Theses of doctoral (PhD) dissertation

Periodic changes in the content characteristics of raw camel and cattle milk determined by FT-MIR spectroscopy and examination of milk quality

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1. Background and objectives of the doctoral thesis

1.1. Introduction

In developed market economies, the continuous monitoring and improvement of the quality characteristics of food is of increased importance, the background of which is above all the building and maintaining of consumer trust, as well as the responsible maintenance of food safety at the social level. Today's customers no longer have the opportunity to independently assess the safe consumption of a food product due to the multi-actor production, distribution and trade chain, so maintaining food quality and safety is the basic task and responsibility of all participants in this chain. This task and responsibility all over the world depends primarily on the care of the participants. But while in developing countries the responsibility mostly remains at this level, in developed countries the food quality and safety requirements of individual food categories have been regulated at higher levels, such as at the level of legislation, codex alimentarius regulations, accepted proposals and guidelines of professional organizations and product councils. This difference depends not only on the level of social and economic development of a given country, but

also on the amount of knowledge we have about specific food. A concrete example of the latter is comparing the amount of scientific knowledge related to the milk of cattle (*Bos taurus taurus*) over several centuries with the amount of scientific knowledge related to the milk of the camel or dromedary (*Camelus dromedarius*). As scientific knowledge material, we mean the "ordered, controlled and systematized set of knowledge" that is "methodically and systematically obtained from the examination, observation, experimentation and analysis of phenomena or facts". So, looking back over several centuries of history, knowledge acquired exclusively through experience and usually only at the level of small communities, which even if they were spread more widely based on oral tradition (e.g. by nomadic shepherds) or rudimentary written memories, cannot be considered as scientific knowledge.

Nowadays, we can witness how the expected food quality and safety characteristics of dromedary milk can be developed and described on the basis of the scientific knowledge acquired in relation to cattle milk, in order to enable to deliver to larger distances and in larger batches this food with special content characteristics and nutritional value, besides the local production and supply.

In addition to the expansion of knowledge materials about the camel, it is also important to periodically summarize and evaluate the results of domestic cattle raw milk certification, to compare them with the information, data, and products existing on the European and world markets in order to position ourselves in the world, and for a similar evaluation in the examined period. This has not yet taken place.

Several decades of domestic experience in improving the quality of raw milk and then in maintaining good quality can contribute to the determination of appropriate qualification parameters for intensively produced camel milk from a food safety point of view, and at the same time to the development of the laboratory control system.

In this thesis, the term "raw milk" is used to name those freshly milked single or mixed milks, which have been sampled by meeting predetermined conditions and are examined, mentioned, and discussed for food safety control and/or scientific purposes.

1.2. Objectives

1.) One of the main goals of this research is the analysis of the milk fat, milk protein, lactose, solids-non-fat content, total bacteria count and somatic cell count values of the raw mixed milk samples from Hungarian producers examined by the domestic raw milk certification laboratory between 2011 and 2020 over a 10-year period, covering the entire country, and comparison of its development by county.

2.) Comparison of the results of the 1st objective with the domestic results obtained in the years preceding the examined period.

3.) We hypothesized that the raw milk components of cattle kept intensively in the continental climate typical of Hungary show significant annual seasonal changes, similar to the raw milk classification data described by the dominant milk-producing countries, as well as the annual seasonal changes described in milking dromedary camels.

4.) Comparison of the results of objectives 1 and 3 with results of other countries.

5.) Another main goal of this research is to monitor the composition of raw camel milk through the entire lactation in the case of dromedaries calved in spring and autumn. The raw milk components were determined using the same FT-MIR technology that was used to determine the composition of Hungarian cattle milk, and whose calibration for camel milk was carried out at the Hungarian Dairy Research Institute as previously published.

1.3. Methods

The examination of the content parameters of both cattle and camel milk was determined using high-capacity laboratory devices operating on the principle of Fourier transform mid-infrared spectroscopy (FT-MIR spectroscopy). The strength of the method lies in its ability to simultaneously analyze several frequency components in a single operation. The procedure is therefore fast, which enables a large number of samples to be examined in a short time.

