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Development of separation techniques for complex characterization of plant proteins and carbohydrates

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1. SUMMARY

In the Research Group of Cereal Science and Food Quality at the Department of Applied Biotechnology and Food Science of BME, separation technique has been part of the methods used for the complex quality assessment of food and food ingredients for a long time. Our colleagues working in our current and predecessor department achieved serious results with the help of their separation technique methods, for example in the analysis of protein and carbohydrate composition, analysis of lipids (fatty acids), quantitative and qualitative evaluation of biogenic amines and amino acids, etc. In addition to determining the composition of the raw material, the impact of different molecules on quality and technological properties was always an important question. It was always possible to investigate this using the modern tools and methods of the time, so the application of gel chromatography, high-performance liquid chromatography, gas chromatography and electrophoretic techniques determined the quality of both research and education. In recent years, the research group has mainly dealt with the quality of grains, their composition, their technological potential and their evaluation from a food safety aspect. For the research of these areas, molecular level (mainly protein and fibre composition) examinations have become essential, for which modern electrophoretic and chromatographic methods are excellent tools. However, their proper application is a great challenge, because in most cases, serious method development and/or method adaptation and partial validation tasks are required for their routine use. In the following, we provide a brief overview of the projects and results achieved in our research group in the field of separation techniques through a few application examples.

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2. Introduction

The range of our plant-based foods is wide, and the consumption of them is necessary to cover our energy intake, they also partially provide our protein and fibre source, but they also have many ingredients that have a beneficial effect on our body. Unfortunately, they may also contain molecules that can be associated with disorders affecting the digestive system (e.g. allergies, celiac disease, irritable bowel syndrome).

In the field of food analytics, we have a number of methods available to characterize foods, but we are faced with a difficult task when we try to analyse and understand such complex material systems like cereal-based dough and bakery products. Because targeted sample preparation, maintaining the native structure during sample preparation, selective separation of different components, or detection of components can be a serious challenge.

In the BME ABÉT Research Group of Cereal Science and Food Quality, within the separation techniques, we mainly deal with the examination of proteins and carbohydrates, which covers the characterization of raw materials or, for example, the interpretation of phenomena experienced during rheological and technological behaviour. Our research goals and tasks can be divided into three large groups: the determination of nutritional value and the quantity of health-supporting ingredients, the characterization of carbohydrates and proteins that fundamentally determine technological behaviour, and the examination of certain ingredients that are critical from a food safety point of view.

3. Characterization of carbohydrates

Among plant carbohydrates, starch has fundamental importance, which is relevant from a nutritional point of view due to our energy needs, and it is a key component in technology, for example, it affects the quality of products made from grains, and in many cases, in its native or modified form, it is an important additive in dough improvement. In the case of non-starch carbohydrates, dietary fibres can play an important role both nutritionally and technologically, depending on their quality. In the case of grains, the most prominent representatives are arabinoxylan, β-glucan and arabinogalactan peptides. Small molecular size carbohydrates are also important for energy intake (e.g. sugars), and can also determine the sensory properties of products. However, some members of this group (FODMAP - fermentable oligo-, di-, monosaccharides and polyols) can pose a food safety problem for certain consumers. **[1, 2]**

Starch, its structure, amylose/amylopectin ratio and size can provide information about its technological properties. The size of the starch particles is related to the water absorption, which in dough systems determines the dough's extensibility and resistance. Among the starch constituents, amylose results in a firmer gel. However, amylose is more involved in the aging process than amylopectin, so its high ratio reduces the shelf life of products. **[1, 3]**

The quantification of starch and its constituents and the characterization of their molecular properties are a serious challenge, as it is hardly possible to apply selective solution and to determine it and its constituents specifically. Enzyme kits are available to determine the total amount of starch and the amylose/amylopectin ratio (amylose content) **[4, 5]**, but their reliability is questionable for the reasons mentioned above. For our research, we perform starch characterization with size exclusion high-performance liquid chromatography (HPLC), which allows us to obtain information on the size of the starch and the amylose/amylopectin ratio. We have used this in many research projects, for example to study wheat with high amylose and high amylopectin ratios produced by plant breeding. In addition, we also participated in a project focusing on the technological implementation of wheat-based starch production, in which case we mainly solved the examination of the variability of raw materials and the monitoring of starch production in this way. **[6, 7]** *(Related projects: GINOP-2.1.1-15-2016-00855; OTKA K112179)*

