New scientific results

Judit Tormási

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Hungarian University of Agriculture and Life Science Institute of Food Science and Technology Departments of Food Chemistry and Analysis

Doctoral (PhD) thesis

Effects of foods containing lipase inhibitory bioactive substances on triglyceride lipolysis and protein digestibility

Judit Tormási Budapest 2023

Name:	Doctoral School of Fo	ood Science	
Discipline:	Food Science		
Head of Doct	toral School:	Dr. Livia Simon-Sarkadi Professor, DSc MATE, Institute of Food Science and Technology Department of Food Chemistry and Nutrition	
Supervisor:		Dr. László Abrankó Professor, PhD MATE, Institute of Food Science and Technology Department of Food and Analytical Chemistry	
Approval o	of the Head of School	Approval of the Supervisor	

Doctoral School:

1. INTRODUCTION

Foods consumable for humans contain three main nutrient groups, i) macronutrients: proteins, lipids, and carbohydrates, ii) various micronutrients and iii) minerals, but also antinutrients and toxic compounds could be present. The purpose of the human digestion process is to efficiently break down foods, to extract and absorb required nutrients and to eliminate waste. In order to understand how the foods that we eat affect the metabolomic processes of human beings, besides the mechanisms of digestive processes i.e., breakdown of foods into molecules able to be absorbed, it is necessary to know the steps of nutrition as well.

The digestion process includes multiple organs, enzymes and chemical fluids (salt solutions, bile and hormones) each with its distinct function to achieve proper nutrition for humans. Digestion starts in the mouth, with the help of the teeth and tongue the bite taken from a food is broken down to get smaller morsels, and mixed with the saliva and amylolytic enzymes. The bolus than is swallowed and through the oesophagus it is transferred into the stomach where the main process is the protein digestion acted out by pepsin. Next to protein digestion, in the stomach lipolysis is started by the action of gastric lipase, acting on triacylglycerols. When the swallowed bolus is degraded enough that the particle size of the chyme in the stomach reaches under 3 mm, it could exit through the pylorus into the small intestine. The first part is the duodenum, this is where the chyme enters from the stomach. Here the acidic gastric secretion is mixed with the alkaline media of the intestine, and a mixture of digestive enzymes secretes into the duodenum by the pancreas. The molecules accessible to be absorbed, go through the intestinal wall (by active or passive transport) into the blood circulation. The unabsorbed material reaches the large intestine, where water and the beneficial molecules – that are created by microbial fermentation – are absorbed, and unnecessary materials are eliminated.

Depending on the level of nutrition three main definition could be introduced: bioaccessibility, bioavailability and bioactivity. Bioaccessibility gives the amount of molecules in the small intestine that are able to pass through the intestinal wall and could be subjected to passive and active transport processes. The part of the bioaccessible molecules that went through the small intestinal wall represent the bioavailable fraction. Those molecules that are in the blood system and hereafter serve a distinct purpose the body became the bioactive molecules.

In light of the complexity of the digestive processes and the levels of nutrition of the human body it is evident that nutrient content and composition of foods consumed are not equal of nutrient content and composition bioaccessible, bioavailable and bioactive in the gut.

Researchers have been trying to learn and understand the underlying mechanisms for more than 100 years. Rapid advancement in this field have been seen in the last two decades which could be stemmed back to the development and widespread of the *in vitro* digestion simulation models. One of the most popular standardized digestion simulation methods recently is the static *in vitro* Infogest protocol.

In order to more deeply understand the mechanisms guiding lipid and protein digestion part of my PhD work focused on implementing analytical methods for determination of bioaccessible lipid and protein content harmonized with the Infogest digestion simulation method. Moreover, modification of lipolytic enzyme activity with several foods containing bioactive substances as well as interplay between lipid and protein digestibility was assessed.

2. AIMS

- 1) Implementing an integrated platform where the effect of bioactive molecules on bioaccessibility of macronutrients could be simultaneously evaluated. The holistic platform is based on an *in vitro* digestion simulation model matched with specific analytical methods for measurement of each macronutrient bioaccessibility.
- 2) Creating systematic and easy-to-use routine procedures to serve industrial projects and functional product development by:
 - a. Determination of nutritional values and scores (e.g., PDCAAS and DIAAS)
 - b. Evaluating effects of known bioactive molecules
 - c. Standardization of protocols that are used to describe nutritional value of foods
- 3) Expanding the knowledge on food science and nutrition, from the point of view of food digestibility.
 - a. Lipid and protein digestibility of foods are determined
 - b. Contribution of gastric lipase during digestion is evaluated
 - c. Effect of certain foods with bioactive compounds on gastric-, and pancreatic lipase activity is tested
 - d. Macronutrient interactions are exposed in co-digestion experiments

3. MATERIALS AND METHODS

3.1. Food samples

Samples were specifically chosen for i) testing the analytical methods designed for evaluating lipid and protein digestibility, ii) evaluating macronutrient interactions during digestion of high fat and protein foods, iii) examine inhibition efficacy of bioactive substances on lipid digestibility of the tested foods. Carp from Akasztó (a PDO food), ground beef (20% fat content; Húsfarm fresh ground beef), cream (30% fat content; TOLLE UHT cream), sour cream (20% fat content; Milfina sour cream; ingredients: cream, bacterial culture), sour cream analogue (20% fat content; Hazai és Finom "Finomföl"; ingredients: skim milk, milk protein concentrate, palm oil, bacterial culture), and durum wheat pasta (Gyermelyi Vita Pasta) was chosen as test matrices. Carp fillets from Akasztó were provided by Fishmarket Ltd. (Budapest, Hungary). Other food products were purchased commercially in local shops. Carp and ground beef were baked before digestion experiments for 20 mins in a 200 °C oven, after cooling baked carp (BC) and baked beef (BB) were homogenized in a meat grinder (Moulinex HV4), three times. Durum wheat pasta was cooked according to the packaging instructions. The 500 g dried pasta was placed in 5 L water boiling water with 1 g/L salt for 8 mins, after cooling cooked pasta (CP) was homogenized in a meat grinder (Moulinex HV4) three times. Baked and cooked samples were stored at -80 °C and thawed before experiments. Cream (C), sour cream (SC), and sour cream analogue (SCA) were used after thorough mixing. Dairy products were always purchased fresh and used right after opening. One of the co-digestion studies on mapping the interplay between lipid and protein digestibility were done with eleven types of edible oils. These oils: sunflower oil (SFO), MCT oil (MCT), pumpkinseed oil (PSO), walnut oil (WO), hemp oil (HO), olive oil (OO), linseed oil (LO), coconut oil (CO), sesame oil (SO), grape seed oil (GSO), rapeseed oil (RO) were bought in local supermarkets. Bioactive rich foods with in vitro assay-proven inhibitory effect on pancreatic lipase were chosen to test their ability in a more complex simulation of digestion i.e., Infogest digestion simulation. Experiments were carried out with either direct addition of bioactive containing food (rosemary, grape seed powder) or after extraction of bioactive compounds with food safe methods (tea, brewed). Effect of rosemary was tested on baked carp lipids. Whole rosemary spice was bought in a local shop and was added as a whole spice. Carp fillets were covered with rosemary (5 w/w%) before baking (200 °C for 20 mins). Effect of grape seed powder (GSP) was tested on cream and baked beef. Grape seed powder was provided by Bock Vineyard Ltd. (Villány, Hungary). Grape seed powder was added to test matrices before digestion experiments, separately. Black tea (Himalayan Spring FF 2022 No.601) with high tannin content were bought commercially. Effect of tea was evaluated as brewed tea (aqueous extract). For the extraction of bioactive compounds from tea 0.2 g of tea leaves were measured into 50-mL round bottom flasks and 50 mL distilled water was added (4 mg/mL). Sample was heated on sand for 1 hour (with water cooler system attached to prevent evaporation). After cooling extracts were sieved on paper sieve and collected filtrate was completed to 50 mL. Black tea brew (BTB) was stored in -80°C until use. Thawed BTB was added separately to fat sources before digestion experiments.