The raw milk tests were performed at the Raw Milk Qualification Laboratory Department of the Food Research, Testing and Raw Milk Qualification Laboratory of the Hungarian Dairy Research Institute Ltd. (H-1093 Budapest, Bakáts utca 8.). Sampling, sample collection

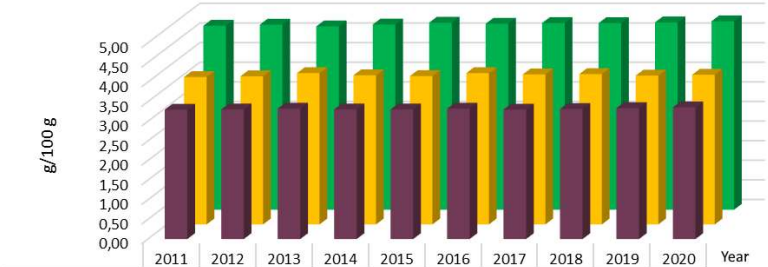
and transport, as well as the conduct of laboratory tests, are regulated by law. In the period from 2011 to 2020, a total of 261151 raw milk samples from 1273 dairy farms were examined. The results were analyzed using Microsoft Excel spreadsheet and Microsoft Access database management programs.

The camel milk tests were carried out in the United Arab Emirates, 25 km from Dubai, at the animal farm of the Emirates Industry for Camel Milk & Products (EICMP) company, in the development and operation of which the contribution of Hungarian specialists has been of decisive importance from the very beginning. The group of camels that calved in spring included 18 animals (Group I), and the group of camels that calved in autumn included 23 animals (Group II). Milk samples were taken during morning milking starting from the day of calving for 122 weeks in Group I, and for 112 weeks in Group II. A total of 6711 samples in Group I. and 9775 samples in Group II. were collected and tested in the laboratory. The tested content parameters of camel milk: milk fat, milk protein, lactose and solids-non-fat content.

2. New scientific results

2.1. Raw milk examinations

As one of the main objectives of this research (1st objective) – on the basis of a large number of raw milk sample tests carried out on a national level over a 10-year period – we determined that the content of milk fat, milk protein, lactose and solids-non-fat (Figure 1-3) changes seasonally within a year, which cyclicality repeats annually (3rd objective). The concentration of milk fat, milk protein and solids-non-fat content is lowest in summer and highest in winter, but in the case of lactose – of which the periodic changes are milder – the minimum concentration can be measured in autumn and the maximum concentration in spring.



	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
AVG/Milk Protein	3,30	3,30	3,32	3,31	3,30	3,32	3,30	3,32	3,34	3,36
AVG/Milk Fat	3,76	3,78	3,86	3,80	3,78	3,86	3,82	3,83	3,79	3,82
AVG/Lactose	4,69	4,72	4,67	4,72	4,77	4,74	4,76	4,76	4,78	4,81

Figure 1: Development of national average milk protein, milk fat and lactose content (g/100 g) between 2011-2020 by year

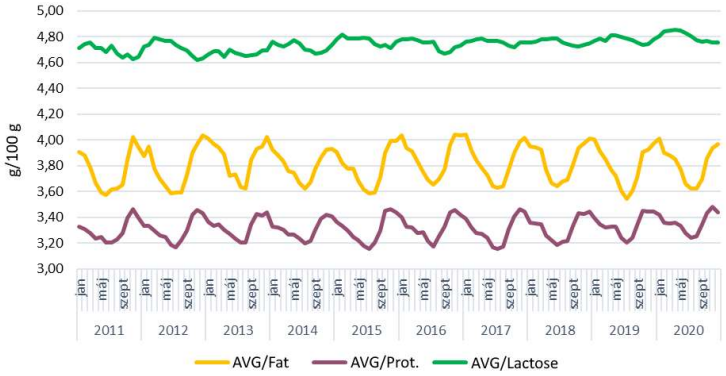


Figure 2: National average milk fat, milk protein and lactose content (g/100g) by month