Fibres play a role in forming the technological properties of cereal based products, but their positive effects on our bodies are also crucial. For assessing the role of fibres in shaping nutritional and technological behaviour, it is important to characterize them from several points of view. We are currently characterizing arabinoxylans in detail using separation techniques, whose composition and size provide valuable information. The ratio of xyloses, which form the backbone of the molecule, and arabinoses, which make up the side chains, basically determines the properties of the polymer, e.g. solubility and size **[8]**. Information on the composition and ratio of the monomers that make up the polymer, as well as their absolute amount, is obtained using the gas chromatography method. In addition to the composition, it is also important to map the size of the fibre molecules in order to examine their effect on the technological properties. For this, the polymer structure is left unchanged, and the size distribution can be examined by size exclusion liquid chromatography. It is currently an unsolved problem that, it is only possible to determine the properties of soluble fibres in this way. It is still questionable how to characterize the insoluble fibres present in the vast majority of cereals. We encounter similar methodological problems for example in the case of examination of HMW glutenins (high molecular weight gluten proteins).

Numerous publications are available in the literature on the evolution of the total fibre content, but much less on the amount of individual fibre constituents. In our group, several projects are connected to the determination of arabinoxylans, during which we mainly mapped the effect of genetic and environmental factors, thus the variability of these molecules (***Figure 1***). **[9, 10, 11]**. *(Related project: OTKA K112179)*

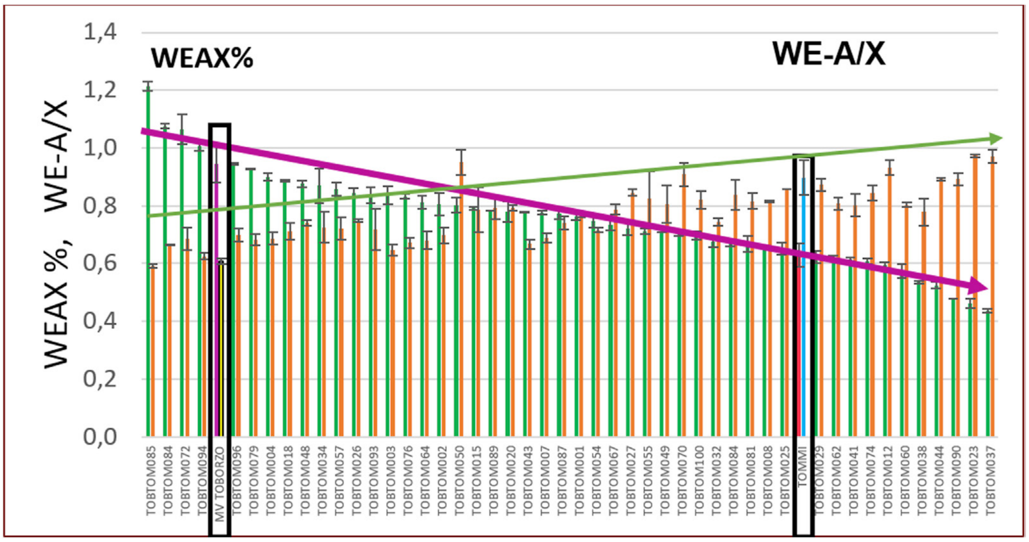

Figure 1. Soluble arabinoxylan content (WEAX%) and arabinose/xylose ratio (WE-A/X) of experimental breeding lines produced by targeted plant breeding ***[11]***

