005
Proline	59.02 ^a ± 5.54	61.89 ^a ± 2.17	41.85 ^b ± 6.08	62.75 ^a ± 4.10	0.0336
Serine	78.84 ^a ± 4.84	50.51 ^b ± 3.88	23.46 ^c ± 5.98	63.17 ^b ± 2.96	0.0001
Tyrosine	83.47 ^a ± 4.13	37.19 ^c ± 3.29	6.20 ^d ± 8.43	65.10 ^b ± 3.50	0.0001
Total Amino Acids	81.34 ^a ± 4.24	63.72 ^b ± 1.67	38.75 ^c ± 5.39	68.35 ^{ab} ± 3.13	0.0001

Means within a row not sharing a common superscript differ ($P < 0.05$).

quantified, which precludes precise estimation of their nutritional quality through DIAAS calculation. We showed that the TID of the cruciferin fraction is very high (95 %), similar to the value reported for milk proteins (Gaudichon et al., 2002), whereas the TID of the napin fraction appeared somewhat lower (about 87 %). In a previous study in humans, starting from a rapeseed meal obtained from similar conditions as that used in the present study, the TID of the whole protein fraction extracted from the meal was reported to be even lower 84 % (Bos et al., 2007). Besides the fact that protein TID is generally slightly higher in pigs than in humans (Deglaire et al., 2009), by more or less eliminating anti-nutritional factors, the protein extraction process could explain the difference between the two studies. The present study highlights that the digestibility of napin is much lower than that of cruciferin, which could probably be related to napin's compact structure and low solubility.

In the present study we used two extraction processes to produce the napin fraction. When applying a similar post-extraction purification step (tangential filtration, 5 kDa-MwCO), the acidic and neutral processes gave similar results in terms of extraction yield and protein TID. The acidic process has the advantage of being easier to implement at the industrial scale, and of producing a napin fraction with low contamination by cruciferin. However, as applied in the present work it had the

Table 3

Ileal flow of endogenous amino acids with cruciferin, napin I (neutral extraction), napin II (acid extraction), and napine I-TT (neutral extraction + heating) in minipigs ($n = 5$). Values are means \pm SEM.

	Cruciferin	Napin I	Napin II	Napin I-TT	P
Indispensable amino acids, g					
Histidine	0.11 ^b \pm 0.02	0.18 ^b \pm 0.02	0.36 ^a \pm 0.05	0.10 ^b \pm 0.01	0.0001
Isoleucine	0.15 ^b \pm 0.03	0.26 ^b \pm 0.02	0.51 ^a \pm 0.07	0.14 ^b \pm 0.01	0.0001
Leucine	0.27 ^b \pm 0.05	0.46 ^b \pm 0.04	0.91 ^a \pm 0.13	0.25 ^b \pm 0.02	0.0001
Lysine	0.16 ^b \pm 0.03	0.28 ^b \pm 0.03	0.55 ^a \pm 0.08	0.15 ^b \pm 0.01	0.0001
Methionine	0.01 ^b \pm 0.00	0.02 ^b \pm 0.00	0.05 ^a \pm 0.01	0.01 ^b \pm 0.00	0.0001
Phenylalanine	0.16 ^b \pm 0.03	0.28 ^b \pm 0.03	0.56 ^a \pm 0.08	0.15 ^b \pm 0.01	0.0001
Threonine	0.28 ^b \pm 0.06	0.49 ^b \pm 0.05	0.96 ^a \pm 0.14	0.26 ^b \pm 0.03	0.0001
Tryptophan	0.12 ^b \pm 0.03	0.21 ^b \pm 0.02	0.42 ^a \pm 0.06	0.11 ^b \pm 0.01	0.0001
Valine	0.21 ^b \pm 0.04	0.37 ^b \pm 0.04	0.73 ^a \pm 0.11	0.20 ^b \pm 0.02	0.0001
Dispensable amino acids, g					
Arginine	0.15 ^b \pm 0.03	0.26 ^b \pm 0.02	0.52 ^a \pm 0.07	0.14 ^b \pm 0.01	0.0001
Alanine	0.21 ^b \pm 0.04	0.37 ^b \pm 0.03	0.72 ^a \pm 0.10	0.20 ^b \pm 0.02	0.0001
Aspartic acid/Asparagine	0.35 ^b \pm 0.07	0.59 ^b \pm 0.06	1.18 ^a \pm 0.17	0.32 ^b \pm 0.03	0.0001
Cysteine	0.12 ^b \pm 0.02	0.20 ^b \pm 0.02	0.40 ^a \pm 0.06	0.11 ^b \pm 0.01	0.0001
Glutamic acid/Glutamine	0.54 ^b \pm 0.11	0.93 ^b \pm 0.09	1.85 ^a \pm 0.27	0.50 ^b \pm 0.05	0.0001
Glycine	0.50 ^b \pm 0.10	0.87 ^b \pm 0.08	1.72 ^a \pm 0.25	0.47 ^b \pm 0.04	0.0001
Proline	0.41 ^b \pm 0.09	0.71 ^b \pm 0.07	1.41 ^a \pm 0.20	0.38 ^b \pm 0.04	0.0001
Serine	0.23 ^b \pm 0.05	0.39 ^b \pm 0.04	0.78 ^a \pm 0.11	0.21 ^b \pm 0.02	0.0001
Tyrosine	0.12 ^b \pm 0.02	0.20 ^b \pm 0.02	0.40 ^a \pm 0.06	0.11 ^b \pm 0.01	0.0001
Total Amino Acids	4.10 ^b \pm 0.85	7.05 ^b \pm 0.67	13.99 ^a \pm 2.01	3.79 ^b \pm 0.36	0.0001