Moisture-, fat-, fatty acid-, and protein content of food samples were determined as follows in Table 1.

Table 1. Methods used for determination of moisture-, fat-, fatty acid-, and protein content of food samples.

Parameter Food sample		Method of determination	
Moisture	Baked carp, baked beef	ISO 1442:2000	
content	Cream, sour cream,	Carrier Araba India at 102 100	
	sour cream analogue	Gravimetry by drying at 103±1°C	
Fat content	Baked carp, baked beef	ISO 1444:2000	
	Cream, sour cream,	ISO 2450:2008	
	sour cream analogue		
Fatty acid	Baked carp, baked beef, cream, sour	ISO 12966-2:2017	
content	cream, sour cream analogue	"Rapid" method	
Protein content	Baked carp, baked beef, cream, sour	Kjeldahl method	
	cream, sour cream analogue, cooked	Correction factor: meat products: 6,25;	
	pasta	dairy products: 6,38; cooked pasta: 5,83	

Additionally, fat content and fatty acid composition were also determined after fat extraction with the Bligh and Dyer method using Folch extraction.

3.3. Digestion simulation and analytical assessment of digestibility

Digestion simulations were made according to the Infogest protocol v1.0 ("PL"; using amylase, pepsin and pancreatin; (Minekus et al. 2014) and Infogest v2.0 ("GL+PL"; using amylase, rabbit gastric lipase (RGE) and pancreatin; (Brodkorb et al. 2019). All digestion experiments were conducted in triplicates. Blank digestions were also made for each triplicate using 5 g (± 0.001 g) of distilled water (lipid digestibility experiments) or 5 g (± 0.001 g) of protein free biscuits (protein digestibility experiments) as sample.

3.3.1. Single food digestions

First, digestion experiments were carried out to test and validate the method for assessment of bioaccessible fatty acid content. Then, lipid and/or protein digestibility of these foods were defined i) to determine lipid and/or protein digestibility of test foods, ii) to use as control in lipase inhibitory experiments with bioactive rich foods, iii) to use as control in co-digestion experiments with other foods with high fat and/or protein content. Single product digestions of BC were carried out with 5, 4, 1, 0.5 g (± 0.001 g) samples. From BB 0.9 g (± 0.001 g), from C 0.5 g (± 0.001 g), from SC and SCA 1 g (± 0.001 g), and CP 4 g (± 0.001 g) sample were digested. All samples below 5 g were diluted with distilled water to reach proper sample size according to consensus (5 g).

3.3.2 Co-digestions – Lipase inhibitory studies with bioactive rich foods

These types of experiments focused on revealing the effects of simultaneous consumption of high fat foods and foods with proven lipase inhibitory effects on lipid digestibility of said high fat food. Effect of rosemary was tested on baked carp lipids. Rosemary spiced baked carp ($1\pm0.001~g$) was digested alone. Effect of grape seed powder and black tea brew was tested on cream, and baked beef. First, dose-dependency tests were done where GSP was added to 0.5 g ($\pm0.001~g$) cream at three levels, 5, 10, and 15 w/w% and BTB was added to 0.5 g ($\pm0.001~g$) cream at three levels, at 1:1, 1:2 and 1:3 (cream: BTB) weight ratios. Levels were chosen based on recommended intake. GSP was added around the typical concentrations as suggested for this food supplement to mix with foods and BTB was added to cream in ratios that would be during consumption of English tea. Further experiments, with different substrate, were adjusted to fat content of cream (150 mg) thus to 0.9 g ($\pm0.001~g$) of baked beef the lowest effective level of GSP (5 w/w%) and BTB (1:2) were added.

3.3.3. Co-digestion of foods – interplay between lipid and protein digestion

Two sets of co-digestion experiments were conducted on revealing the interplay between fat and protein digestion, with the addition of different sources of fat to protein containing meals. To reveal how co-consumption changes the lipid digestibility of the high fat toppings (sour cream and sour cream analogue) "sour cream pasta" experiment was designed. Co-digestions were made using 4 g (± 0.001 g) CP and either 1 g (± 0.001 g) of SC or 1 g (± 0.001 g) of SCA for this purpose. Since in these experiments the addition of different type of fats seemed to impact protein digestibility next experiment was designed to see the how some edible oils affect protein digestibility of cooked pasta. Eleven types of oils were chosen, namely, sunflower oil, MCT oil, pumpkinseed oil, walnut oil, hemp oil, olive oil, linseed oil, coconut oil, sesame oil, grape seed oil, rapeseed oil and were added to 4 g (± 0.001 g) of CP at 5 w/w% in co-digestion simulations.