In addition to the raw material characterization, we also examined the influence of the fibre-forming agents on the technological behaviour. One of the defining research tasks of our group in recent years was the forming of a dough structure similar to gluten-containing grains in the development of gluten-free products. Since the gluten-free raw materials lack the proteins that give the appropriate structure of the dough, this must be replaced somehow. During our research, we attempted to at least partially replace this with carbohydrates, namely by forming an arabinoxylan network (***Figure 2***). Our liquid chromatography method proved to be suitable for tracking this, proving the formation of the network and the changes that occur in the polymers as a result of enzyme treatment (***Figure 3***). **[12, 13, 14]** *(Related projects: OTKA ANN-114554; TÉT_15-1-2016-0066)*

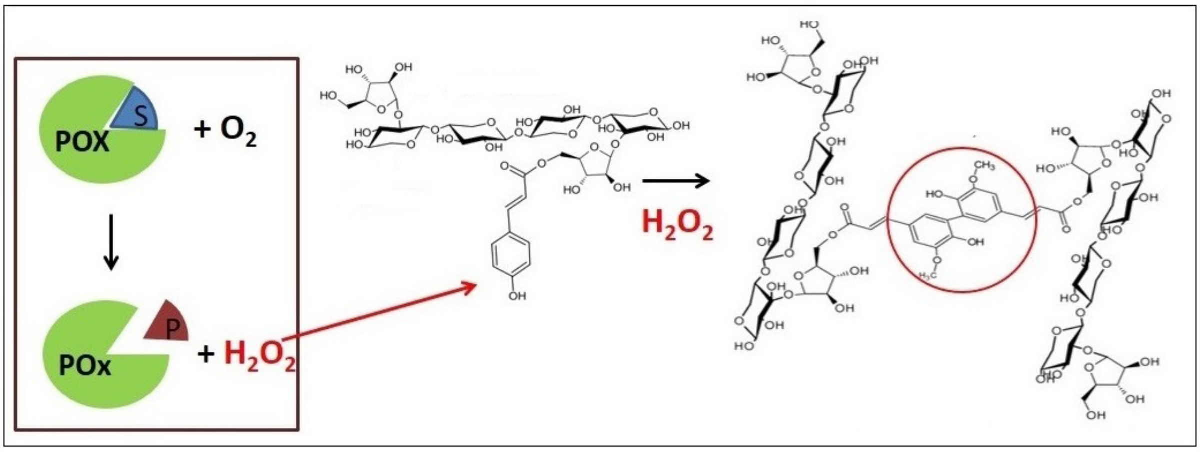

Figure 2. A possible mechanism of the effect of enzyme treatment on the formation of the arabinoxylan network (POx – pyranose oxidase; S (substrate) – mono- and disaccharides; P (product) – dicarbonyl derivatives) ***[15]***