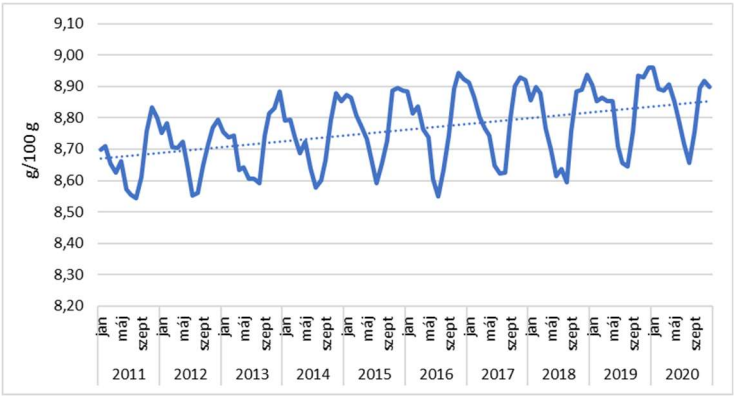


Figure 3: National average solids-non-fat content (g/100 g) between 2011-2020 by month

Based on the analysis of the trend lines, it was established that, in contrast to the constant development of milk fat and milk protein, the annual average values of

lactose and solids-non-fat content show an increasing trend between 2011 and 2020. Given that lactose is part of the solids-non-fat content, we are looking for the possible reasons behind the rising trend in the case of lactose, which requires further investigations (possible reasons: increase in the proportion of first calving animals; genetic change).

Among the milk hygiene characteristics, the total bacteria count and the somatic cell count were analyzed. The monthly average values of the total bacteria count also showed significant differences, but no cyclical pattern repeating every year could be discovered (Figure 4).

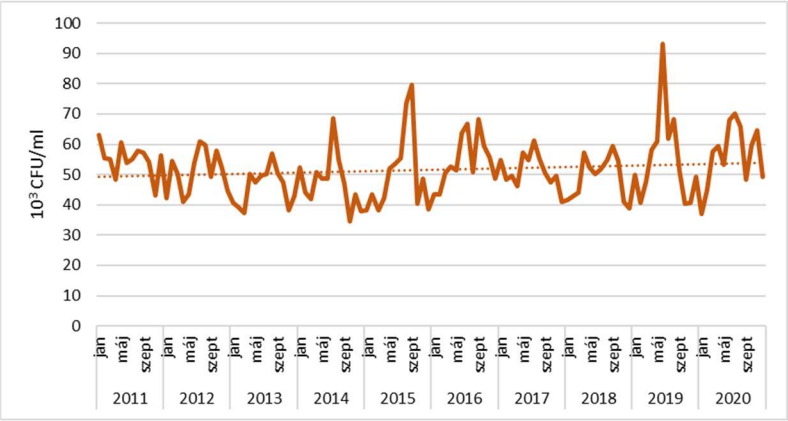


Figure 4: National average monthly values of the total bacteria count (10^3 CFU/ml) between 2011-2020

The somatic cell count changes seasonally within a year, which cycle repeats annually (Figure 5). The amount of somatic cell count is highest in summer, while the lowest quantitative values can be measured from late autumn to spring.

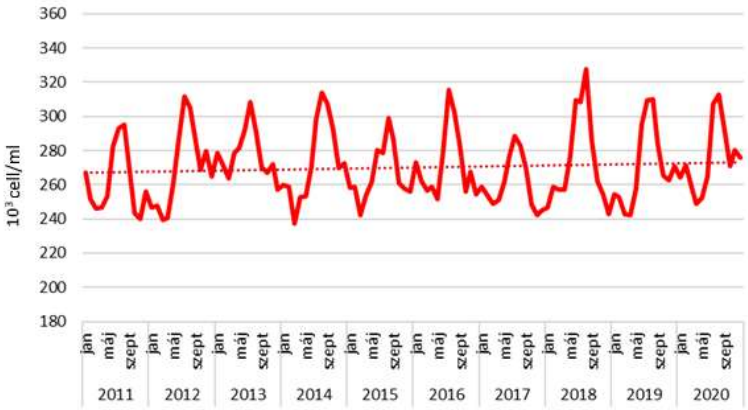


Figure 5: National average monthly values of the somatic cell count (10^3 cells/ml) between 2011-2020

The cyclicity of milk content parameters and somatic cell count within a year can be the result of the influence of many factors and their interaction with each other. Such influencing factors can be the breed, genetic stock, feeding system, animal husbandry conditions, lactation period, milking systems (daily milking frequency, milking technology), climatic effects, etc.

