DOCTORAL (PhD) DISSERTATION THESES

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Budapest

2022

Hungarian University of Agriculture and Life Sciences

The development of a gluten-free flour mixture and the examination of the final product with regard to the consumer requirements

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2022

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1. INTRODUCTION

Bakery products -like bread- have played an important role in everyday meals for thousands of years, but the consumption of wheat, barley or rye flour based products cause health problems for a growing part of population. These people have to follow a gluten-free products, which differ from their wheat-based counterparts. Despite the ever-expanding range of gluten-free ingredients, technologies and products, the taste, colour, texture and shelf-life of gluten-free breads and the rheological properties of gluten-free flour mixtures still different from their wheat-based counterparts.

This PhD dissertation was prepared on behalf of Táplálékallergia Centrum (TAC). The purpose was complex, covering several different but at the same time equally important areas. The number of local and international publications on gluten-free bakery products, as well as consumer need for high quality gluten-free raw materials and finished goods are growing, but unfortunately basic information are missing: detailed and accurate market demand assessment, the rheological, textural and sensorial testing of commercially available gluten-free flours and breads, just as the agreement on methods to be used during the evaluations.

Based on the above mentioned points, aims of the research were:

1. perform market research among gluten-free bread consumers, revealing the exact market needs, priorities, problems regarding the gluten-free flours and breads
2. examination of the commercially available gluten-free flours by farinograph method, and compare the results to wheat and rice flour
3. investigation the presence and effect on the farinograph curve of hydrocolloids (xanthan and hydroxypropyl-methylcellulose)
4. based on literature data and own measurements developing gluten-free flour mixtures using optimalisation approach, which gets similar farinograph parameters to wheat flour as much as possible
5. Physical analysis (hyperspectral imaging and texture profile analysis) and sensory evaluation of gluten-free bread samples, based on the developed gluten-free flour mixtures
6. review the currently used method of sensory evaluation method for breads, and develop proposals for amendments when gluten-free bread is investigated

2. MATERIAL AND METHOD

2.1. Primary and secondary market research

To collect proper and detailed information on consumer needs and habits primary and secondary market research were performed. During the secondary research, relevant publications from the scientific databases (Scopus, Web of Knowledge, PubMed, Google Scholar) were selected to provide information on the following: rootcause of following gluten-free diet, opinion on gluten-free products and raw materials, what are the biggest quality disadvantages of gluten-free flours and breads. Primary research was carried out by an online questionnaire. Number of responders was calculated by the Yamane formula (HALMOS et al., 2018), determined in a minimum of 400 people.

2.2. Farinograph measurements

As there is no agreed method for analysing gluten-free flour mixtures, previous studies were used (GIRI and SAKHALE, 2021; BEITANE et al., 2015; LAZARIDOU et al., 2007) during analysing commercial gluten-free flour. I also investigated the effect of xanthan and hydroxypropyl-methylcellulose (HPMC) addition into Táplálékallergia Centrum (TAC) gluten-free bread flour mix by Farinograph method (LAZARIDOU et al., 2007; BEITANE et al., 2015): what effect does the usage of HPMC and xanthan separately or combined have on the dough rheology, as well as regarding the optimum quantity which should be added to have the best result. During the measurements development time (DT, min), softening (FU), stability time (ST, min) and water adsorption (WA, %) were determined.

2.3. Materials

The hydrocolloids (HPMC and xanthan), pseudocereals and gluten-free flour mixture bases used during the experiments and for product development were known to TAC, as they produce and/or distribute them. All ingredients used were gluten-free, which was confirmed by accredited laboratory test (ELISA method). TAC bread flour was suitable for white bread production, but in my thesis pseudocereals were added to be suitable for brown bread production as well. As a preliminary experiment, sensory test was performed with breads containing increasing amount of buckwheat and amaranth flour. Based on the results, in the final brown-bread base four 1% amaranth and 5% buckwheat flour was added to the TAC base flour mixture. Thus, two bread flours were used: TAC base flour mix for white bread, and TAC base with pseudocereals for brown- and peasant bread (hereafter brown-bread flour).

2.4. Optimalization

In this study TAC bread flour mixtures (for white bread and for brown bread) were used, which were prepared for this purpose without hydrocolloid addition. Aim was to identify the optimal mixing ratio of HPMC and xanthan. For that purpose, with Statistica (Tibco Statistica, California, USA) program design of experiment (DoE) was created separately for the two bread flours (white and brown): 3^2 full factorial experimental design (3 levels, 2 factors). Minimum and maximum amount of hydrocolloids were determined based on previous publications, and were set to be between 0-3% (LAZARIDOU et al., 2007, SAHIN et al., 2020, HORSTMANN et al., 2018). Water absorption of the flour mixtures were determined with Farinograph by reaching the maximum consistency and stability (FARKAS et al., 2021). Dough development time (DT, min), stability (ST, min), softening (SO, min) and water absorption were included as dependent variables, while level of HPMC and xanthan were present as independent variables.

Based on the Farinograph results a model equation was created for the dependent variable results based on the hydrocolloid addition level. Regression calculation (R^2) was used to check the fit of the model equations. Using the model equations, a prediction tool was created (Microsoft Excel 2018). Validation of the model equations were based on comparing the prediction and measured values (RMSE) of 6 flour mixes. Based on the final results, 2 different mixes were created for white and brown loaf breads as well as peasant bread.