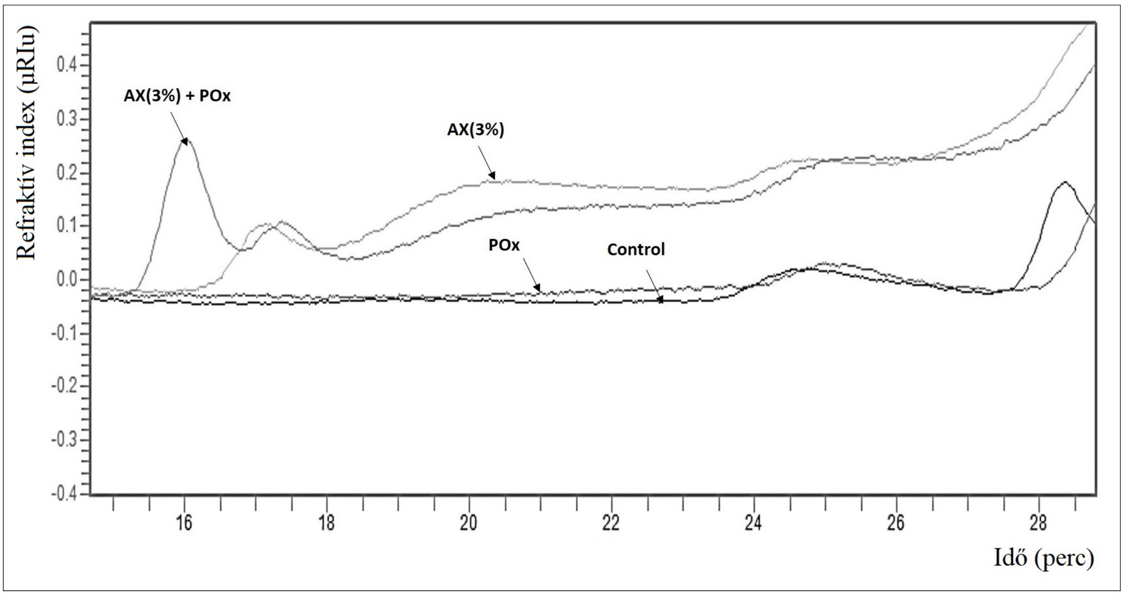

Figure 3. Examination of the enzyme treatment and the formation of the arabinoxylan network using the SE-HPLC method on a whole grain millet sample (AX – arabinoxylan; POx – pyranose-oxidase) ***[15]***

Irritable bowel syndrome, which can be classified as a disorder affecting the digestive system, can be associated with the consumption of short-chain carbohydrates whose collective name is FODMAP **[16]**. In the past period, we have focused on mapping these in cereals, the qualitative and quantitative analysis of them can be solved using liquid chromatography methods.

There are many plant-based foods that can be considered high risk in terms of FODMAP content. Most of the cereals can be classified here, but we know almost nothing about the genetic and environmental variability of the FODMAP content of cereals. In recent years, during research work with our partners in the breeding and milling industry, we have begun to develop analytical methods suitable for the determination of these components in cereals. As a result, with adapted and improved methods, we are now able to provide information on the small molecular carbohydrate composition of different grain species and varieties, as well as different milling fractions. This enables mapping the quantitative and qualitative variability of these components. *(Related project: 2017-1.3.1-VKE-2017-000)*

4. Examination methods of plant proteins

The classification of plant proteins is traditionally based on the Osborne fractionation, which separates individual proteins according to their solubility. Although this does not always mean homogeneous groups in terms of function, size and composition, so, for example, in the case of cereal proteins, other classification including other aspects has also been developed. **[17, 18]**

In our group, we mainly deal with the understanding of the techno-functional properties of different proteins, and with the characterization and development of the analytical environment of proteins which are critical in food safety aspect. We mainly use liquid chromatography methods based on size exclusion and reversed-phase separation to examine the protein composition.

In case of wheat, the number of sources related to protein composition available in the literature is large, but interestingly the amount of knowledge about very similar and closely related grains (e.g. rye, barley, oat) is much smaller. Therefore, dealing with these kind of grains is a much more difficult task in our research, because we can rely less on the methods and data described in the literature. So the interpretation of the results and the identification of the obtained chromatographic peaks are often difficult due to the lack of knowledge. The development and application of liquid chromatography methods takes place in parallel with the mapping of the detailed compositional, rheological and technological behaviour of oats and rye. As a result, we can get a more detailed picture of the protein composition and its variability of these grains (***Figure 4***). **[19]** *(Related project: 2017-1.3.1-VKE-2017-000)*