Means within a row not sharing a common superscript differ ($P < 0.05$).

disadvantage of leaving more phytates and fibre in the extracted fraction. The presence of phytates is detrimental in terms of nutrition, because it decreases mineral absorption. It has also been shown in vitro that phytates can decrease the digestibility of proteins from leguminous seeds (Rosa-Sibakov, Re, Karsma, Laitila, & Nordlund, 2018). In the present in vivo study, the presence of phytates in Napin II did not appear to affect protein digestion, since the protein TID was not different from that of Napin I. Nevertheless, to improve the nutritional quality of the napin fraction, phytate content can be significantly reduced by incorporating a phytase treatment into the purification process (Defaix et al., 2019). The presence of fibre could be of nutritional interest in terms of its effect on health (Veronese et al., 2018). In the present study, their presence in Napin II did not affect the digestibility of napin (no difference of TID between Napin I and II), however this probably explains the large increase ($\times 2$) in endogenous amino acid losses observed between Napin I and II. Indeed, it was shown that fibre increases endogenous amino acid losses (Mariscal-Landin, Seve, Colleaux, & Lebreton, 1995). Three different mechanisms may underlie the short-term effect of fibre on endogenous amino acid losses: 1/ an increase in endogenous protein secretion, 2/ the adsorption of amino acids to fibre limiting their absorption, 3/ an accelerated transit rate.

In vitro pre-tests (data not shown) had indicated very low

Table 4

True ileal digestibility (TID) of amino acids of cruciferin, napin I (neutral extraction), napin II (acid extraction), and napine I-TT (neutral extraction + heating) in minipigs ($n = 5$). Values are means \pm SEM.

