In vitro protein digestibility – selected rapeseed protein products

Jens Christian Sørensen, Marcel Jøhnke, Hilmer Sørensen, Susanne Sørensen, Keld Ejdrup Markedal
FOOD – University of Copenhagen, Denmark

UNIVERSITY OF COPENHAGEN

In Vitro Digestibility of Selected Rapeseed Protein Products

Jens Christian Sørensen, Marcel Jøhnke, Susanne Sørensen, Hilmer Sørensen, Keld Ejdrup Markedal

Department of Food Science, University of Copenhagen, Frederiksberg, Denmark

Exploring the full nutritional potential of proteins within a food or feed product is related to the digestibility of the proteins. In addition a well-balanced amino acid profile is key for optimal nutritional value of the protein. Rapeseed protein as a whole is considered to have a high nutritional value. In addition the two major groups of rapeseed storage proteins show considerable differences with respect to functionality thus providing a potential for value addition by fractionation. Processing and fractionation is expected to influence the digestibility of the specific protein fractions and digestibility is thus an important quality parameter to include in design of optimal processing procedures, and the use of fast characterization methods are needed for iterative process optimization.

In the present work, several rapeseed protein products rich in 2S napin protein were prepared using various mild pilot-scale processing schemes based on acidic aqueous extraction, ion-exchange chromatography, and membrane processing. The napin-rich rapeseed protein products were post-process defatted yielding final products with variable content of proteins and fibers. The aim was to investigate the effect of processing on *in vitro* protein digestibility (IVPD) and trypsin inhibitor activity (TIA) level of the napin-rich rapeseed protein products. Presence of protease inhibitors (e.g. trypsin inhibitors) in rapeseed can adversely affect the protein digestibility. Many processes have been developed to inactivate protease inhibitors (e.g. thermal treatments). However, these processes often alter the protein structure of also non-target proteins, which may lead to poor nutritional value of plant-based protein products due to increased protein denaturation, reduced protein solubility, and decreased protein digestibility. In addition, different compounds apart from proteins as e.g. glucosinolate transformation products and phenolics also have the capacity to bind or interact with proteins causing diminished or slower rate of proteolysis.

The IVPD and TIA levels were assessed using analytical methods developed in our laboratory. IVPD values were subsequently compared to expected values for maximal enzymatic cleavage of napin protein isoforms based on AA sequences obtained from protein databases. In addition to the specific cleavage site specificity of digestive enzymes, in vitro enzymatic digestibility is also influenced by the amino acids in proximity of the reactive site. Preliminary results showed that napin protein products are less susceptible to peptic digestion irrespective of the processing method used for their purification. This limited digestion behavior exerted by pepsin may be associated with a large proportion (25% to 31.3%) of potential peptic amino acid sites being blocked due to the presence of adjacent proline residues in the amino acid sequences of napin protein isoforms. Pancreatic digestion was demonstrated to elicit higher IVPD compared to pepsin in all products, but the overall IVPD values were significantly different amongst the napin protein products ranging from 8.9% to 17.7%. These differences are possibly due to a combined effect of variations in the TIA level, as well as the degree of process-induced protein modifications. The products were found to contain significantly different TIA levels, with a clear link to the employed processing method.

Outline

- Why in vitro digestibility
- What would we like to test
  - What is the effect of processing on protein digestibility
  - Other components affecting the outcome
- What do we test
- Results
- Conclusion

In vitro digestion (pros and cons)

- Possibility of testing many samples
- Less costly relative to in vivo studies
- Challenges include – mimicked mechanical movement, mixing, resident time, controlled secretion of enzymes, pH fluctuations, doesn't include availability …
- Does not substitute in vivo studies
- But may supplement these with other possibilities

Materials and Methods

- Protein samples 5 mg/mL in 0.05 M HCl (U)
- Pepsin treatment: 1:50 mass ratio pepsin (porcine) :protein
  - 1 hour at 37.5 C stirred (pH 1.7-2) (P)
- Pancreatin treatment 1:10 mass ratio pancreatin (porcine):protein
  - 1 hour at 37,5 C stirred in sodium cholate, bicarbonate (pH 7.5) (PT)