There are several reasons behind the increase in the proportion of objectionable milk samples from 5.5% to 7.5% at national level (Figure 6). The most important of which is that the proportion of farms sending 3 samples per month decreased from 80.7% to 63.4% during the 10-years-period, while the proportion of those submitting only 2 samples per month increased by 17.3%. This shows that the examination of a larger number of samples that better represent the quality of milk – which also serves to improve quality – has increasingly been pushed into the background by dairy farms.

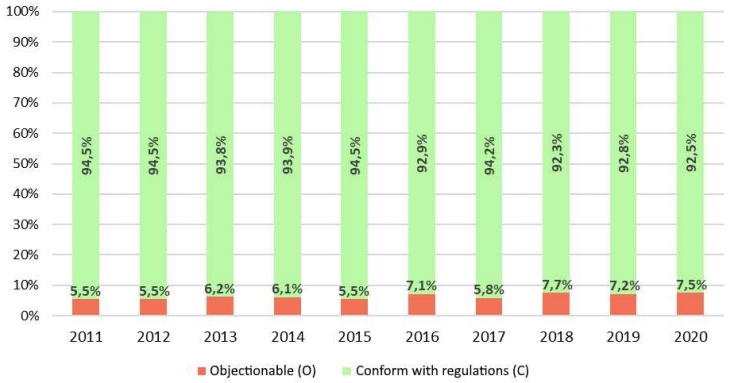


Figure 6: The ratio (%) of milk samples conforming to the regulations (C) and objectionable (O) to the total number of samples in the years 2011-2020

According to our studies, the udder health status of the cows was worse at the end of the examined period than

in 2011. In 2011, 52.6% of the objectionable milk samples were classified as "objectionable" due to the somatic cell count exceeding the limit, this ratio was 62.0% in 2020.

In the period from 2011 to 2020, by analyzing the distribution of objectionable samples according to milk classification by county, we found that the proportion of objected samples was between 2-5% in 13 counties, and between 6-16% in 6 counties of the 19 counties (Figure 7). In these counties, but especially in the four counties with an objectionable sample rate of over 10%, it is extremely important to explore the causes, which can be done in cooperation with the competent animal health authority.

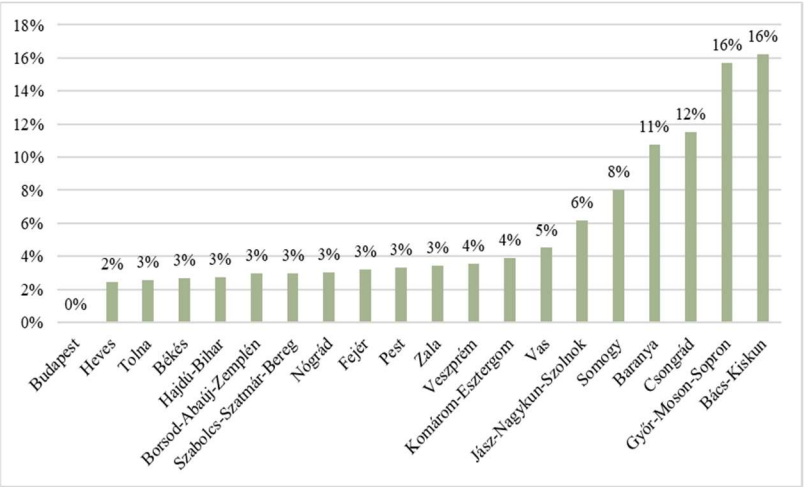


Figure 7: The proportion of objectionable milk samples (%) by county in the period 2011-2020

In accordance with our 2nd objective, we compared the results of our research with previous domestic results. Annual national level data have been available in Hungary since 1984, based on the analysis of which we found that, in contrast to the results before the turn of the millennium, the period from 2011 to 2020 shows a great similarity to the annual average values of the previous decade (2001-2010). Since the values of the qualifying samples correspond to the legal requirements, it can be stated that the quality of domestic raw milk was also excellent during the examined period.

Comparing our results (4th objective) with the results found in the literature of other European and non-European countries that are dominant in milk production in the world, we found that similar studies were conducted in several countries, but in these (with a few exceptions) they usually focused on a shorter period and/or a smaller territory. Although the data and results are very different, the annual cyclicity of milk content parameters and milk hygiene characteristics has been verified in most cases, however, in several cases with different seasons of the minimum and maximum average values.