3.3.4. Assessment of digestibility

Development of the *in vitro* lipid digestibility method is part of the results. *In vitro* protein digestibility was determined from the small intestinal digesta after methanolic isolation, from the supernatant containing the digested protein fraction (tri-, dipeptides and amino acids) with three methods: i) based on free amino group content with the OPA (ortho-phtalaldehyde) method, ii) after acidic hydrolysis based on free amino group content with the OPA method; iii) based on amino acid composition after acidic hydrolysis with HPLC-UV method. Additionally protein quality indicators such as proxy–PDCAAS and *in vitro* DIAAS were calculated. Structural analysis was done in order to evaluate changes during gastric phase that could modify digestibility. Products and samples taken after chemical digestion were dyed with Coomassie blue (protein) and Nile red (lipid) and microscopic images were taken using an Olympus BX41 (40x lens).

4. RESULTS

4.1. Harmonized protocol to evaluate bioaccessibility of foods

My goal was to create a systematic and routine way to determine both the quantity and the quality of the bioaccessible lipid fraction at the same time. In addition, the developed method contains built in quality control points which serves simultaneous verification of the results. During method development there were a few key questions that are needed to be addressed to establish the final protocol. These questions were directed at the i) appropriate fat extraction method; ii) determination of fatty acid release; iii) normalisation of derivatization methods using internal standardization. In order to clarify these aspects i) fat content and fatty acid composition – of the foods and their digesta – gathered after ISO standard extraction methods and the chosen Bligh and Dyer method were compared and accepted; ii) two standard derivatisation method were chosen to assess total fatty acid content (TFA) and esterified fatty acid content (EFA) from which free fatty acid content could be derived; iii) recovery experiment was designed and carried out to test applicability of the chosen glyceryl trinonadecanoate (C19:0 TAG) internal standard as an way to normalise between the two derivatisation methods used.

In the proposed routine workflow, fat content of the tested food could/should be determined after fat extraction with the Bligh & Dyer method – if ISO stated standard method is not routinely used or unavailable. After in vitro digestion simulation – according to the Infogest protocol – is carried out, all lipid content is extracted from the small intestinal digesta with the Bligh & Dyer method. During extraction, polar components are separated from the non-polar components, which are transferred into the chloroform phase already spiked with internal standard (C19:0 TAG). From the total volume of chloroform (12.5 mL), two times 5 mL aliquots (containing 100 µg C19:0 TAG) are taken for derivatization, one with the total fatty acid method and the other with the esterified fatty acid method (ISO 12966-2:2017). For separation of fatty acid methyl esters (FAMEs) gas chromatography is used. Qualitative and quantitative analysis is based on a fourlevel calibration (0, 10, 20, 40 µg/mL approximate concentration) with a FAME mixture composed of 37 FAMEs spiked at 100 µg/mL at each level with methyl nonadecanoate (C19:0 ME). Fatty acid content and composition from TFA method derivatized aliquot should be compared with B&D extracted fatty acid content and composition of the food sample as a built-in quality control step. Bioaccessible fatty acid content and individual release ratio of fatty acids are gotten after subtracting EFA results from TFA results.

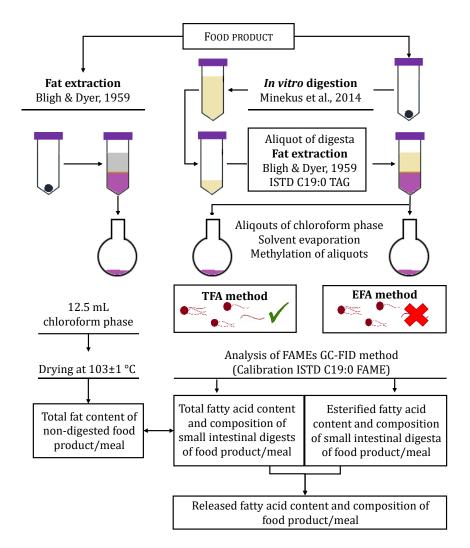


Figure 1. Protocol outline for the harmonized sample preparation of *in vitro* digesta for the assessment of free fatty acid release in food samples along with the protocol for fat content determination of the same food sample (*Original image*).

4.2. Application of the lipid digestibility method

The method was used to determine the lipid digestibility of baked carp, baked beef, cream, sour cream and sour cream analogue. Based on the results role of gastric lipase and food matrix were highlighted. In addition, co-digestion experiments were conducted with i) foods containing lipase inhibitory bioactive substances, and ii) foods with substantial amount of protein content.

4.2.1. Single food digestions and conclusions

Lipid digestibility of the chosen test foods; baked carp, baked beef, cream, sour cream analogue were determined using the established protocol after *in vitro* digestion simulation according to the Infogest protocol (Table 2).

Table 2. *In vitro* lipid digestibility of the chosen test foods using two versions of the Infogest digestion simulation protocol.

Test food	Lipid digestibility (%) using GL+PL*	Lipid digestibility (%) using PL*
Baked carp	72.3 ± 0.9	62.8 ± 1.5
Baked beef	67.2 ± 1.6	67.7 ± 2.5
Cream	77.1 ± 5.0	69.2 ± 6.1
Sour cream	61.1 ± 3.9	52.6 ± 3.7
Sour cream analogue	66.2 ± 2.5	58.5 ± 4.5

^{*}GL: gastric lipase; PL: pancreatic lipase. Results are given in average \pm deviation format, n=6.

Using the two versions of the Infogest protocol i.e., addition of both gastric lipase and pancreatic lipase (GL+PL) or only pancreatic lipase (PL), the role and effect of gastric lipase can be highlighted and linked to the fatty acid composition of the digested food. My results showed that during digestion of foods with more diverse fatty acid composition, such as cream and sour cream containing milk fat, having both short-, medium-, and long chain fatty acids, addition of gastric lipase results in a higher extent of lipolysis, mainly caused by the more efficient release of short-, and medium chain fatty acids by the action of gastric lipase. Simultaneously, it was shown that in lipid sources without significant amounts of short-, and medium chain fatty acids e.g., baked beef, the inclusion of gastric lipase does not result an additional increase in lipid digestibility compared PL-only digestions. These results also highlight the inefficiency of pancreatic lipase of cleaving short-, and medium chain fatty acids from asymmetric triacylglycerides without the pre-digestion of gastric lipase.

Moreover, based on the lipid digestibility of the test foods role of the food matrix could be highlighted. On the example of sour cream and sour cream analogue, it was shown that during lipid digestion of food with similar matrix but different fatty acid composition the drive of lipid digestion is modified by the structural properties. Sour cream containing milk fat in its native form (covered with milk fat globule membrane) is prone to flocculation and aggregation during gastric digestion which consequently lowers lipid digestibility caused by the decrease in enzyme-available surface area. However, this effect is relevant for sour cream analogue since this imitate product contains palm oil droplets without protective layer therefore these droplets do not tend to aggregate under gastric conditions. In addition, in the case of sour cream analogue the palm oil based emulsion is more stable due to coverage by milk proteins.