IVPD method

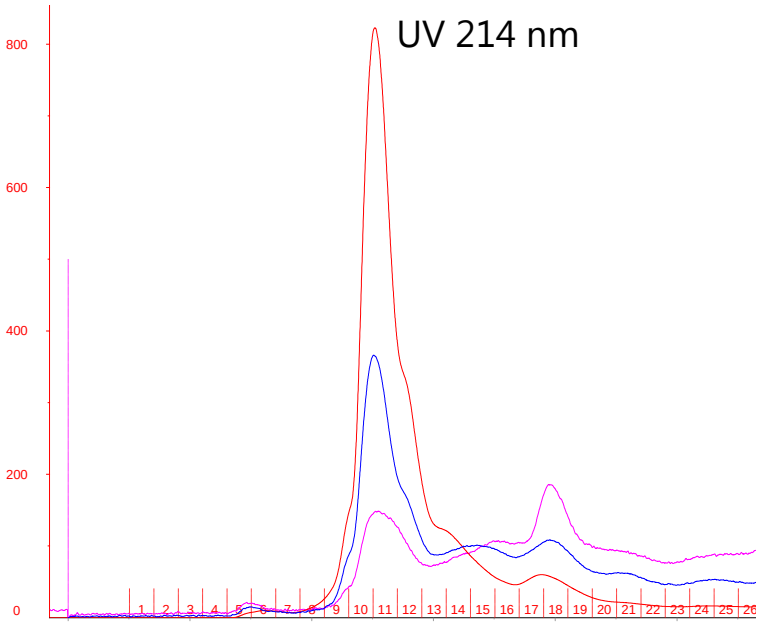
- Analysis of released (free) amino groups after reaction with TNBS (Trinitrobenzenesulfonic acid)
- Alanine as standard (yielding 100 %)
- Analysis of in vitro digestibility by SDS-PAGE

Protein products to be tested

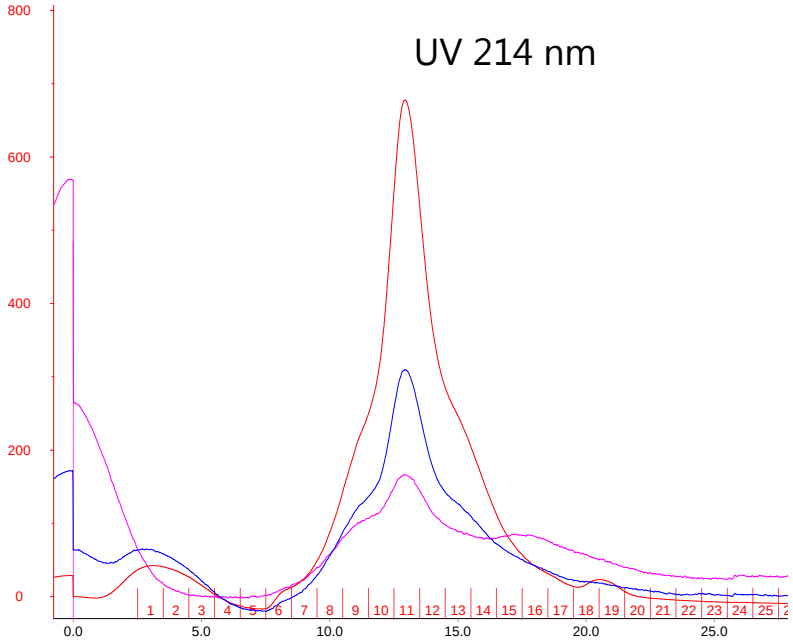
- Rapeseed proteins produced in pilot scale
- 2 products (Ultrafiltration or ion exchange chromatrography)
- Bovine serum albumin (BSA)
- Selected milk proteins (casein, α-lactalbumin, β-lactoglobulin)

Rapeseed protein product characteristics

- Protein profile (size exclusion: Superdex 75)