	Cruciferin	Napin I	Napin II	Napin I-TT	P ^a
Indispensable amino acids, %					
Histidine	91.30 \pm 2.85	85.57 \pm 2.26	87.03 \pm 3.42	86.53 \pm 2.43	0.4604
Isoleucine	96.60 ^a \pm 1.65	83.27 ^b \pm 3.04	80.43 ^b \pm 2.65	78.05 ^b \pm 3.12	0.0010
Leucine	99.48 ^a \pm 1.16	89.00 ^b \pm 3.91	85.73 ^b \pm 3.33	90.19 ^b \pm 1.78	0.0078
Lysine	97.03 ^a \pm 1.67	86.03 ^b \pm 1.52	93.68 ^a \pm 3.08	86.89 ^b \pm 1.44	0.0031
Methionine	98.47 ^a \pm 0.88	94.92 ^{ab} \pm 3.16	90.48 ^b \pm 3.19	97.02 ^{ab} \pm 1.18	0.0001
Phenylalanine	97.20 ^a \pm 1.59	88.03 ^b \pm 2.18	89.92 ^b \pm 3.24	87.98 ^b \pm 1.87	0.0134
Threonine	93.02 \pm 2.72	92.36 \pm 3.01	89.56 \pm 2.97	83.30 \pm 2.42	0.0520
Tryptophan	87.45 \pm 9.88	98.65 \pm 4.80	87.41 \pm 4.99	83.02 \pm 5.62	0.4713
Valine	96.52 ^a \pm 1.70	87.23 ^b \pm 3.2	86.27 ^b \pm 4.07	79.56 ^b \pm 2.42	0.0063
Dispensable amino acids, %					
Arginine	96.99 ^a \pm 1.32	84.75 ^b \pm 2.50	85.73 ^b \pm 3.68	87.17 ^b \pm 1.87	0.0130
Alanine	93.70 \pm 2.86	87.41 \pm 4.54	84.68 \pm 4.87	78.31 \pm 2.96	0.1187
Aspartic acid/Asparagine	93.17 ^a \pm 2.26	64.67 ^{ab} \pm 4.20	76.13 ^{ab} \pm 7.56	27.65 ^b \pm 8.84	0.0001
Cysteine	86.59 \pm 5.51	87.37 \pm 4.16	75.29 \pm 3.35	73.83 \pm 4.64	0.1300
Glutamic acid/Glutamine	97.34 ^a \pm 1.56	88.55 ^b \pm 3.27	82.42 ^b \pm 3.07	83.49 ^b \pm 2.54	0.0107
Glycine	86.08 ^{ab} \pm 10.36	96.23 ^a \pm 5.95	90.92 ^{ab} \pm 5.86	73.34 ^b \pm 6.41	0.0410
Proline	91.04 ^a \pm 4.71	91.86 ^a \pm 2.35	98.82 ^a \pm 4.65	78.87 ^b \pm 3.53	0.0031
Serine	94.06 ^a \pm 2.68	81.40 ^b \pm 4.21	86.32 ^{ab} \pm 4.78	79.79 ^b \pm 2.91	0.0370
Tyrosine	96.34 ^a \pm 1.76	75.18 ^b \pm 2.48	80.67 ^b \pm 4.67	85.53 ^b \pm 3.47	0.0039
Total Amino Acids	95.14 ^a \pm 2.21	87.63 ^b \pm 2.59	86.66 ^b \pm 2.67	81.21 ^b \pm 2.60	0.0085

Means within a row not sharing a common superscript differ ($P < 0.05$).

digestibility of the napin fraction. Suspecting a link with the conformation of the protein, heat treatment was applied to this fraction (Napin I-TT). The treatment conditions chosen derived from previous publications (Wu & Muir, 2008; Yang, Wang, Vasanthan, & Chen, 2014), evidencing the high thermal stability of napin, and the need for drastic heat treatment to induce its denaturation. As high temperature heat treatment can lead to the formation of protein aggregates detrimental to protein digestion (Bax et al., 2012), we ensured that under our conditions (1 h at 120 °C), the aggregates did not represent more than 5 % of the total napin. Nonetheless, the heat treatment applied in our study did not improve the TID of napin. The low digestibility of plant proteins can also be attributed to the presence of trypsin inhibitors in the protein fraction (Sarwar Gilani, Wu Xiao, & Cockell, 2012). In the present study trypsin inhibitors were mainly co-extracted from the rapeseed meal with the napin fraction. Antitrypsin activity was greater for Napin I than for Napin II. This difference could probably be explained by the partial denaturation of trypsin inhibitors upon acidic conditions. However, as documented in the literature (Li, Huang, Luo, & Tamer, 2020), the strong heat treatment applied to Napin I-TT totally abolished the anti-trypsin activity. Since this had no consequence on Napin TID, it may be speculated that the presence of trypsin inhibitors in the napin fraction was not the reason for its low digestibility. Finally, the presence of