4.2.2. Co-digestion with foods containing bioactive substances

Fat-free foods containing bioactive substances were selected that have previously been shown to exhibit lipase inhibitory effect with *in vitro* enzyme activity studies. Chosen materials were rosemary (Slanc et al. 2009), grape seed powder (Moreno et al. 2003) and black tea brew (Sellami et al. 2017; Jamous et al. 2018). In addition, applicability of method for evaluating enzyme inhibition were tested with Orlistat as a positive control.

Rosemary was tested as a whole spice using baked carp as a lipid source however results gathered from *in vitro* digestion simulations showed that in this setting rosemary spice have no influence on overall lipolysis of baked carp meal (t test p=0.557). Moreover FA-specific evaluation revealed no effect on release of individual FAs (t test p>0.05). Therefore it can be stated that the results of the *in vitro* enzyme assays may not be relevant in a more realistic model, i.e., the Infogest model, and that the investigation of organic extracts may not necessarily reflect the effect during conventional consumption (seasoning), but their use may optimise the effect in order to make food supplements.

Grape seed powder (GSP) and black tea brew (BTB) were co-digested first with cream in a dosedependency test at three levels, 5, 10 and 15 w/w% for GSP and 1:1; 1:2 and 1:3 weight ratios for BTB. Results showed that both bioactive containing foods could lower lipid digestibility of cream, GSP was able to lower extent of lipolysis at the 5 w/w% addition by 12% meanwhile BTB at the 1:2 ratio by 22%. The results also showed that further addition did not increase the expressed effect. The fatty acid-specific results also highlighted that the effect of both bioactive containing foods are more pronounced on pancreatic lipase. This effect is showed by the systematic decrease of the release ratio of the short-, and medium chain fatty acids compared to the long chain fatty acids. The effect of GSP and BTB were also tested on baked beef substrate therefore the two fatfree foods were added to baked beef at the effective levels (5 w/w% for GSP and 1:2 ratio for BTB). On baked beef, neither GSP nor BTB showed inhibitory effect on lipolysis. The reason for this could be explained by differences in the characteristics of fat sources. In baked beef, relative abundancy of short-, and medium chain fatty acids are negligible and consequently, asymmetric triacylglycerols in which these fatty acids are present are also negligible. Thus, the dispreference shown by PL towards such substrates as well as the reduced contribution of GL to pre-digest these substrates as a consequence of the inhibition does not result in the apparent decrease in overall digestibility of beef fat.

4.2.3. Interplay between lipids and proteins – sour cream pasta study

Since consumption of food products – especially the type of high fat toppings such as sour cream – usually happens as part of a meal, additional ingredients could modify digestibility

behaviour of the products. Therefore, revealing and understanding the effect of co-consumption on the lipid digestibility, the two tested high fat toppings, SC and SCA were studied during co-digestion with cooked pasta (CP). Cooked pasta was chosen as a low fat test product (<1% fat content) and an important element of western and Mediterranean diet (Simonato et al. 2015), usually consumed with simple toppings or sauces. Lipid digestibility of the two products was studied in co-consumption experiments, therefore both products were co-digested with cooked pasta to simulate a real meal.

It was observed that lipid digestibility of SC in the pasta dish increased compared to the single food digestion by 9% (GL+PL: p=0.021; $61.1\pm3.9\%$ to $66.8\pm3.2\%$), however, this increase was only observed when both lipolytic enzymes were present. In the simulation with only PL, the addition of cooked pasta did not significantly ameliorate lipid digestibility (PL: p=0.565; $52.6\pm2.7\%$ to $54.0\pm4.8\%$). Moreover, this was not the case for SCA where the extent of lipolysis did not change in either versions (GL+PL: p=0.454; PL: p=0.599). To reveal the reasons behind these differences fatty acid-specific evaluation and structural analysis were done. Based on these results, it can be concluded that the ameliorating effect during SC+CP co-digestion caused by the presence of pasta has happened in the gastric phase is twofold: i) pasta proteins inhibit structural disintegration of MFGs during gastric conditions that would result in coalescence and increased lipid droplet size. Thus, the reserved smaller globule size provides more efficient lipolysis for gastric lipase, and ii) the increased efficiency of gastric lipase (compared to single food digestion) manifested in the increased release of GL-preferred FAs (SCFAs and MCFAs).

4.3. *In vitro* assessment of protein digestibility

During my PhD studies, I had the opportunity to take part in an international ring trial on standardization of a protein digestibility assessment method based on the Infogest digestion simulation method, called "In vitro digestion protocol for the analysis of protein digestibility and in vitro DIAAS in dairy products". Since I have already been working on a protein digestibility assessment method my work focused on bringing the two alternative methods together and to expand the use of the method for non-dairy products. This combined method was used to determine protein digestibility of baked carp, baked beef and cooked pasta, as well as to reveal the effect of edible oils on protein digestibility of cooked pasta in co-digestion experiments.

4.3.1. Modification of the protein digestibility method

In addition of the assessment of the lipid digestibility of foods the harmonized protocol could be used to determine protein digestibility of foods from the same small intestinal digesta. The modification of the chosen protein digestibility method (Sousa et al., 2023) in order to be

applicable to simultaneous determination of lipid and protein digestibility evaluation by sampling was tested and validated using a reference material. The results showed that the sampling method instead of the one-step precipitation approach included in the original method is a good alternative, since the results gathered with the two preparation methods are not differ from each other. The amino acid based protein digestibility results are also sufficient to calculate approved protein quality indicators, such as *proxy*-PDCAAS and *in vitro* DIAAS.

4.3.2. Protein digestibility of selected test foods

Some test foods with higher protein content (baked carp, baked beef) and cooked pasta were chosen for the purpose of protein digestibility determination. Protein content of the test foods were determined with Kjeldahl method (relevant conversion factor was used). After *in vitro* digestion simulation bioaccessible protein content of these foods were isolated with methanolic precipitation. According to Sousa et al., the supernatant obtained by this method contains the bioaccessible protein content, which could be absorbed in the small intestine (Sousa et al. 2023). The extract will contain smaller proteins with different degree of polymerization, i.e., amino acids, di- and tripeptides will be present. Determination of protein content in the supernatant was carried out in three ways: i) unhydrolysed supernatant based on free amino group with the OPA method, ii) hydrolysed supernatant based on free amino group with the OPA method, and iii) hydrolysed supernatant based on amino acids with AQC derivatization and HPLC-UV analysis. Measurement of the unhydrolysed supernatant will give information on the degree of digestion, additionally to the overall protein digestibility (IVPD%) obtained from the other two methods. Protein content (%) of test foods determined with Kjeldahl method and *in vitro* protein digestibility (%) measured with different methods described above and calculated protein quality scores are shown in Table 3.