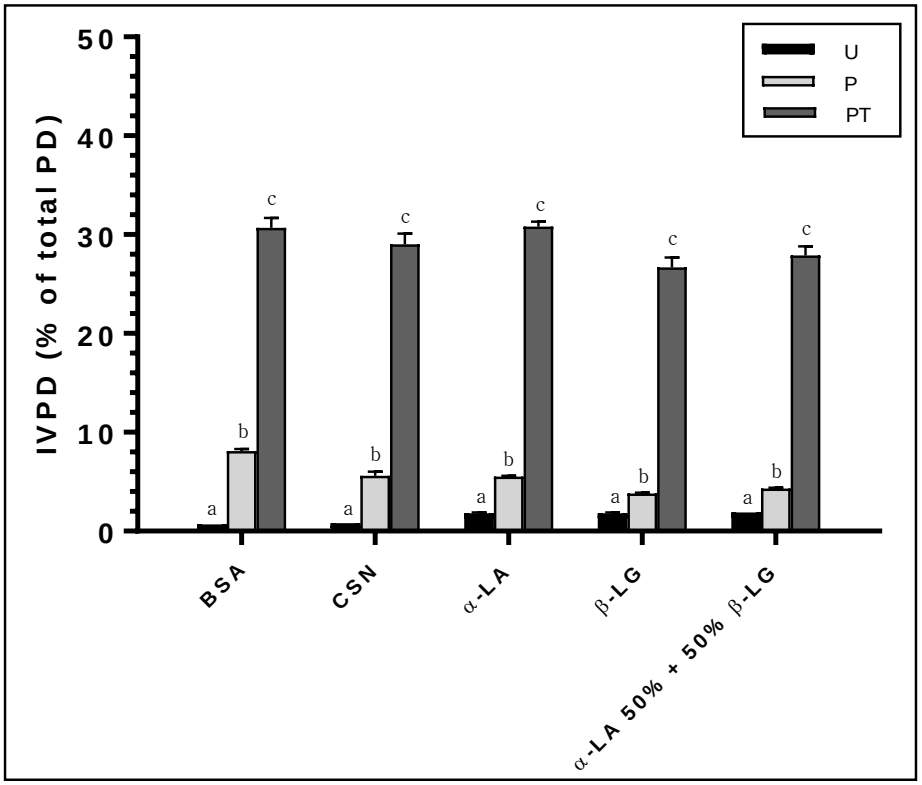


RP1: 63 % protein (N x 6.25)
Calculated Mw approximately (18 kDa)