Table 5

Digestible indispensable amino acids (g/ 100 g AA) of cruciferin, napin I (neutral extraction), napin II (acid extraction), and napine I-TT (neutral extraction + heating) in minipigs (n = 5). Values are means ± SEM.

	Cruciferin	Napin I	Napin II	Napin I-TT	P
Histidine	2.32 ^b ± 0.07	2.83 ^a ± 0.07	3.01 ^a ± 0.12	2.86 ^a ± 0.08	0.0005
Isoleucine	3.91 ^a ± 0.07	2.73 ^b ± 0.10	2.53 ^b ± 0.08	2.56 ^b ± 0.10	0.0001
Leucine	7.37 ^a ± 0.09	5.40 ^{bc} ± 0.24	4.99 ^c ± 0.19	5.48 ^b ± 0.11	0.0001
Lysine	5.61 ^c ± 0.10	6.66 ^b ± 0.12	7.55 ^a ± 0.25	6.73 ^b ± 0.11	0.0001
Methionine	2.02 ^c ± 0.02	2.79 ^a ± 0.09	2.43 ^b ± 0.09	2.86 ^a ± 0.03	0.0001
Cysteine	1.56 ^c ± 0.10	3.99 ^a ± 0.19	3.47 ^b ± 0.15	3.37 ^b ± 0.21	0.0001
Phenylalanine	5.64 ^a ± 0.09	3.32 ^c ± 0.08	3.60 ^b ± 0.13	3.32 ^c ± 0.07	0.0001
Tyrosine	2.50 ^a ± 0.05	1.35 ^c ± 0.04	1.48 ^{bc} ± 0.09	1.54 ^b ± 0.06	0.0001
Threonine	5.98 ^a ± 0.18	5.09 ^b ± 0.17	4.80 ^{bc} ± 0.16	4.59 ^c ± 0.13	0.0001
Tryptophan	1.25 ± 0.14	1.07 ± 0.05	1.24 ± 0.07	0.90 ± 0.06	0.0600
Valine	5.58 ^a ± 0.10	4.32 ^b ± 0.16	4.15 ^b ± 0.20	3.94 ^b ± 0.12	0.0001

Means within a row not sharing a common superscript differ ($P < 0.05$).

Table 6

Digestible indispensable amino acid scores (DIAAS, %), and limiting amino acid for cruciferin, napin I (neutral extraction), napin II (acid extraction), and napine I-TT (neutral extraction + heating).

	Cruciferin	Napin I	Napin II	Napin I-TT
Infant (birth to 6 months)	71 (Ile)	50 (Ile)	46 (Ile)	46 (Ile)
Child (6 months to 3 years)	99 (Lys)	82 (Leu)	76 (Leu)	80 (Ile)
Older child, adolescent, adult	117	89 (Leu)	82 (Leu)	85 (Ile)

Scores were calculated using the recommended amino acid scoring patterns for three age groups (FAO, 2013).

phenolic compounds, mainly sinapin in rapeseed protein extracts (Le et al., 2021), may interact with napins and decrease their digestibility. However, in this study a diafiltration step was carried out during napin purification that removes more than 90 % of the phenolic compounds initially present (data not shown).