Table 3. Protein content (%) of test foods: baked carp, baked beef and cooked pasta, determined with Kjeldahl method and *in vitro* protein digestibility (%) measured with different methods: unhydrolysed OPA, hydrolysed OPA and hydrolysed AA. Protein quality scores: *proxy*-PDCAAS and *in vitro* DIAAS, calculated from amino acid-based evaluation of *in vitro* protein digestibility after simulated digestion.

		Test food		
	Mode of determination	Baked carp	Baked beef	Cooked pasta
Protein content [%]	Kjeldahl method	22.3±0.3	29.9 ± 0.6	4.9±0.2
· '/ D / '	Unhydrolysed OPA	36.8	33.1	21.7
in vitro Protein digestibility [%]	Hydrolysed OPA	n.a.	n.a.	54.2
digestibility [/0]	Hydrolysed AA	76.3	89.8	100.0

n.a. - not applicable

		proxy-PDCAAS*	
Test food	Preschool child	Schoolchild	A do:16
	(2-5 year)	(10-12 year)	Adult
Baked carp	69 (Leu)	87 (Ile)	100 (SAA)
Baked beef	55 (Trp)	68 (Trp)	100 (Trp)
Cooked pasta	30 (Lys)	39 (Lys)	100 (Lys)

		in vitro DIAAS*	
Test food	Infant	Child	Older child,
	(0-6 month)	(6-36 month)	adolescent, adult
Baked carp	50 (AAA)	90 (AAA)	103 (Leu)
Baked beef	40 (Trp)	80 (Trp)	103 (Trp)
Cooked pasta	17 (Trp)	27 (Lys)	32 (Lys)

^{*}Calculated based on hydrolysed AA results. PDCAAS values above 100 were truncated.

4.3.2. Co-digestion of cooked pasta with edible oils

Addition of cooked pasta seemed to impact the lipid digestibility of high fat toppings containing milk fat and palm oil. Therefore, the question arises that if cooked pasta could affect the release of fatty acids form different fat sources, could different fat sources (i.e., edible oils) modify protein digestibility of cooked pasta? To test this theory, experiment was designed to see the how edible oils affect protein digestibility of cooked pasta. Eleven types of oils were chosen, namely, sunflower oil (SFO), MCT oil (MCT), pumpkinseed oil (PSO), walnut oil (WO), hemp oil (HO), olive oil (OO), linseed oil (LO), coconut oil (CO), sesame oil (SO), grape seed oil (GSO), rape oil (RO), and were co-digested with CP at 5 w/w% addition. Bioaccessible protein content of small intestinal digesta was isolated with methanolic precipitation and supernatant was used to determine *in vitro* protein digestibility (IVPD%) before and after acidic hydrolysis (AOAC 2018.06) as shown before, and amino acid-based results were gathered from the supernatant after microwave

assisted hydrolysis. Moreover, fatty acid composition of oils was determined after derivatization with GC-FID, and lipid composition indicators were created to assess the effect of fatty acid composition on protein digestibility. *In vitro* protein digestibility results were correlated with lipid composition indicators, however strong correlation (r=0.89) was only detected with IVPD% results gathered with the OPA analysis but not with results calculated based on amino acid composition (r=~0.00). Based on these results it is hypothesized that oleic acid could form complexes with proteins and enhanced during thermal denaturation. Thermal denaturation increases chance of protein-oleic acid interactions due to unfolding and opening of interaction sites. In the bioaccessible fraction, a part of the protein content is present as peptides, therefore interaction sites are already available to bind to oleic acid, which reaction might be amplified by the heat treatment during the hydrolysis process. Thus peptide-oleic acid complexes might form, which could hinder peptide hydrolysis towards amino acids.

5. NEW SCIENTIFIC RESULTS

- 1) I established and validated a new harmonised sampling and analytical protocol suitable for simultaneous determination of lipid and protein digestibility from the same Infogest *in vitro*, static digestion simulation.
 - It was proven that total lipid content of a digesta, containing a mixture of hydrolysed and intact lipid species (TAG, DAG, MAG, FFA) cannot be determined after solvent evaporation and weight determination, since some of the lipid species formed during digestion are lost during evaporation.
 - It was concluded that a sample size containing not more than 150 mg lipids is to be used in the developed protocol.
 - I proved that the developed sampling method is a more effective substitute of the currently accepted standardized method, which sacrifices the entire sample for studying only one nutrient.
- 2) Fatty acid-specific lipid digestibility results of cream, sour cream, and sour cream analogue as well as the prepared, ready-to-eat meal forms of baked carp (PDO from Akasztó) and baked beef were presented for the first time using the Infogest digestion simulation method and it was shown that TAGs containing short and medium chain fatty acids are non-preferred substrates for pancreatic lipase (PL) however this specificity is not characteristic for gastric lipase (GL).
 - It was shown for the first time on the example of cream and sour cream that GL plays a key role in ameliorating the digestibility of short and medium chain fatty acids from milk fat.
 - It has been shown, on baked beef as an example, that for lipid sources without significant amounts of short- and medium-chain fatty acids, the inclusion of GL does not result in an additional increase in lipid digestibility compared to PL-only digestions.
- 3) Using fatty acid-specific lipid digestibility assessment with the Infogest *in vitro* digestion simulation and microscopic structural analysis, I proved that divergent droplet size formed during gastric digestion is a key determinant of the difference in the extent of lipolysis of sour cream and sour cream analogue (containing palm oil).
 - It was shown that on the contrary to sour cream, where milk fat globules are naturally covered by milk fat globule membrane, palm oil droplets of sour cream analogue

(without membrane) are not prone to flocculation and aggregation under the conditions typical during gastric digestion, thus the original lipid droplet size is not increasing during gastric digestion.