2.2. Camel milk examinations

The concentration of milk fat, milk protein and solids-non-fat content of dromedary milk – under the same animal husbandry, feeding and milking conditions – is much more strongly related to environmental effects that vary within the year, typical of the climate, than to the time since calving (Figures 8-10). In contrast, the concentration of lactose decreases steadily as lactation progresses and shows no correlation with environmental effects that vary within a year, typical of the climate (Figure 11). These results confirm the assumption that the strong functioning of the central and peripheral circadian biological clock (the "biological clock of the udder") is behind the marked seasonal variation in the composition of camel milk, despite the fact that environmental factors (the number of hours of light per day) only show a moderate change in the natural environment of dromedaries.

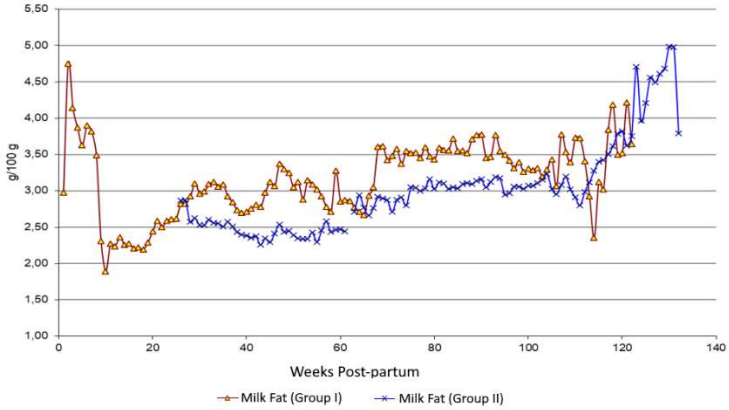


Figure 8: The average milk fat content (g/100 g) of camels calved in the spring (Group I) and calved in the fall (Group II) corrected by the seasonal effect (the results of the Group II were indicated with a shift of 26 weeks).

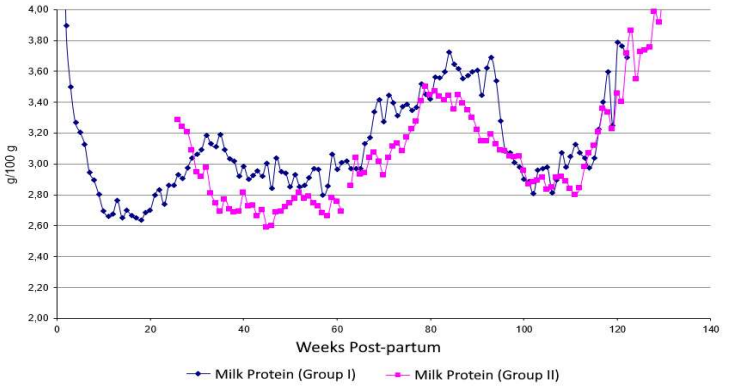


Figure 9: The average milk protein content (g/100 g) of camels calved in the spring (Group I) and calved in the autumn (Group II) corrected by the seasonal effect (the results of the Group II were indicated with a shift of 26 weeks).

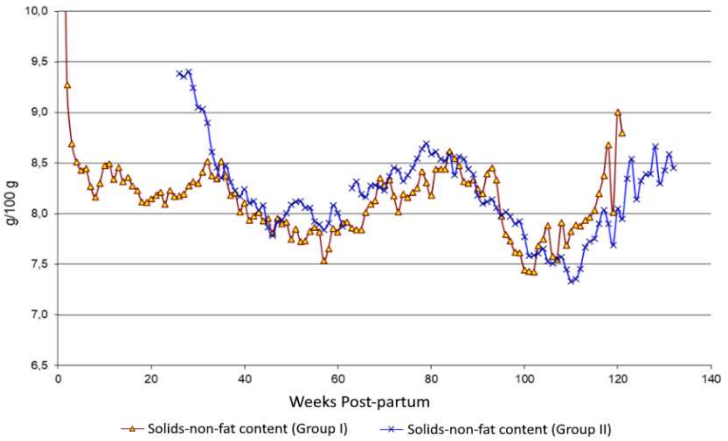


Figure 10: The average solids-non-fat content (g/100 g) of the milk of camels calved in the spring (Group I) and calved in the autumn (Group II) corrected by the seasonal effect (the results of the Group II were indicated with a shift of 26 weeks).