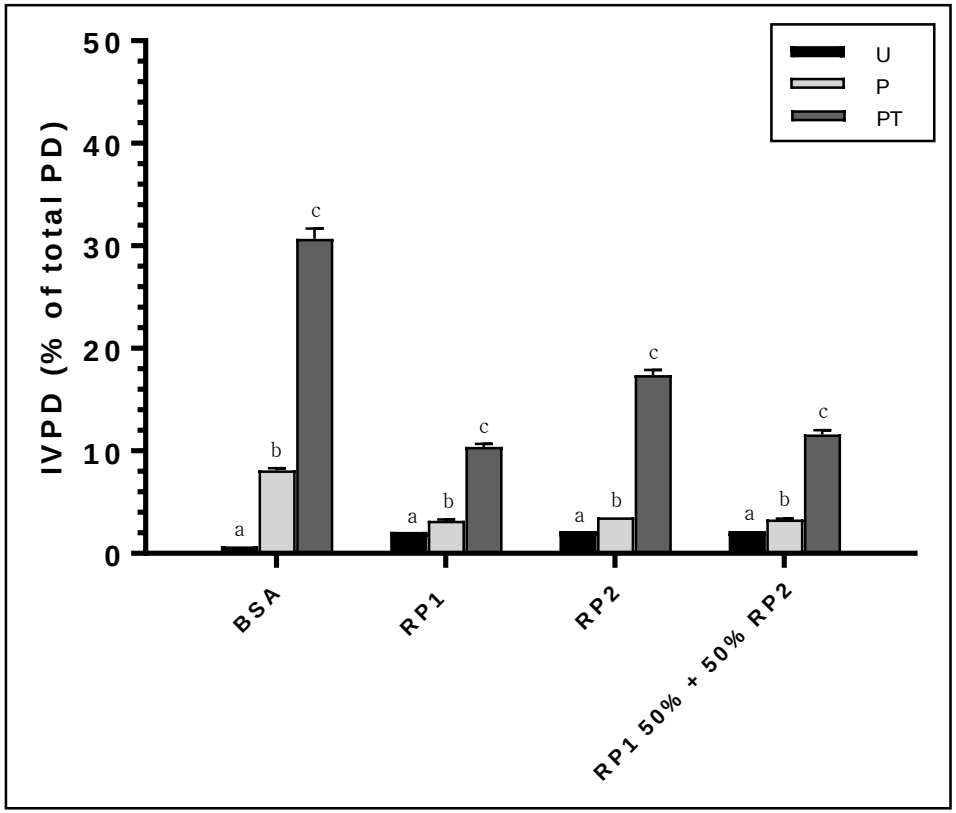


RP2: 70 % protein (N x 6.25)
Calculated Mw approximately (15 kDa)