Despite their low digestibility, total rapeseed protein (cruciferin and napin) has been shown to have a high nutritional potential for human nutrition. This was attributed to a balanced supply of amino acids and a low postprandial catabolism of the absorbed amino acids (Bos et al., 2007). Using the averaged intestinal digestibility of rapeseed AA (based on standard intestinal digestibility in pig), the DIAAS of total rapeseed protein can be estimated from the literature (Herreman, Nommensen, Pennings, & Laus, 2020). Reported values ranged from 41 to 56 for 0–0.5 y, from 53 to 75 for 0.5–3 y and from 36 to 89 above 3 y. The present study is the first to report the DIAAS of the two main fractions of rapeseed proteins. Due to its balanced amino acid composition and its high digestibility, the cruciferin fraction presents higher nutritional quality. For an intake covering protein requirements, it satisfies the indispensable amino acid requirements from the age of 3 years old. The nutritional quality of the napin fraction is penalized by its lower digestibility and its poor content in branched-chain amino acids, the level of which is limiting at all ages.

Although the extraction methods led to relatively low CML contents (< 1 %), reflecting a low Maillard reaction intensity. This low amount is

most probably explained by fact the hexane used for rapeseed cake deoiling was achieved at relatively low temperature (around 50 °C). It is interesting to note that the TID of lysine evolved in a comparable way to the CML content. An intriguing result of this study is the difference in CML content observed between Napin I and II. Napin I and Napin II were extracted from the meal at different pH (pH 7 and 2 respectively). It is well established that proteins are most extracted far from their isoelectric point. Napin isoelectric point (pI) is reported to be very high (around pH 11, Nioi et al., 2012). CML formation should strongly reduce napin isoelectric point since it neutralizes lysine positive charges. So, it can be hypothesised that pH 7 allows an effective extraction of low CML high pI napin while pH 2 is favourable to the extraction of high CML low pI napin. The increase in CML content between Napin I and Napin I-TT was consistent with the additional heat treatment applied.

Whatever the case, it should be kept in mind that rapeseed storage proteins represent a very good source of sulphur amino acids and lysine that can be combined with other sources of proteins like leguminous seeds for instance. In addition, new technological processes could be implemented to improve its digestibility. Although heat treatment at 120 °C for 1 h was not effective in improving napin digestibility, other treatments such as partial enzymatic hydrolysis was shown to improve it, while preserving its functional properties (Beaubier et al., 2023).

5. Conclusion

While low in vivo digestibility of total rapeseed protein has been consistently reported in the literature, this study highlights for the first time the difference in digestibility between cruciferin and napin, the two main fractions of rapeseed protein. Whereas the cruciferin fraction is highly digestible, the true ileal digestibility of the napin fraction is lower by about eight points. This digestibility was not affected by the napin extraction/purification process. Nevertheless, the process leading to the napin fraction containing the highest amount of fibre induced a greater loss of endogenous amino acids, which can negatively impact amino acid peripheral bioavailability. Whereas cruciferin has good nutritional quality (DIAAS > 100 for children and adults), the napin fraction is penalized by its low digestibility and its low content in branched-chain amino acids.

Funding

This work was performed in partnership with the SAS PIVERT, in the framework of the French Institute for the Energy Transition (Institut pour la Transition Énergétique (ITE) P.I.V.E.R.T. (www.institut-pivert.com), selected as an Investment for the Future (“Investissements d’Avenir”). This work was supported by the French Government as part of the Investments for the Future program under reference ANR-001-01. Partial funding was also provided by the INRAE (France).

CRedit authorship contribution statement

Romain Kapel: Supervision, Conceptualization. **Celine Niquet-Léridon:** Methodology, Investigation, Conceptualization. **Frédéric Tessier:** Supervision, Conceptualization. **Jean-Luc Messio:** Writing – original draft, Methodology, Investigation. **Caroline Buffière:** Methodology, Investigation. **Nouredine Hafnaoui:** Methodology. **Carole Migné:** Methodology. **Bérénice Houinsou-Houssou:** Methodology. **Alain Riaublanc:** Supervision, Investigation, Conceptualization. **Véronique Solé:** Methodology, Investigation. **Didier Rémond:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work

reported in this paper.

