

Master of Science HES-SO in Life Sciences

**Soybeans as a valuable alternative source
of proteins: potential contribution of fermentation
on digestibility.**

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DESCRIPTION

In Switzerland, a part of the population are flexitarians, vegetarians or vegans according to a market study by COOP [1]. These diets consists of a reduction of the meet consumption or stopping meat and fish consumption or stopping the consumption of all animal products, respectively. The motivation for adopting these diets are health reasons, a desire for variety, environmental concern or animal welfare. To meet the demand for alternatives to meat, more and more plant-based proteins are being offered in supermarkets. In view of the growth of these diets, and in the context of human nutrition and health, it is important to study the digestibility of these plant-based proteins. Indeed, the indispensable amino acids (IAA : phenylalanine, leucine, methionine, lysine, isoleucine, valine, threonine, tryptophane and histidine) present in proteins are not synthesised by the human body and must be obtained through the food.

Considering this key element and in contrary to animal protein, which are easy to digest and release AA; in plant-based proteins, there are antinutrients like proteases inhibitors, lectins and tannins that reduce the digestibility and therefore the access to IAA. To overcome this obstacle, treatment before the consumption of food can be implemented like fermentation or extrusion. The fermentation process could allow better availability of IAA. In the case of extrusion, the combination of high temperature, pression and shear force, proteins undergo a change in their structure than can potentially increase digestibility.

To assess the impact of these treatments on protein digestibility, a protocol of *in vitro* digestion developed by INFOGEST [2], and scores can be calculated. This score, developed by the FAO [3] in 2013 is the digestible indispensable amino acids score (DIAAS) which consider each IAA individually and is determined by the lowest score after *in vitro* digestion.

Therefore, to investigate the effect of fermentation on the digestibility of plant-based proteins, two food models were chosen. These were tempeh and natto from soybeans.

OBJECTIFS

The first objective of this project was to optimise the fermentation process of soybeans with *Rhizopus oligosporus* and *Bacillus subtilis* as microorganisms in order to obtain tempeh and natto respectively, which could be used as food model. Four times points were chosen for each fermentation (tempeh : 0h, 24h, 40h and 48h and natto: 0h, 16h, 24h and 48h).

Secondly, the aim was to determine whether the fermentations reduced the presence of antinutrients such as trypsin inhibitor. This was tested by measuring trypsin activity in the presence of the different time points of the fermented soybeans.

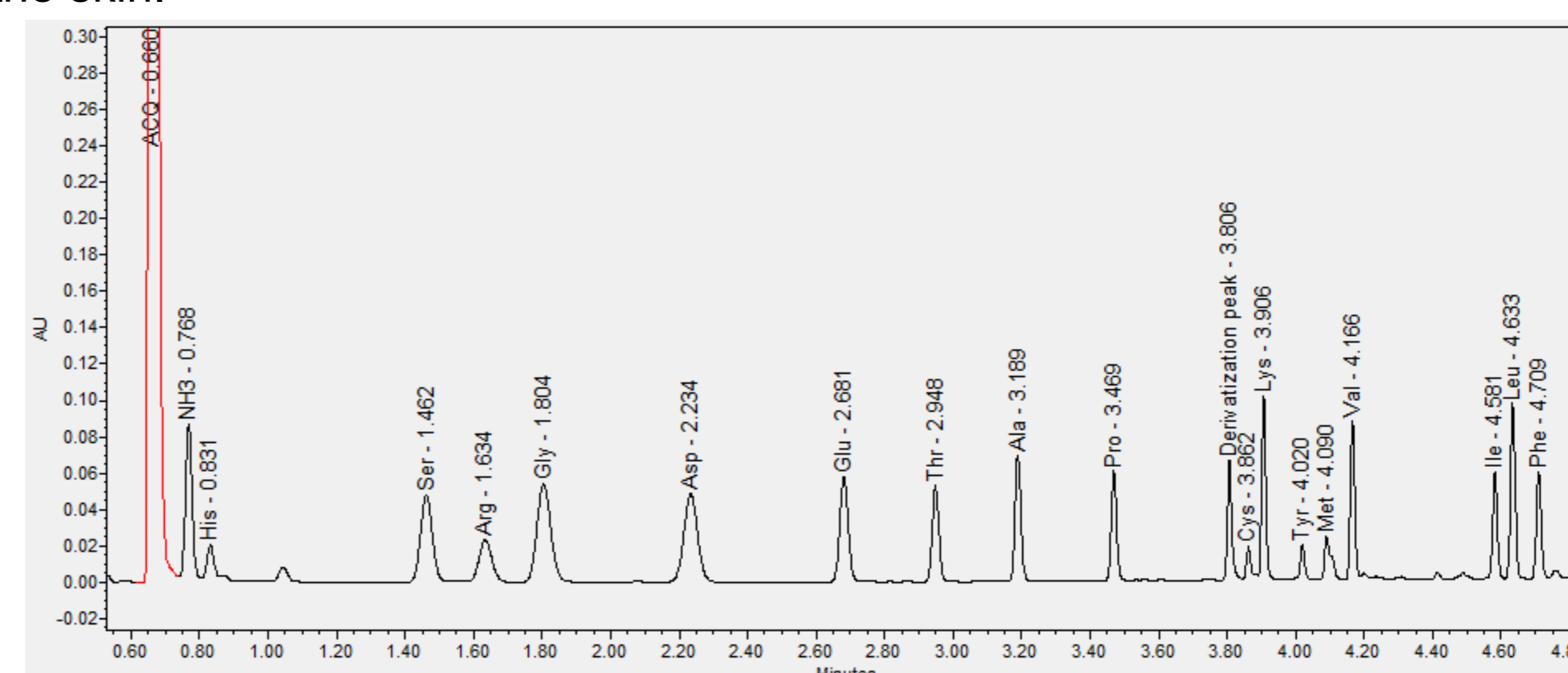
Thereafter, the purpose was to observe whether fermentation of soybeans increased the digestibility of the proteins. These digestibility were determined following *in vitro* digestion of the fermentations and acid hydrolysis by R-NH₂ quantification (OPA) and by quantification of each IAA by UPLC/UV.