- 4) *In vitro* digestion simulation of baked carp meal with rosemary spice (5 g dried commercially available rosemary/100 g baked carp) showed no effect on the digestibility of baked carp lipids.
- 5) Using fatty acid-specific lipid digestibility assessment with the Infogest *in vitro* digestion simulation, I proved that co-consumption of grape seed powder and black tea brew can inhibit lipid digestibility in some, but not any types of food.
 - Direct addition of GSP (Bock Hungary) in 5 w/w% concentration and black tea brew (Himalayan Spring FF 2022 No.601) to cream in 1 (cream):2 (tea) ratio is sufficient to significantly decrease extent of lipid digestion of cream, whereas it did not affected digestibility of baked beef lipids.
 - I have shown that GSP and BTB primarily affect the lipolysis of short- and mediumchain fatty acids, and thus may reduce the lipid digestibility of foods with triacylglycerols containing significant amounts of these fatty acids.
- 6) I proved that both GSP and BTB have similar *in vitro* lipase inhibitory effects when consumed together with cream. Namely, GSP and BTB selectively decrease the release of short- and medium chain fatty acids, which indicates an inefficient pancreatic lipase function.
- 7) Using fatty acid-specific lipid digestibility assessment with the Infogest *in vitro* digestion simulation, I proved that co-consumption sour cream and sour cream analogue with cooked pasta, increased the extent of lipid digestion of sour cream but not sour cream analogue.
 - It was shown that presence of pasta protein inhibits structural disintegration of milk fat
 globules during gastric conditions that would result in coalescence and increased lipid
 droplet size. Thus, the reserved smaller globule size provides more efficient lipolysis
 for gastric lipase, which manifested in the increased release of gastric lipase-preferred
 short- and medium chain fatty acids.

- 8) Using amino acid-specific assessment of protein digestibility with the Infogest *in vitro* digestion simulation, I proved on the example of eleven different edible oil types that co-consumption of edible oils with cooked pasta generally reduces protein digestibility of pasta proteins.
 - Correlation of fatty acid composition with the *in vitro* protein digestibility results proved that observed proteolysis-reducing effect of edible oils could not be directly stemmed back to their fatty acid composition.

In the above text, "Infogest in vitro digestion simulation method" means the currently accepted standardized method published in Brodkorb, A., Egger, L., Alminger, M. et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc 14, 991–1014 (2019). https://doi.org/10.1038/s41596-018-0119-1 (2023.03.16)

7. PUBLICATIONS

Journal articles linked tot he thesis:

- Tormási, Judit; Abrankó, László; Assessment of Fatty Acid-Specific Lipolysis by In Vitro Digestion and GC-FID; NUTRIENTS 13: 11 p. 3889 (2021) DOI: https://doi.org/10.3390/nu13113889
- Tormási, Judit; Abrankó, László; **Impact of grape seed powder and black tea brew on lipid digestion. An in vitro co-digestion study with real foods;** (Accepted manuscript)
- Tormási, Judit; Abrankó, László; **Lipid digestibility of sour cream and its analogue during in vitro digestion simulation and co-consumption with cooked pasta**; (Submitted manuscript)

Journal articles:

- Nath, Arijit; Ahmad, Abubakar Saleh; Amankwaa, Abraham; Csehi, Barbara; Mednyánszky, Zsuzsanna; Szerdahelyi, Emőke; Tóth, Attila; Tormási, Judit; Truong, Duy Hoàng; Abrankó, László et al.; Hydrolysis of Soybean Milk Protein by Papain: Antioxidant, Anti-Angiotensin, Antigenic and Digestibility Perspectives; BIOENGINEERING 9:9 p.418 (2022) DOI: https://doi.org/10.3390/bioengineering90904
- Zhang, Miaomiao; Simon Sarkadi, Livia; Üveges, Márta; Tormási, Judit; Benes, Eszter; Vass, Réka Anna; Vári, Sándor; Gas chromatographic determination of fatty acid composition in breast milk of mothers with different health conditions; ACTA ALIMENTARIA: AN INTERNATIONAL JOURNAL OF FOOD SCIENCE 51: 4 pp. 625-635., 11 p. (2022) DOI: https://doi.org/10.1556/066.2022.00120
- Jakab, Ivett; Tormási, Judit; Dhaygude, Vinod; Mednyánszky, Zsuzsanna; Sipos, László; Szedljak, Ildikó; Cricket flour-laden millet flour blends' physical and chemical composition and adaptation in dried pasta products; ACTA ALIMENTARIA: AN INTERNATIONAL JOURNAL OF FOOD SCIENCE 49: 1 pp. 4-12., 9 p. (2020) DOI: https://doi.org/10.1556/066.2020.49.1.2
- Szedljak, Ildikó; Tóth, Viktória; Tormási, Judit; Kovács, Anikó; Somogyi, László; Sipos, László; Kiskó, Gabriella; Effects of Different Heat Treatments on the Chemical and Microbiological Characteristics of Egg-free and Quail Egg Dried Pasta.; HUNGARIAN JOURNAL OF INDUSTRY AND CHEMISTRY 46: 2 pp. 85-90., 6 p. (2018) DOI: https://doi.org/10.1515/hjic-2018-0024

Conference material – full paper:

- Seel, Mariella; Tormási, Judit; Schmotzer, Christoph; "How might we use digital technologies for improving health literacy on cardiovascular diseases?" Highlights from an interdisciplinary, interprofessional and international Design Thinking Lab in the E³UDRES² project; LERNEN ÜBER DEN TELLERRAND HINAUS; GOOD PRACTICES ZU INTERDISZIPLINARITÄT, INTERNATIONALISIERUNG UND FUTURE SKILLS; pp. 63-77. (2023) ISBN: 978-3-99123-210-0
- Tormási, Judit, Abrankó, László; Biológiailag hasznosítható zsír mennyiségi meghatározása in vitro emésztményekben (determination of bioaccessible fat content

of in vitro digests); IFJÚ TEHETSÉGEK TALÁLKOZÓJA; Budapest, Szent István Egyetem, Budai Campus (2019) ISBN: 978-963-269-886-1

Conference material – Abstract:

In English:

- Üveges, Márta; Zhang, Miaomiao; Tormási, Judit; Benes, Eszter; Simon Sarkadi, Livia; Vass, Réka; Vari, Sándor; Fatty acid profile of human milk from healthy mothers at various lactation periods; In: Edward Prunchunas; Sandor G. Vari; Simona Lauerova; Csaba Vladar (szerk.); Abstract Book 5th RECOOP International Student and 18th RECOOP Bridges in Life Sciences Conferences; Konferencia helye, ideje: Budapest, Magyarország 2023.04.20.–2023.04.21. Los Angeles (CA), Budapest: Cedars-Sinai Medical Center, Recoop HST Association, p. 117. (2023)
- Zhang, Miaomiao; Simon Sarkadi, Livia; Üveges, Márta; Tormási, Judit; Benes, Eszter; Vass, Réka; Vari, Sándor; **Gas chromatographic determination of fatty acid composition in breast milk of mothers at different lactation periods;** In: Edward Prunchunas; Sandor G. Vari; Simona Lauerova; Csaba Vladar (szerk.); Abstract Book 5th RECOOP International Student and 18th RECOOP Bridges in Life Sciences Conferences; Konferencia helye, ideje: Budapest, Magyarország 2023.04.20.–2023.04.21. Los Angeles (CA), Budapest: Cedars-Sinai Medical Center, Recoop HST Associationp. 66. (2023)
- Nath, Arijit; Muriithi, Grace Wanjugu; Szerdahelyi, Emőke; Berki, Mária; Kónya-Lengyelné, Éva; Tömösköziné Farkas, Rita; Tormási, Judit; Abrankó, László; Bioaccessibility of amino acids and antioxidant properties in heat- and lactase-treated milk during in vitro digestion; In: Szalóki-Dorkó, Lilla; Batáné Vidács, Ildikó; Pradeep, Kumar; Pomázi, Andrea; Gere, Attila (szerk.) 4TH FOODCONF INTERNATIONAL CONFERENCE ON FOOD SCIENCE AND TECHNOLOGY. Book of Abstracts; Bicske, Magyarország: Élelmiszertudományért OKF Alapítvány (2022) p. 4155; p. 119; ISBN: 978-615-01-5422-0
- Bilkei, Fanni; Berki, Mária; Lengyelné Kónya, Éva; Tömösköziné Farkas, Rita; Tormási, Judit; Abrankó, László; Digestibility of powdered milk protein concentrate an in vitro study; In: Szalóki-Dorkó, Lilla; Batáné Vidács, Ildikó; Pradeep, Kumar; Pomázi, Andrea; Gere, Attila (szerk.) 4TH FOODCONF INTERNATIONAL CONFERENCE ON FOOD SCIENCE AND TECHNOLOGY. Book of Abstracts; Bicske, Magyarország: Élelmiszertudományért OKF Alapítvány (2022) p. 4056; p. 73; ISBN: 978-615-01-5422-0
- Tormási, Judit; Berki, Mária; Lengyelné Kónya, Éva; Tömösköziné Farkas, Rita; Nagy, Katalin; Abrankó, László; Assessment of nutrient bioaccessibility by digestion simulation a potential tool for functional food development; In Szalóki-Dorkó, Lilla; Batáné Vidács, Ildikó; Pradeep, Kumar; Pomázi, Andrea; Gere, Attila (szerk.) 4TH FOODCONF INTERNATIONAL CONFERENCE ON FOOD SCIENCE AND TECHNOLOGY. Book of Abstracts; Bicske, Magyarország: Élelmiszertudományért OKF Alapítvány (2022) pp. 86-86., ISBN: 978-615-01-5422-0
- Zhang, Miaomiao; Simon, Sarkadi Livia; Üveges, Márta; Tormási, Judit; Benes, Eszter; Vass, Réka; Vári, Sándor; Gas chromatographic determination of fatty acid composition in breast milk; In Szalóki-Dorkó, Lilla; Batáné Vidács, Ildikó; Pradeep, Kumar; Pomázi, Andrea; Gere, Attila (szerk.) 4TH FOODCONF INTERNATIONAL CONFERENCE ON FOOD SCIENCE AND TECHNOLOGY. Book of Abstracts; Bicske,

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- Simon, Sarkadi Livia; Üveges, Márta; Zhang, Miaomiao; Tormási, Judit; Benes, Eszter; Vass, Réka; Vári, Sándor; Fatty acid composition of human milk of pregnant women with obesity and Gestational Diabetes; In: Prunchunas, Edward; Vari, Sandor, G.; Lauerova, Simona; Vladar, Csaba (szerk.) 4TH RECOOP INTERNATIONAL STUDENT AND 17TH RECOOP BRIDGES IN LIFE SCIENCES CONFERENCES; Prague, Csehország: Recoop HST Association (2022) p. 166; p. 88; ISBN: 978-615-60-0603-5
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- Tormási, Judit ; Abrankó, László; Protein digestibility of cooked wheat pasta affected by coconsumption with fatty cream toppings; In: 7TH INTERNATIONAL CONFERENCE ON FOOD DIGESTION ICFD2022; Book of Abstracts; Cork, Ireland (2022) p. 12; p. 60.
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 INFOGEST protocol; In: 7TH INTERNATIONAL CONFERENCE ON FOOD DIGESTION ICFD2022; Book of Abstracts; Cork, Ireland (2022) p. 21.
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- Tormási, Judit; Abrankó, László; Quality control of fatty acid profiling in vitro digests;
 In: Zsomné, Muha Viktória; Márki, Edit; Baranyai, László (szerk.) BIOSYSFOODENG
 2019 PROCEEDINGS: 3rd International Conference on Biosystems and Food Engineering; Budapest, Magyarország: Szent István University (2019) p. e302.

In Hungarian:

- Tormási, Judit; Abrankó, László; **Lipázgátló bioaktív anyagokat tartalmazó** élelmiszerek zsíremésztésre gyakorolt hatásának vizsgálata in vitro emésztésszimulációval; In: Hungalimetaria Konferencia kiadvány, 2023

