УДК 664.38

A comparative analysis of research methods for determining digestibility of high-protein products

Negasi Tsighe K. (ITMO-National Research University, Saint Petersburg, Russia)
Scientific supervisor - Associate Professor, Doctor of Technical Sciences Nadtochii Liudmila Anatolevna (ITMO-National Research University, Saint Petersburg, Russia)

The present review summarizes on digestibility of high protein products and comparative analysis of factors affecting their values. High protein products of animal origin had more protein digestibility values than plant origin; in vitro methods are more rapid with variability, less expensive, less labour intensive with no ethical restrictions than more accurate in vivo; the true ilea protein digestibility most accurate developed quantification method. This initiates more comparative studies to evaluate and validate in vitro digestibility with in vivo data to minimize variation to standardize in vitro method.

Typically meat, bean and dairy products are nutritionally characterized high-protein foods because of their high protein quality measured after protein digestibility and their ability to provide all the amino acids required for growth and maintenance of body functions. Among the various protein quality determination, is protein digestibility that measures available proteins and its products after gastrointestinal enzymatic digestion to utilize as diet after absorption. In vitro simulated digestion methods try to mimic physiological conditions of in vivo such as presence of digestive enzymes and their concentrations, pH, digestion time, and salt concentrations, among other factors in the oral, gastric and small intestinal phases. The expensive in vivo method uses target species or suitable animal model (rat/pig) to obtain fecal digestibility from feces; true ileal digestibility using naso-intestinal intubation or through co-operation of ileostomates; growth, nitrogen balance and hematological methods. The average protein digestibility reports are for insect 76-96%, for milk proteins 92-100%; for egg protein 95% or beef 85-98% and pulse 89-. In numerous literatures, a highly profound variability of results between in vitro protein digestibility methods used was observed than in vivo that might be the use of different bioassay parameters, mechanism of determination of digestion products, sample type used and origin, internal and external factors of test protein, calculation methods among the many factors. To deepen specific factors role, this review will generally describe protein digestibility methods of high protein products and provide a comparative analysis of the main source of their variation especially in vitro and concluding with a few future research priorities based on the current state of knowledge.

Of the current big research interest of food industry on indirectly measurement protein quality by protein digestibility is explained with comparative analysis. Several research methods have been demonstrated varying in protein digestibility values of high protein products may be due to high protein product origin, in vivo/in vitro methods, age of organism, digestion process condition, digested protein end products determination methods, test protein its internal and external factors, indigestible protein. Clearly, the in vivo protein digestibility is more accurate with lesser variation of similar samples compared to in vitro method. Comprehensive studies as determined using human subjects had demonstrated high true ileal digestibility of protein in milk proteins 95%, hydrolyzed casein based diet 92.3% compared to values of soy 91%, pea 89%, rapeseed protein isolate 87.1% and 80–85% meat based proteins. Using naso-intestinal intubation method, true ileal amino acid digestibility ranged from 92% for glycine to 99% for tyrosine in cows milk, whereas, for soy bean, digestibility ranged from 89% for threonine to 97% for tyrosine. In ileostomates also found to range from 98% for aspartate to 100% for cysteine in sodium caseinate; from 93% for threonine to 99% for cysteine in whey protein concentrate; from 95% for glycine to 99% for arginine in soy protein isolate; and from 91% for cysteine to 100% for arginine in soy protein concentrate. The findings indicates, milk based proteins have superior protein digestibility values over vegetable based proteins even upon application of heat treatment. Younger age rats had shown higher true ileal protein digestibility than older once. Throughout the course of gastrointestinal digestion process, appropriate type and amount of enzymes in appropriate physiological condition must be maintained. For instance, variation in

physiological pH hastens to the hydrolysis of proteins thereby low protein digestibility. Test proteins internal factors include protein amino acid profile (proline rich stretches) and protein folding (secondary beta-conformation) and crosslinking (high disulfide bridges) cause lower protein digestibility due to reduced effectiveness of peptidases. External factors of test protein include pH, temperature and ionic strength conditions, physical entrapment (chitin), presence of secondary molecules emulsifiers and antinutritional factors (trypsin and chymotrypsin inhibitors in legumes). Effect of processing to obtain test proteins mostly tends to increase nutritional value by particle size reduction and physical size separation (refinement), heat and pressure treatments, extrusion, state of protein hydrolysis, and fermentation. Particle size reduction and physical size separation, heat and pressure treatments improves protein digestibility in single or their combined effect, but paradoxically, intense heat and high pressure leads to low protein digestibility. For instance, milk derived products of such infant formula of 91% true ilea lysine digestibility to 100% UHT milk, prolonged heating affect lysine bioavailiability. Among the in vivo protein digested products quantification methods; the more accurate true ileal protein digestibility method is preferred than fecal measurement; because it determines unabsorbed amino acids amino acids, peptides and proteins at the small bowel terminal ileum that are not metabolized by colonic microbiota. Thus, as the digestible indispensable amino acid score (DIAAS) utilizes true ileal digestibility is considered superior to the popular Protein Digestibility Corrected Amino Acid Score (PDCAAS). To date, the method of in vitro protein digestibility quantification are shown to vary among research methods, for instance extrusion of raw black soldier fly larvae with wheat flour demonstrated a high level of crude protein digestibility (94%) compared to whey protein isolate (91.7%), such variability may be due to method of calculation employed. In another study, using standardized INFOGEST in vitro digestion protocol, protein digestibility of reference whey protein isolate quantified by o-phthaldialdehyde (OPA) method was obtained 35% but 85% in mealworm isolate proteins, which is comparatively much lower than whey protein isolate (88.4-91.7%) in other report employed similar digestion condition but used trichloroacetic acid nitrogen soluble (SN-TCA) method for digestibility quantification and calculated differently. These contradictory results may be due to first the specificity, amount, type of enzyme used; that leads to suggest to use optimized determinant factors. Similar paper suggests supporting of in vitro protein digestibility quantified by OPA method with protein molecular size measurement and identification to indicate availability of target proteins. SN-TCA and OPA with limitation are described the most common methods of protein degree of hydrolysis (DH) to quantify in vitro digestibility with superiority over pH-stat, trinitrobenzenesulfonic acid (TNBS), and formol titration. SN-TCA method measures amount of TCA-soluble nitrogen rather than DH; OPA method measures amino groups generated and directly determine DH, but the assumption that the response factor for all derivatized N-terminal amino acids is similar may lead to inaccuracies. It is noted that no best method to be chosen for determining DH of protein hydrolysates. This lead to a standardized approach to do inter-study comparisons. Moreover, it can also be suggested to the use of similar categories of high proteins products with similar origin to minimize variation in digestibility.

The increasing developments of various research methods for protein digestibility helped to visualize factors causing variation among results presented and chose suitable one. Based on the stated of knowledge superiority of in vivo over in vitro method is noticed. With the inherent limitation of in vitro methods can be used to rank food proteins based on digestibility, in understanding of protein structure under digestion conditions, and with more refinement could help to predict in vivo nutritive value. To maximize this, a systemized optimization by selecting the key determinant source of variation of protein digestibility research results is needed. Moreover, harmonization of international scientific research communities is also a paramount to consolidate digestion models to improve the comparability of experimental results between sample types used and other parameters of assumption in future.

Negasi Tsighe K. (Author):_____

Nadtochii Liudmila Anatolevna. (Scientific Supervisor):_____