Finally, the aim was to investigate the potential of fermentation to improve the amount of IAA and to provide a sufficient daily intake according to the recommendations. From the quantification of each IAA by UPLC/UV, digestible indispensable amino acid ratio (DIAAR) and DIAAS were calculated to evaluate this. For each IAA, DIAAR is calculated and the lower DIAAR represent the DIAAS, which consider the most limiting IAA.

RESULTS

After several trials to produce tempeh and natto, products conforming to the commercial ones were obtained. The final product of tempeh is soybeans surrounded by with mycelium with a fresh mushroom and nutty smell. For natto, soybeans covered with sticky filaments are obtained. However, the result is less sticky than commercial natto. The reason of the weaker aspect of the sticky filament could be linked to the too small size of the sample which does not allow proper development of the bacteria.

Concerning the decrease of antinutrients in fermented soybeans, for tempeh, there was no significant change between the different fermentation times points. However, a different phenomenon is observed in the presence of the natto. While the T0h displayed an inhibition of trypsin activity, this activity seems to be, at least partially, recovered at the early stage of the fermentation(16h).This could indicate that the fermentation could potentially decrease the antinutrients effect on trypsin activity. Nevertheless, the activity of the trypsin could not be fully restored under these conditions. In natto, contrary to tempeh, the skin of the soybeans is preserved. The observed inhibition of the trypsin seems to confirm that antinutrients are mainly present in the skin.



Chromatogram of derivatized amino acids presents in pellet of natto 0h after *in vitro* digestion.

As shows in the chromatogram, the peak of each amino acids as a good resolution and a good peak shape which allow to easily quantify each of them.

The digestibility of tempeh and natto, shown in the table, does not display significant variation. This observation is not surprising as soybeans has been described as highly digestible. The fermentation does not improve the digestibility.

For tempeh as for natto and for the three age groups, the limiting amino acid in the calculation of DIAASs is leucine. According to the DIAAS in the table and the DIAAR, tempeh and natto would appear to be good source of IAA but needs to be combined with another protein source that can compensate for the lack of IAA. For some IAA, the DIAAR are lower than 100 which indicate that this IAA are not present in sufficient quantity to respond to the daily recommendation intake.

Digestibility [%]	Tempeh 0h	Tempeh 24h	Tempeh 40h	Tempeh 48h
	99,6	99,5	98,7	97,3
DIAAS	Tempeh 0h	Tempeh 24h	Tempeh 40h	Tempeh 48h
Infant (birth to 6 month)	LEU: 38.8	LEU: 44	LEU: 41.1	LEU: 44.3
Child (6 month to 3 year)	LEU: 56.4	LEU: 64	LEU: 59.8	LEU: 64.4
Older child, adolescent, adult	LEU: 61	LEU: 69.3	LEU: 64.7	LEU: 69.7
Digestibility [%]	Natto 0h	Natto 16h	Natto 24h	Natto 40h
	99,5	99,5	96,5	96,4
DIAAS	Natto 0h	Natto 16h	Natto 24h	Natto 40h
Infant (birth to 6 month)	LEU: 41.1	LEU: 46.8	LEU: 45	LEU: 33.2
Child (6 month to 3 year)	LEU: 59.7	LEU: 68.1	LEU: 65.4	LEU: 48.3
Older child, adolescent, adult	LEU: 64.6	LEU: 73.7	LEU: 70.8	LEU: 52.2

Digestibility and DIAAS of tempeh and natto at different time point

CONCLUSION

In conclusion, investigation on the reduction of antinutrients showed that natto could partially reduce the presence of trypsin inhibitor mainly present in the hull of soybeans. After *in vitro* digestion, the different fermentation times of tempeh and natto show high protein digestibility, especially for crude soybeans. This means that soya proteins are easy digestible plant-based protein, but the crude soybeans, with a hull could benefit from a fermentation process. Regarding DIAARs and DIAAS, the different fermentation times of tempeh show high values for most of the IAA but remain below the recommended daily intake of IAA and should be complemented with other protein source. For natto, too long fermentation time shows a decrease in DIAARs which could be due to microorganisms that consume part of the IAA and release ammonia. Among the fermentation times performed, T16h gives the best results.

With the aim of providing enough IAA to people consuming plant-based proteins, research on the mix of various sources of proteins of plant origin could be interesting. Moreover, more detailed studies of antinutrients and possible treatments, in addition to the fermentation, to reduce them seems to be an essential point concerning the supply of nutrients. Indeed, antinutrients do not only interfere with the bioavailability of proteins but also on other nutrients bioavailability such as minerals and vitamins, that can form complex with antinutrients.