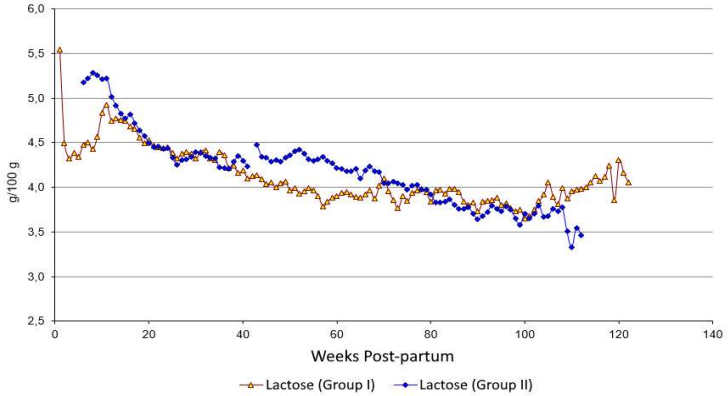


Figure 11: The average lactose content (g/100 g) of the milk of camels calved in spring (Group I) and calved in autumn (Group II) during lactation (data seasonally not adjusted)

2.3. Summary of new scientific results

1. Based on Hungarian mixed raw milk tests, the content of milk fat, milk protein, lactose and solids-non-fat varies seasonally within the year, and this cycle repeats annually. The concentration of milk fat, milk protein and solids-non-fat content is lowest in summer and highest in winter, but in the case of lactose, the minimum concentration can be measured in autumn and the maximum concentration in spring.

2. Based on Hungarian mixed raw milk tests, among the milk hygiene characteristics, the somatic cell count changes seasonally within the year, which cycle repeats every year. The amount of somatic cell count is highest in summer, while the lowest quantitative values can be measured from late autumn to spring.

3. The concentration of milk fat, milk protein and solids-non-fat content of dromedary milk – under the same animal husbandry, feeding and milking conditions – is much more strongly related to environmental effects that vary within the year, typical of the climate, than to the time since calving. In contrast, the concentration of lactose decreases steadily as lactation progresses and shows no correlation with environmental effects that vary within a year, typical of the climate.

3. Publications on the topic of the dissertation

1. Kocsis, R., Süle, J., Nagy, P., Gál, J., Tardy, E., Császár, G., Rács, B.: **Annual and seasonal trends in cow's milk quality determined by FT-MIR spectroscopy in Hungary between 2011 and 2020**, Acta Vet. Hung., (*in press*)

2. Hejel P., Kocsis R., Könyves L., Helyes K.: **Biológiailag aktív peptidek a tejben: Irodalmi összefoglaló (Bioactive peptides in milk: Literature review)**, Magyar Állatorvosok Lapja (Hungarian Veterinary Journal), 143. 47-55, 2021.

3. Nagy, P., Juhász, J., Reiczigel J., Császár, G., Kocsis, R., Varga, L.: **Circannual changes in major chemical composition of bulk dromedary camel milk as determined by FT-MIR spectroscopy, and factors of variation**, Food Chem., 278. 248-253, 2019.

Presentations on the topic of the dissertation

1. Kocsis R.: **Milk quality in Hungary**, Presentation at „11th European Mastitis Panel" conference, Budapest, 17-18 May 2018.

2. Kocsis, R., Nagy, P.: **The developments potential of the camel dairy industry: Innovative methods and technologies to improve dromedary camel milk quality and extend the shelf-life of camel milk products**, Presentation at „Salon International de l'Agriculture du Maroc – SIAM 2017." event, Meknes, Morocco, 20 April 2017.

3. Kocsis, R., Nagy, P.: **Hungarian know-how transfer to North Africa and Middle East – The role of Hungarian Dairy Research Institute in innovative camel milk processing technologies**, Plenary presentation at „18th Wellmann International Scientific Conference" (online event), Szeged, 13 May 2021.

4. Kocsis R.: **Miért fontos a tej? Tények, tévhitek a tejjel és a tejfogyasztással kapcsolatban (Why is milk important? Facts, misconceptions about milk and milk consumption)**, Presentation at Pensioner University of Széchenyi István University, Faculty of Agricultural and Food Sciences, Szechenyi Istvan University, 17 February 2022.