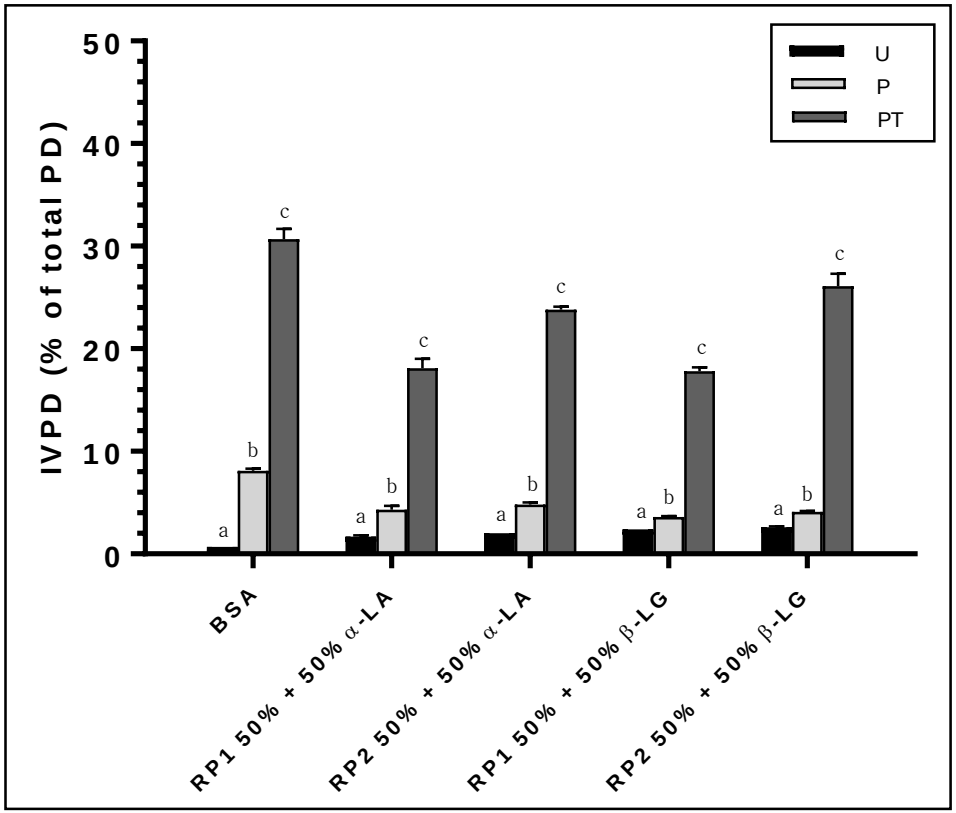
In vitro digestibility of milk proteins



In vitro digestibility of rapeseed protein products + mix

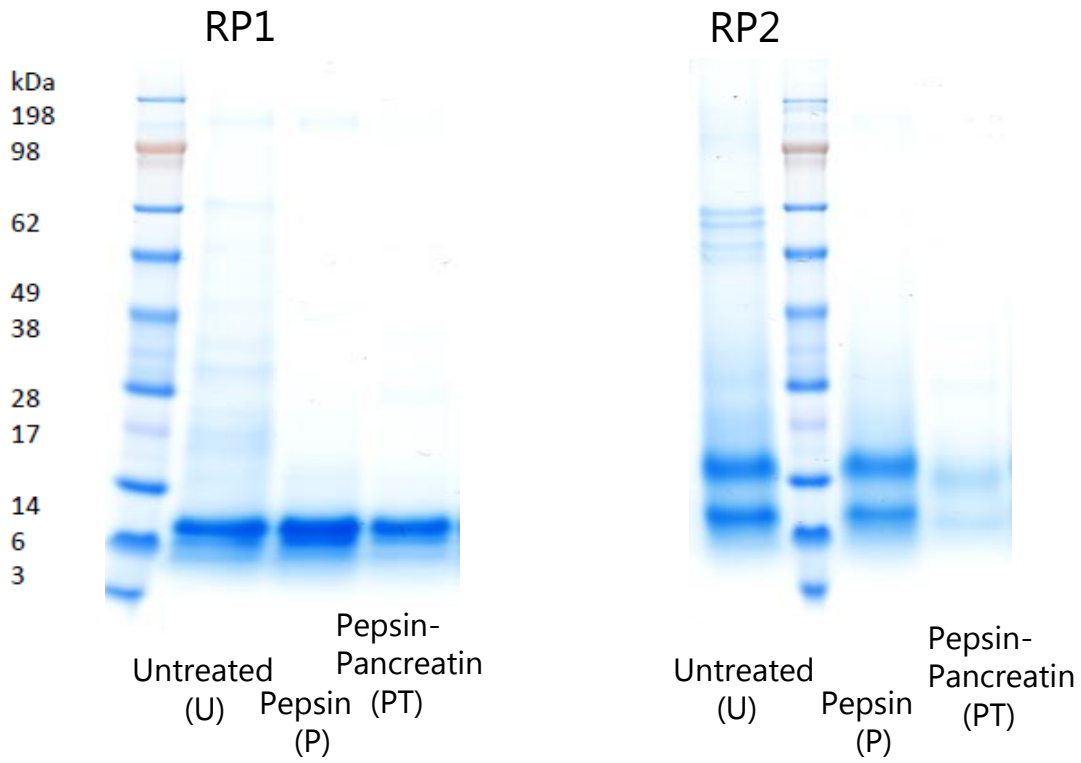


In vitro digestibility of rapeseed protein products combined with milk proteins



- RP1 and RP2 seems to be acting differently on digestibility relative to which milk protein it is combined with – interestingly digestibility is almost raised to the level of β-lactoglobulin