Acknowledgments

The authors thank D. Durand (UMRH, INRAE) for the surgery performed on minipigs, and B. Cohade (UNH, INRAE) for animal care.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.143161>.

Data availability

Data will be made available on request.

References

- AACC 08—01.01. Ash—Basic Method. AACC *Approved Methods of Analysis, 11th Ed. Cereals & Grains Association, St. Paul, MN, U.S.A.* Doi: <https://doi.org/10.1094/AACCIntMethod-08-0.1.01>.
- AACC 32—05.01. Total Dietary Fiber. AACC *Approved Methods of Analysis, 11th Ed. Cereals & Grains Association, St. Paul, MN, U.S.A.* Doi: <https://doi.org/10.1094/AACCIntMethod-32-05.01>.
- AACC 44—15.02. Moisture—Air-Oven Methods. AACC *Approved Methods of Analysis, 11th Edition. Cereals & Grains Association, St. Paul, MN, U.S.A.* Doi: <https://doi.org/10.1094/AACCIntMethod-44-15.02>.
- AACC 46—16.01. Crude Protein—Improved Kjeldahl Method. AACC *Approved Methods of Analysis, 11th Ed. Cereals & Grains Association, St. Paul, MN, U.S.A.* Doi: <https://doi.org/10.1094/AACCIntMethod-46-16.01>.
- Bacon, J. R., Wanigatunga, S. C. D. R., & Fenwick, G. R. (1995). A microassay for the analysis of trypsin inhibitor activity in peas. *Food Chemistry, 52*, 77–80. [https://doi.org/10.1016/0308-8146\(94\)P4184-H](https://doi.org/10.1016/0308-8146(94)P4184-H)
- Bax, M. L., Aubry, L., Ferreira, C., Daudin, J. D., Gatellier, P., Rémond, D., & Sante-Lhoutellier, V. (2012). Cooking temperature is a key determinant of in vitro meat protein digestion rate: Investigation of underlying mechanisms. *Journal of Agricultural and Food Chemistry, 60*(10), 2569–2576. <https://doi.org/10.1021/jf205280y>
- Beaubier, S., Pineda-Vadillo, C., Mesieres, O., Framboisier, X., Galet, O., & Kapel, R. (2023). Improving the in vitro digestibility of rapeseed albumins resistant to gastrointestinal proteolysis while preserving the functional properties using enzymatic hydrolysis. *Food Chemistry, 407*, Article 135132. <https://doi.org/10.1016/j.foodchem.2022.135132>
- Berot, S., Compoin, J. P., Larre, C., Malabat, C., & Gueguen, J. (2005). Large scale purification of rapeseed proteins (*Brassica napus* L.). *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 818*(1), 35–42. <https://doi.org/10.1016/j.jchromb.2004.08.001>
- Bos, C., Airinei, G., Mariotti, F., Benamouzig, R., Berot, S., Evrard, J., ... Gaudichon, C. (2007). The poor digestibility of rapeseed protein is balanced by its very high metabolic utilization in humans. *The Journal of Nutrition, 137*(3), 594–600. <https://doi.org/10.1093/jn/137.3.594>
- Bos, C., Mahe, S., Gaudichon, C., Benamouzig, R., Gausseres, N., Luengo, C., ... Tome, D. (1999). Assessment of net postprandial protein utilization of 15N-labelled milk nitrogen in human subjects. *The British Journal of Nutrition, 81*(3), 221–226. <https://www.ncbi.nlm.nih.gov/pubmed/10434848>.
- Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., & Rosicka-Kaczmarek, J. (2021). Canola/rapeseed protein - nutritional value, functionality and food application: A review. *Critical Reviews in Food Science and Nutrition, 61*(22), 3836–3856. <https://doi.org/10.1080/10408398.2020.1809342>
- Defaix, C., Kapel, R., & Galet, O. (2019). Protein isolates and process for the production thereof. *European Patent Office, Article EP3494797A1*.