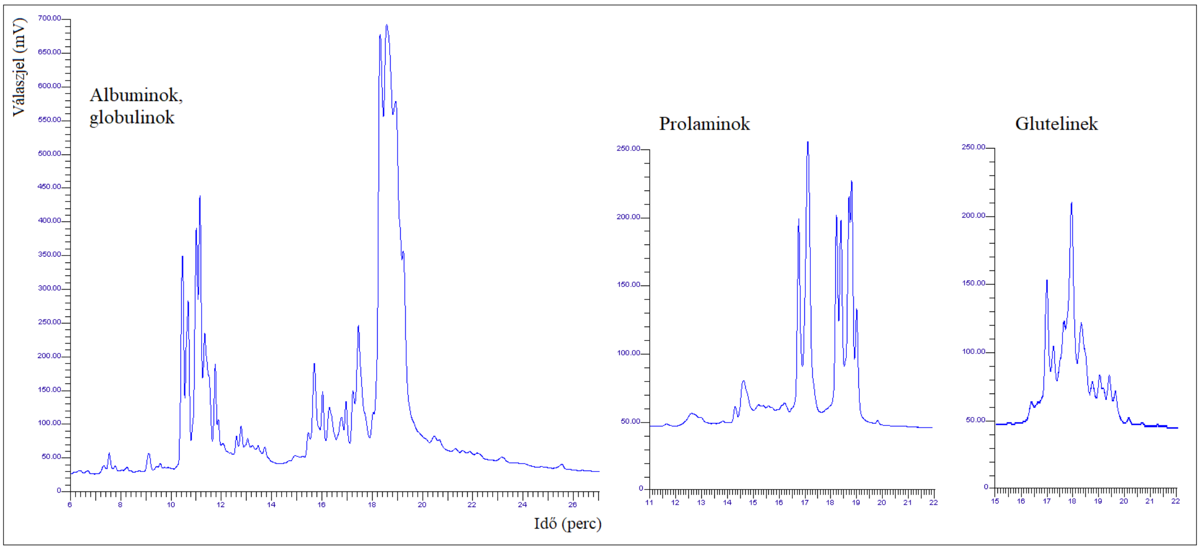

Figure 4. Profile of oat proteins separated by Osborne fractionation and reversed-phase liquid chromatography (own figure)

However, we can use these methods not only for the characterization of raw materials. When developing products with appropriate technological characteristics, it is essential to understand the interactions between the components in the dough system. The researches at our department has contributed to understanding of the gluten complex formation **[20]**. But there are still many questions about the relationship between proteins and carbohydrates, the role of fibres and the integration of molecules added from an external source into the dough system. Understanding the exact mechanism of them is essential for product development aimed at improving technological properties, and chromatographic methods can be a good tool for it. They are well suited for monitoring the effect of various technological treatments in product development as well. An example of this is the examination of the effects of the kneading, dough rising and baking steps, as well as the arabinoxylan addition in terms of protein composition in gluten-free product development. **[21]** *(Related project: OTKA ANN-114554)*

Last, but not least, components critical to food safety (e.g. proteins that cause celiac disease) can also be examined with the help of separation techniques. The lack of reference materials is a serious problem with regard to the reliability of analytical methods suitable for detecting/determining gluten contamination. For several years now, we have been working with companies producing immunoanalytical rapid tests and foreign partner research groups to solve this problem. An essential part of the development is the mapping of the genetic and environmental variability of the proteins that cause celiac disease, as well as the changes in the physico-chemical properties of the proteins during food production. The former was mainly investigated using reversed-phase HPLC, while the latter was studied using size-exclusion liquid chromatography. A more detailed analysis of the proteins was first carried out in case of wheat, however, rye and barley proteins are also involved in the disorder, which are currently being investigated in our research group. **[22, 23, 24, 25]** *(Related project: FOODCT-2006-036337)*

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- TKP2021 funding programme, BME-EGA-02 and BME TKP-BIO 2020 projects
- "GalgaGabona project: Developments to improve the conditions of human utilization of oats and rye in terms of food safety, agrotechniques, processing technology and nutritional value" project (2017-1.3.1-VKE-2017-00004)

- “Improving gluten-free dough by a novel hemicellulose network” (OTKA ANN 114554) (FWF I1842-N28)
- “Fundamental study on the structure, rheological and functional properties of model gluten-free dough and products based on modified carbohydrate systems” (TÉT_15-1-2016-006)
- “New aspects in wheat breeding: improvement of the bioactive component composition and its effects” (OTKA 11279)(FWF-I1842-N28)
- “Vállalatok K+F+I tevékenységének támogatása: Minőségorientált komplex ipari termelési rendszer és modell kifejlesztése, új módosított keményítő kialakítása, illetve új rostalapú feldolgozott termék hasznosításának kutatása” című projekt (GINOP-2.1.1-15)
- “Health Promotion and Tradition: Development of raw materials, functional foods and technologies in cereal-based food chain” (TECH_08_A/2-2008-0425)
- “Development of quality oriented, harmonized educational and R+D+I strategy and operational model at the Budapest University of Technology and Economics” (TÁMOP-4.2.1/B-09/1/KMR-2010-0002)
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5. References