SDS-PAGE to illustrate in vitro digestibility



- RP1 clearly less digested than RP2 and subunits still visible

Content of rapeseed proteinase inhibitors

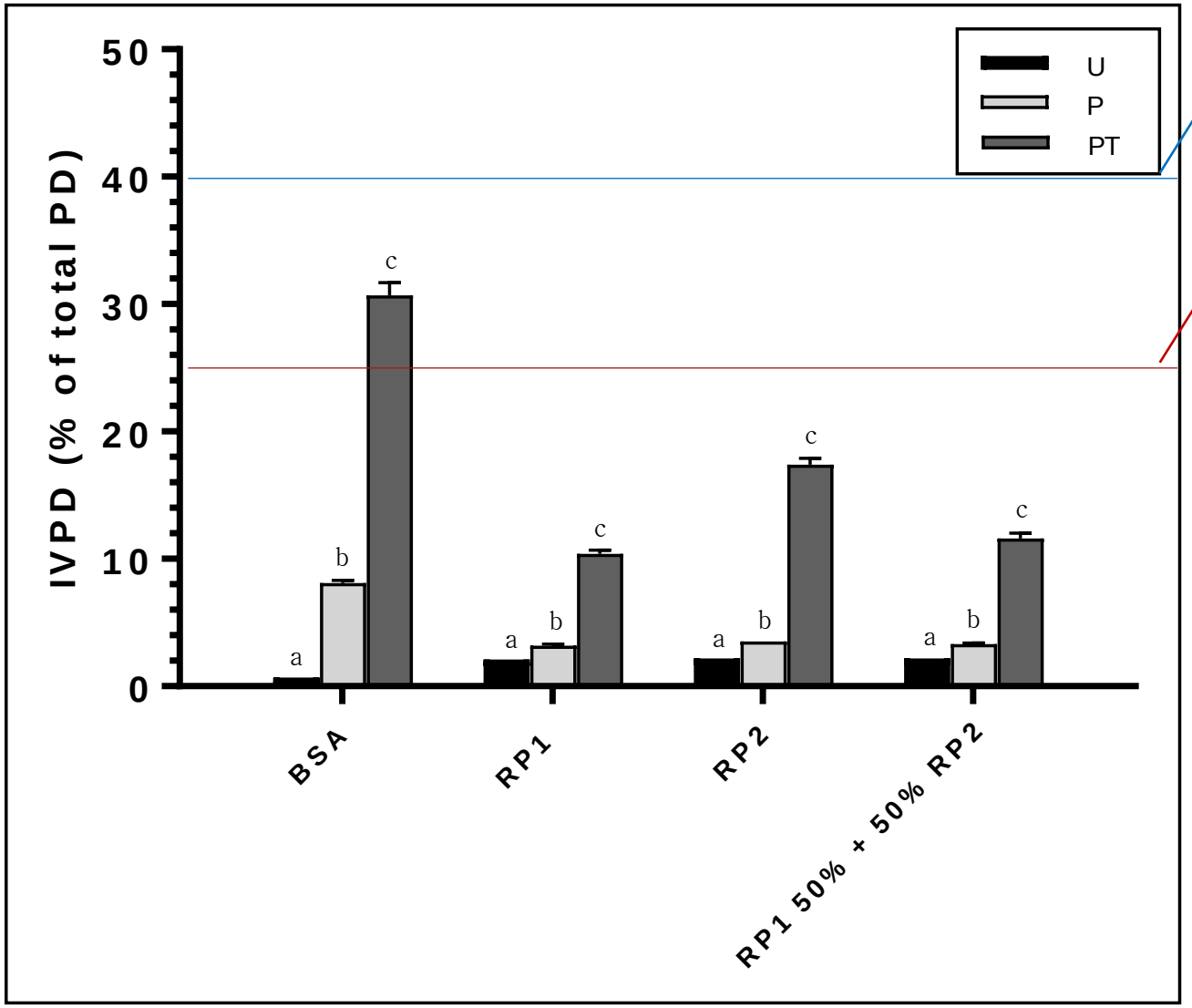
- Trypsin inhibitor level:
- RP1: 8 TIA/g
- RP2: less than 1 TIA/g

Despite the difference in inhibitor level, this does not fully account for the difference between RP1 and RP2 digestibility

Specificity of pepsin and pancreatin

- Pepsin cleaves at N side of Leu, Phe, Trp, Tyr
- Pancreatin (trypsin and chymotrypsin) cleaves at C-side of Lys and Arg or Phe, Trp, Tyr respectively

Proteins	Pepsin sites [%]	Trypsin sites [%]	Chymotrypsin sites [%]	Total pancreatic sites [%]	Total sites [%]
Napins	8.8 ± 0.9 [a]	11 ± 0.6 [b]	5 ± 1.6 [a]	15.9 ± 1.4 [a]	24.7 ± 2.1 [a]
Cruciferins	14.1 ± 1.3 [b]	7.8 ± 0.3 [a]	6.9 ± 0.6 [a]	14.8 ± 0.7 [a]	28.7 ± 1.9 [a]
Whey proteins	19.3 ± 1.1 [c]	11.5 ± 1.5 [b]	8.1 ± 1.8 [a]	19.6 ± 2.5 [a]	38.9 ± 2.6 [b]
BSA	18.2	13.3	8.3	21.5	39.8
α-LA	20.5	10.7	9.8	20.5	41
β-LG	19.3	10.6	6.2	16.8	36
Caseins	13.6 ± 2.3 [b]	9.1 ± 3 [ab]	6.4 ± 2.7 [a]	15.5 ± 5.3 [a]	29.1 ± 7.1 [a]


IVPD (% of total PD)
50
40
30
20
10
0
U
P
PT
a
b
c
BSA
RP1
RP2
RP1 50% + 50% RP2
BSA max
Napin max

Conclusion

- Digestibility is not a guaranty of availability of the amino acids
- Protein composition in products may vary from seed/meal/pressed cake
- Effects from trypsin inhibitors, matrix effects, other compounds, heat treatment .....
- A test system may help in elucidating effects from other and non-protein compounds
- Differences has been observed in in vitro digestibility of rapeseed protein products (both mainly napin type products) related to differences in processing