- Tormási, Judit; Nagy, Katalin; Tömösköziné, Farkas Rita; Abrankó, László; Rozmaring fűszer szerepének feltárása sült ponty étel emészthetőségében in vitro emésztésszimulációs modell alkalmazásával; In: Fodor, Marietta; Bodor-Pesti, Péter; Deák, Tamás (szerk.) LIPPAY JÁNOS ORMOS IMRE VAS KÁROLY (LOV) TUDOMÁNYOS ÜLÉSSZAK: Összefoglalók; Budapest, Magyarország: Magyar Agrárés Élettudományi Egyetem, Budai Campus (2021) p. 137; p. 133-133., 1 p. ISBN: 978-615-01-3738-4
- Tormási, Judit; Abrankó, László; **In vitro emésztésszimulációs módszer alkalmazása DIAAS meghatározására**; In: MET, METT25 A MAGYAR
 ELVÁLASZTÁSTUDOMÁNYI TÁRSASÁG JUBILEUMI KONFERENCIÁJA:
 Végleges program, előadás- és poszterkivonatok; Pécs, Magyarország : Magyar
 Elválasztástudományi Társaság (2021) p. 05; **ISBN: 978-615-52-7066-6**
- Tormási, Judit; Nagy, Katalin; Abrankó, László; Élelmi tápanyagok biológiai hozzáférhetőségének vizsgálata in vitro emésztésszimulációval; In: MET, METT25 A MAGYAR ELVÁLASZTÁSTUDOMÁNYI TÁRSASÁG JUBILEUMI KONFERENCIÁJA: Végleges program, előadás- és poszterkivonatok; Pécs, Magyarország: Magyar Elválasztástudományi Társaság (2021) p. 53, 1 p. ISBN: 978-615-52-7066-6
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 KÖRNYEZETVÉDELMI ANALITIKAI ÉS TECHNOLÓGIAI KONFERENCIA & 62.
 MAGYAR SPEKTROKÉMIAI VÁNDORGYŰLÉS TUDOMÁNYOS PROGRAMJA;
 Balatonszárszó, SDG Családi Hotel és Konferencia-központ (2019)
- Kovács, Anikó; Tormási, Judit; Szedljak, Ildikó; Somogyi, László; Kereskedelmi forgalomban kapható zsiradékok és keverékeik termikus tulajdonságainak és termékfejlesztési célokra való alkalmasságának vizsgálata; In: Balogh, András; Klein, Mónika (szerk.) MŰSZAKI KÉMIAI NAPOK; Veszprém, Magyarország: Pannon Egyetem (2018) p. 54.
- Kovács, Anikó; Tóth, Viktória; Tormási, Judit; Somogyi, László; Kiskó, Gabriella; Sipos, László; Szeldjak, Ildikó; Különböző hőkezelések hatása tojásnélküli és fürjtojásos tészta kémiai és mikrobiológiai jellemzőire; In: Balogh, András; Klein, Mónika (szerk.) MŰSZAKI KÉMIAI NAPOK; Veszprém, Magyarország: Pannon Egyetem (2018) p. 77.

R&D Projects:

Subject leader of national scientific and R&D proposals and research contracts:

- MEC-R 140813 kódszámú "Tudományos Mecenatúra Pályázat" MEC_R_21 kódszámú alprogramja keretében nemzetközi konferencián való részvétel támogatásának elnyerése.
 Részvétel a 7. Nemzetközi Élelmiszeremésztési Konferencián, részvétel 3 db absztrakttal Vezető kutató/Lead researcher NKFIH; 2022
- EGYETEMI-ÖKO_POC-2021-002 **INFOGEST statikus in vitro emésztés** szimulációs modell validálása élelmiszerek **DIAA** értékének meghatározására/ Validation of INFOGEST static in vitro digestion simulation model for DIAAS

- determination of foods 2019-1.2.1-EGYETEMIÖKO-2019-00006; Vezető kutató/Lead researcher; 2021
- ÚNKP-21-3-2 Élelmiszerek biológiailag hozzáférhető fehérjetartalmának és a szabad fehérjefrakció biológiai értékének meghatározása/Determination of the bioavailable protein content and biological value of foods Vezető kutató/Lead researcher NKFIH; 2021

Participant (non-subject leader) in national scientific and R&D proposals and research contracts:

- OTKA K135294 Az élelmiszer-összetevők biológiai hozzáférhetőségének in vitro vizsgálata emésztést szimuláló modellben/In vitro investigation of the bioaccessibility of food components in digestion simulation model Kutató/Researcher NKFIH, Projekt vezető/Project Leader: Abrankó, László; 2020-2024
- ÚNKP-19-4-SZIE-26 Bólyai plusz "Élelmi zsírok biológiai hozzáférésének csökkentése természetes bioaktív összetevőkkel" című pályázat által támogatott kutatócsoport tagja
- ÚNKP-20-5-SZIE-1 Bólyai plusz "Élelmi fehérjék biológiai hozzáférésének vizsgálata mesterséges emésztés szimulációval" című pályázat által támogatott kutatócsoport tagja
- GINOP_PLUSZ-2.1.1-21-2022-00048 Tejfehérje alapú, speciális célra szánt élelmiszer és tápszerösszetevők fejlesztése/Development of milk protein-based special purpose food and nutritional ingredients Kutató/Researcher NKFIH; Projekt vezető/Project Leader: SOLE-MiZo Ltd.; Szakmai vezető/Scientific Leader: Abrankó, László; 2022-2025
- EGYETEMI-ÖKO_POC-2022-009 Szénhidrát anyagcserét kedvezően befolyásoló funkcionális élelmiszerek, összetevők hatásának igazolására alkalmas vizsgálati platform kialakítása/Development of a test platform to verify the effects of functional foods and ingredients that favourably influence carbohydrate metabolism Kutató/Researcher, Projekt vezető/Project Leader: Abrankó, László; 2022-2023
- E³UDRES² Engaged And Entrepreneurial European University as Driver For European Smart And Sustainable Regions Oktató/Educationer Erasmus+; Vezető/Leader: St. Pölten University of Applied Sciences; 2020-2023

Project leader for student work:

- Bilkei, Fanni, Élelmiszerbiztonsági- és minőségi mérnök MSc nappali tagozatos hallgató társtémavezetése "Tejfölök-és tejföl imitátumok fogyasztói megítélése és biológiailag hozzáférhető zsírtartalma" (2021) OTDK 1. helyezést elért dolgozat
- Bognár, Kitti Annamária, Élelmiszerbiztonsági- és minőségi mérnök MSc nappali tagozatos hallgató társtémavezetése "Az akasztói szikiponty és brokkoli köret in vitro emésztésének vizsgálata" (2020)
- Fekete, Szimonetta, Élelmiszerbiztonsági- és minőségi mérnök MSc levelező hallgató témavezetése "Különböző eredetű növényi olajok hatása főtt tészta emészthető fehérjetartalmára" (2022) Intézményi TDK 2. helyezést elért dolgozat
- Illés Klaudia, Élelmiszerbiztonsági- és minőségi mérnök MSc nappali tagozatos hallgató témavezetése "Szőlőmag-őrlemény zsír- és fehérjeemészthetőségre gyakorolt hatásának vizsgálata in vitro emésztésszimulációs modellben" (2023)

- Anas Al Halabi, Food Engineering MSc, nappali tagozatos hallgató társtémavezetése "Characterization of plant protein powders: functional and digestive properties" (2023)

Total points for PhD procedure

1. Publications and their response	Required	Reached
1.1. Publication in an IF journal	min. 20	50
1.2 In a conference publication		46
1.3 Book, note	-	-
1.4 References (MTMT)	-	10
2. Specific scientific works	=	-
3. External research sources	=	17
4. Education of young scientists	=	4,5
5. Other scientific activities	-	-
1-5. Total	min. 40	127,5