[1] Goesaert H., Brijs K., Veraverbeke W.S., Courtin C.M., Gebruers K., Delcour J.A. (2005): Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology* **16** (1-3) pp. 12-30. https://doi.org/10.1016/j.tifs.2004.02.011

[2] Khan K., Shewry P.R. (2009): *Wheat: Chemistry and Technology*. 4th ed. AACC International, Inc.

[3] Gray J.A., Bemiller J.N. (2003): Bread Staling: Molecular Basis and Control. *Comprehensive Reviews in Food Science and Food Safety* **2** (1) pp. 1-21. https://doi.org/10.1111/j.1541-4337.2003.tb00011.x

[4] McCleary B.V., Charnock S.J., Rossiter P.C., O’Shea M.F., Power A.M., Lloyd R.M. (2006): Measurement of carbohydrates in grain, feed and food. *Journal of the Science of Food and Agriculture* **86** (11) pp. 1648-1661. https://doi.org/10.1002/jsfa.2497

[5] McCleary B.V., Charmier L.M.J., McKie V.A. (2018): Measurement of Starch: Critical Evaluation of Current Methodology. *Starch – Stärke* **71** (1-2) 1800146. https://doi.org/10.1002/star.201800146

[6] Jaksics E., Paszerbovics B., Egri B., Rakszegi M., Tremmel_Bede K., Vida Gy., Gergely Sz., Németh R., Tömösközi S. (2020): Complex rheological characterization of normal, waxy and high-amylose wheat lines. *Journal of Cereal Science* **93** 102982 pp. 1-11. https://doi.org/10.1016/j.jcs.2020.102982

[7] Fekete D. (2021): A fajtahatás vizsgálata a búzakeményítő előállítás laboratóriumi modellezése során nyert termékekben. *MSc Diplomamunka*. Budapesti Műszaki és Gazdaságtudományi Egyetem, Budapest.

[8] Saulnier L., Sado P.-E., Branlard G., Charmet G., Guillon F. (2007). Wheat arabinoxylans: Exploiting variation in amount and composition to develop enhanced varieties. *Journal of Cereal Science* **46** (3) pp. 261-281. https://doi.org/10.1016/j.jcs.2007.06.014

[9] Török K., Szentmiklóssy M., Tremmel-Bede K., Rakszegi M. Tömösközi, S. (2019): Possibilities and barriers in fibre-targeted breeding: Characterisation of arabinoxylans in wheat varieties and their breeding lines. *Journal of Cereal Science* **86** pp. 117–123. https://doi.org/10.1016/j.jcs.2019.01.012

[10] Tremmel-Bede K., Szentmiklóssy M., Tömösközi S., Török K., Lovegrove A., Shewry P.R., Láng L., Bedő Z., Vida Gy., Rakszegi M. (2020): Stability analysis of wheat lines with increased level of arabinoxylan. *PLoS ONE* **15** (5) pp. 1–15. https://doi.org/10.1371/journal.pone.0232892

[11] Szentmiklóssy M., Török K., Pusztai É., Kemény S., Tremmel-Bede K., Rakszegi M., Tömösközi S. (2020): Variability and cluster analysis of arabinoxylan content and its molecular profile in crossed wheat lines. *Journal of Cereal Science* **95** 103074 pp. 1-8. https://doi.org/10.1016/j.jcs.2020.103074

[12] Bender D., Nemeth R., Cavazzi G., Turoczi F., Schall E., D’Amico S., Török K., Lucisano M., Tömösközi S., Schoenlechner, R. (2018): Characterization of rheological properties of rye arabinoxylans in buckwheat model systems. *Food Hydrocolloids* **80** pp. 33-41. https://doi.org/10.1016/j.foodhyd.2018.01.035