- Deglaire, A., Bos, C., Tome, D., & Moughan, P. J. (2009). Ileal digestibility of dietary protein in the growing pig and adult human. *The British Journal of Nutrition, 102*(12), 1752–1759. <https://doi.org/10.1017/S0007114509991267>
- Dong, X. Y., Guo, L. L., Wei, F., Li, J. F., Jiang, M. L., Li, G. M., ... Chen, H. (2011). Some characteristics and functional properties of rapeseed protein prepared by ultrasonication, ultrafiltration and isoelectric precipitation. *Journal of the Science of Food and Agriculture, 91*(8), 1488–1498. <https://doi.org/10.1002/jsfa.4339>
- FAO. (2013). *Dietary protein quality evaluation in human nutrition* (p. 2013). Rome: Food and Agriculture Organisation.
- García-Estépa, R. M., Guerra-Hernández, E., & García-Villanova, B. (1999). Phytic acid content in milled cereal products and bread. *Food Research International, 32*, 217–221.
- Gaudichon, C., Bos, C., Morens, C., Petzke, K. J., Mariotti, F., Everwand, J., ... Metzger, C. C. (2002). Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. *Gastroenterology, 123*(1), 50–59. <https://doi.org/10.1053/gast.2002.34233>
- Ghodsvall, A., Haddad Khodaparast, M. H., Vosoughi, M., & Diosady, L. L. (2005). Preparation of canola protein materials using membrane technology and evaluation of meals functional properties. *Food Research International, 38*, 223–231.
- Guillin, F. M., Gaudichon, C., Guerin-Deremaux, L., Lefranc-Millot, C., Airinei, G., Khodorova, N., ... Calvez, J. (2022). Real ileal amino acid digestibility of pea protein compared to casein in healthy humans: A randomized trial. *The American Journal of Clinical Nutrition, 115*(2), 353–363. <https://doi.org/10.1093/ajcn/nqab354>
- Herremans, L., Nommensen, P., Pennings, B., & Laus, M. C. (2020). Comprehensive overview of the quality of plant- and animal-sourced proteins based on the digestible indispensable amino acid score. *Food Science & Nutrition, 8*(10), 5379–5391. <https://doi.org/10.1002/fsn3.1809>
- Le Bourgout, C., Liu, X., Buffiere, C., Hafanaoui, N., Salis, L., Pouyet, C., ... Remond, D. (2023). Development of a protein food based on texturized wheat proteins, with high protein digestibility and improved lysine content. *Food Research International, 170*, Article 112978. <https://doi.org/10.1016/j.foodres.2023.112978>
- Le, T. T., Framboisier, X., Aymes, A., Ropars, A., Frippiat, J. P., & Kapel, R. (2021). Identification and capture of phenolic compounds from a rapeseed meal protein isolate production process by-product by macroporous resin and valorization their antioxidant properties. *Molecules, 26*(19). <https://doi.org/10.3390/molecules26195853>
- Li, Q., Huang, L., Luo, Z., & Tamer, T. M. (2020). Stability of trypsin inhibitor isolated from potato fruit juice against pH and heating treatment and in vitro gastrointestinal digestion. *Food Chemistry, 328*, Article 127152. <https://doi.org/10.1016/j.foodchem.2020.127152>
- Mariscal-Landin, G., Seve, B., Colleaux, Y., & Lebreton, Y. (1995). Endogenous amino nitrogen collected from pigs with end-to-end ileocecal anastomosis is affected by the method of estimation and altered by dietary fiber. *The Journal of Nutrition, 125*(1), 136–146. <https://doi.org/10.1093/jn/125.1.136>
- Nioi, C., Kapel, R., Rondags, E., & Marc, I. (2020). Selective extraction, structural characterisation and antifungal activity assessment of napins from industrial rapeseed meal. *Food Chemistry, 134*, 2149–2155.