[13] Németh R., Bender D., Jaksics E., Calicchio M., Langó B., D’Amico S., Török K., Schoenlechner R., Tömösközi S. (2019): Investigation of the effect of pentosan addition and enzyme treatment on the rheological properties of millet flour based model dough systems. *Food Hydrocolloids* **94** pp. 381–390. https://doi.org/10.1016/j.foodhyd.2019.03.036

[14] Farkas A., Szepesvári P., Németh R., Bender D., Schoenlechner R., Tömösközi, S. (2021): Comparative study on the rheological and baking behaviour of enzyme-treated and arabinoxylan-enriched gluten-free straight dough and sourdough small-scale systems. *Journal of Cereal Science* **101** 103292. https://doi.org/10.1016/j.jcs.2021.103292

[15] Németh R., Bender D., Jaksics E., Calicchio M., Langó B., D'Amico S., Török K., Schoenlechner R., Tömösközi S. (2019): Investigation of the effect of pentosan addition and enzyme treatment on the rheological properties of millet flour based model dough systems. *Food Hydrocolloids* **94** pp. 381-390. https://doi.org/10.1016/j.foodhyd.2019.03.036

[16] Ispiryan L., Zannini E., Arendt E.K. (2020): Characterization of the FODMAP-profile in cereal-product ingredients. *Journal of Cereal Science* **92** 102916. https://doi.org/10.1016/j.jcs.2020.102916

[17] Osborne, T. B. (1907): *The protein of the wheat kernel*. Publication No. 84. Carnegie Institute: Washington, DC

[18] Shewry P.R., Halford N.G., Lafiandra D. (2003): Genetics of wheat gluten proteins, *Advances in Genetics* **49** pp. 111–184. https://doi.org/10.1016/S0065-2660(03)01003-4

[19] Járó K. (2021): Különböző zab minták fehérjeösszetételének jellemzése nagyhatékonyságú folyadékkromatográfiás módszerekkel. *MSc Diplomamunka*. Budapesti Műszaki és Gazdaságtudományi Egyetem, Budapest.

[20] Lásztity R., Békés F., Örsi F., Smied I., Ember-Kárpáti M. (1996). protein-lipid and protein-carbohydrate interactions in the gluten complex. Periodica Polytechnica Chemical Engineering 44 (1-2) pp. 29-40.

[21] Németh R. (2019): Sütőipari minőség meghatározására alkalmas műszer- és módszerfejlesztések és alkalmazásuk búzaalapú és gluténmentes modelltermékek vizsgálatára. *Doktori értekezés*. Budapesti Műszaki és Gazdaságtudományi Egyetem, Budapest.

[22] Török K., Hajas L., Bugyi Z., Balázs G., Tömösközi S. (2015). Investigation of the effects of food processing and matrix components on the analytical results of ELISA using an incurred gliadin reference material candidate. *Acta Alimentaria* **44** (3) pp. 390–399. https://doi.org/10.1556/AAlim.2014.0018

[23] Hajas L., Scherf K.A., Török K., Bugyi Z., Schall E., Poms R.E., Koehler P., Tömösközi, S. (2018): Variation in protein composition among wheat (Triticum aestivum L.) cultivars to identify cultivars suitable as reference material for wheat gluten analysis. *Food Chemistry* **267** pp. 387–394. https://doi.org/10.1016/j.foodchem.2017.05.005

[24] Schall E., Scherf K.A., Bugyi Z., Hajas L., Török K., Koehler P., Poms R.E., D'Amico S., Schoenlechner R., Tömösközi, S. (2020): Characterisation and comparison of selected wheat (Triticum aestivum L.) cultivars and their blends to develop a gluten reference material. *Food Chemistry* **313** 126049. https://doi.org/10.1016/j.foodchem.2019.126049

[25] Schall E., Scherf K.A., Bugyi Z., Török K., Koehler P., Schoenlechner R., Tömösközi S. (2020): Further Steps Toward the Development of Gluten Reference Materials – Wheat Flours or Protein Isolates? *Frontiers in Plant Science* **11** 906. https://doi.org/10.3389/fpls.2020.00906