- Niquet-Léridon, C., & Tessier, F. J. (2011). Quantification of Ne-carboxymethyl-lysine in selected chocolate-flavoured drink mixes using high-performance liquid chromatography—linear ion trap tandem mass spectrometry. *Food Chemistry, 126*(2), 655–663. <https://doi.org/10.1016/j.foodchem.2010.10.111>
- Nogueira Silva Lima, M. T., Howsam, M., Delayre-Orthez, C., Jacolat, P., Jaisson, S., Criquet, J., ... Tessier, F. J. (2023). Glycated bovine serum albumin for use in feeding trials with animal models - in vitro methodology and characterization of a glycated substrate for modifying feed pellets. *Food Chemistry, 428*, Article 136815. <https://doi.org/10.1016/j.foodchem.2023.136815>
- Oberli, M., Marsset-Baglieri, A., Airinei, G., Sante-Lhoutellier, V., Khodorova, N., Remond, D., ... Gaudichon, C. (2015). High true ileal digestibility but not postprandial utilization of nitrogen from bovine meat protein in humans is moderately decreased by high-temperature, Long-Duration Cooking. *J Nutr, 145*(10), 2221–2228. <https://doi.org/10.3945/jn.115.216838>
- Ostbring, K., Tullberg, C., Burri, S., Malmqvist, E., & Rayner, M. (2019). Protein recovery from rapeseed press cake: Varietal and processing condition effects on yield, emulsifying capacity and antioxidant activity of the protein rich extract. *Foods, 8* (12). <https://doi.org/10.3390/foods8120627>
- Pedroche, J., Yust, M. M., Lqari, H., Giron-Calle, J., Alaiz, M., Vioque, J., & Millan, F. (2004). *Brassica carinata* protein isolates: Chemical composition, protein characterization and improvement of functional properties by protein hydrolysis. *Food Chemistry, 88*, 337–346.
- Reynaud, Y., Buffiere, C., Cohade, B., Vauris, M., Liebermann, K., Hafanaoui, N., ... Remond, D. (2021). True ileal amino acid digestibility and digestible indispensable amino acid scores (DIAAS) of plant-based protein foods. *Food Chemistry, 338*, Article 128020. <https://doi.org/10.1016/j.foodchem.2020.128020>
- Rosa-Sibakov, N., Re, M., Karsma, A., Laitila, A., & Nordlund, E. (2018). Phytic acid reduction by bioprocessing as a tool to improve the in vitro digestibility of Faba bean protein. *Journal of Agricultural and Food Chemistry, 66*(40), 10394–10399. <https://doi.org/10.1021/acs.jafc.8b02948>
- Sarwar Gilani, G., Wu Xiao, C., & Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *The British Journal of Nutrition, 108*(Suppl. 2), S315–S332. <https://doi.org/10.1017/S0007114512002371>
- Veronese, N., Solmi, M., Caruso, M. G., Giannelli, G., Osella, A. R., Evangelou, E., ... Tzoulaki, I. (2018). Dietary fiber and health outcomes: An umbrella review of systematic reviews and meta-analyses. *The American Journal of Clinical Nutrition, 107* (3), 436–444. <https://doi.org/10.1093/ajcn/nqx082>
- Wu, J., & Muir, A. D. (2008). Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of Food Science, 73*(3), C210–C216. <https://doi.org/10.1111/j.1750-3841.2008.00675.x>
- Xu, L., & Diosady, L. L. (2000). Interactions between canola proteins and phenolic compounds in aqueous media. *Food Research International, 33*, 725–731.
- Yang, C., Wang, Y. X., Vasanthan, T., & Chen, L. Y. (2014). Impacts of pH and heating temperature on formation mechanisms and properties of thermally induced canola protein gels. *Food Hydrocolloids, 40*, 225–236. <https://doi.org/10.1016/j.foodhyd.2014.03.011>
- Yoshie-Stark, Y., Wada, Y., & Wäsche, A. (2008). Chemical composition, functional properties, and bioactivities of rapeseed protein isolates. *Food Chemistry, 107*, 32–39.