2.5. Rheology - Texture profile analysis

Texture Profile Analysis (TPA) is one of the most commonly used methods for testing bread crumb. The essence of the method is to compress the sample twice with a given force, thus imitating chewing. Several parameters can be determined from the resulting force-time curve, which can be linked to sensory properties of the sample (BOURNE, 2002). For the TPA measurements Stable Micro System TA.XT2 device was used with the following settings (MERETEI, 2012):

- measuring method: relaxation
- Measuring head speed: 0.2 mm/s
- Measuring head speed before and after measurement: 2 mm/s
- Measuring head: a/BE35 35 mm diameter plexi disc
- Measurement time: 60s
- Unloading time: 60s
- Loading force: 5N
- Sample height: 15 mm

- Sample diameter: 35 mm
- Sampling: 200 measurements/s

Hardness, cohesiveness and springiness were the main representative parameters of the sample texture. During the TPA measurements gluten-free samples made from the developed flour mixtures were used, and compared to commercial wheat-based counterparts: Ceres Bükki Peasant bread (Ceres Zrt., Hungary), Ceres Sütő Butter Toast bread (Ceres Zrt., Hungary), Roberto Whole grain slices bread (Roberto Industria Alimentare, Italy). From each bread 7 slices were measured on each day of the 4 day long storage test, always using a previously unopened package. None of the bread samples contained preservatives, modified atmosphere or active packaging.

2.6. Hyperspectral imaging

The purpose of the measurement was to estimate the moisture content of the bread slices, to determine their distribution and to monitor them during storage. For this purpose, Headwall Photonics XEVA-1648 XC134 (Specim spectrograph, Xeneth InGaAs) equipment was used. The instrument recorded the spectra in a push-broom layout in the wavelength range of 1300–1600 nm (TSENKOVA, 2009) with a spectral resolution of 5 nm and a spatial resolution of 0.475 mm per pixel. The measuring system was operated by the Argus software (FIRTHA, 2011). To track the spectral changes during the storage of the bread types, an area of 400 pixels (20 × 20) was selected and manually positioned in the middle of the images, and then exported the 400 spectra belonging to these pixels. Segmentation of the HS images were done with CuBrowser (FIRTHA and ÉDER, 2012). For the noise abatement of NIR spectra, Savitzky-Golay smoothing (sgol) (third order polynomial, 9 data points) was applied, followed by different spectrum pretreatment procedures to optimize subsequent statistical modelling. The first qualitative assessment of multivariate statistical analyses was performed by principal component analysis (PCA). Subsequently, the classification of the samples (observations) with linear discriminant analysis (LDA) was performed. Partial least square regression method (PSLR) was used to estimate the moisture content of the bread samples from the NIR spectra.

2.7. Determination of moisture content

Moisture content of the examined samples (gluten-free and wheat-based white loaf bread, wholegrain brown bread and peasant) and their change during the storage experiment were determined using Kern MLB-50-3 type device (Kern & Sohn, Germany). Method was based on drying the samples on high temperature until constant weight was achieved. Sample weight was 2g for each sample. According to the storage experiment, from each bread type 4 samples were taken from an original, sealed pack every day, which had not been used or opened before. All samples were baked and packed on the same day. On each day of the storage test, 5 slices from each sample were taken to analysis (thickness: 12 mm to determine the moisture content. Measurements were performed from the middle part, close-to-crust part and between the two area of the slice, taking 3 samples per bread type every day. Average values of the measurement were used for further analysis with consideration of bread type, storage time and position.

2.8. Sensory evaluation

Within the sensory evaluation descriptive teste (profile analysis method) and a paired comparison test were used based on the description of MSZ 20501-2:2018. In the profile analysis commercially available gluten-free white and brown bread samples were tested by a group of trained panellist (10 people) using the existing bread aroma-wheel (ZHAW, 2018). The bread slices were placed in front of the participants on a white plate with 3-digit randomly generated code, who firstly individually created a list of attributes regarding colour, smell, texture (by touch and tasting) and taste (KÓKAI and SIPOS, 2020). The results were then discussed as a group exercise with the participants using the aroma-wheel and the descriptions included in it. Aroma-wheel described properties that were clearly perceived by the reviewers as well as those which were not included in the aroma-wheel were determined and discussed. As a result, the list of sensory properties/attributes were listed and linked with an unstructured scale of 100 mm length. Following that, two other groups tested the developed gluten-free samples: group of gluten-free diet followers (50 people) and non-dieting people (50 people) using the developed sensory evaluation sheet. Members of these two groups can be considered as untrained panellists. Aim of the sensory evaluation was to check if any significant difference between the panellist groups are present. ANOVA test, followed by post-hoc test (Tukey, Games-Howell test) were done using IBM SPSS Statistics 25.0.2.2. software. The products obtained as a result of the development were examined by the following groups, diet-follower (n=50) non-dieting (n=50), trained (n=10) people. Latter two groups tested the wheat-based counterparts (which were used in the rheological measurements) as well parallel with the gluten-free samples.

3. RESULTS

3.1. Primary and secondary market research

To the question "Do you have any other food consumption related allergy or intolerance beside Coeliac disease?" only 50.8% of the 500 responders said no (Figure 1). Responds revealed, that during gluten-free product development it is beneficial to ignore soy, milk protein and lactose usage, thus the final good can serve and satisfy wider range of consumers.

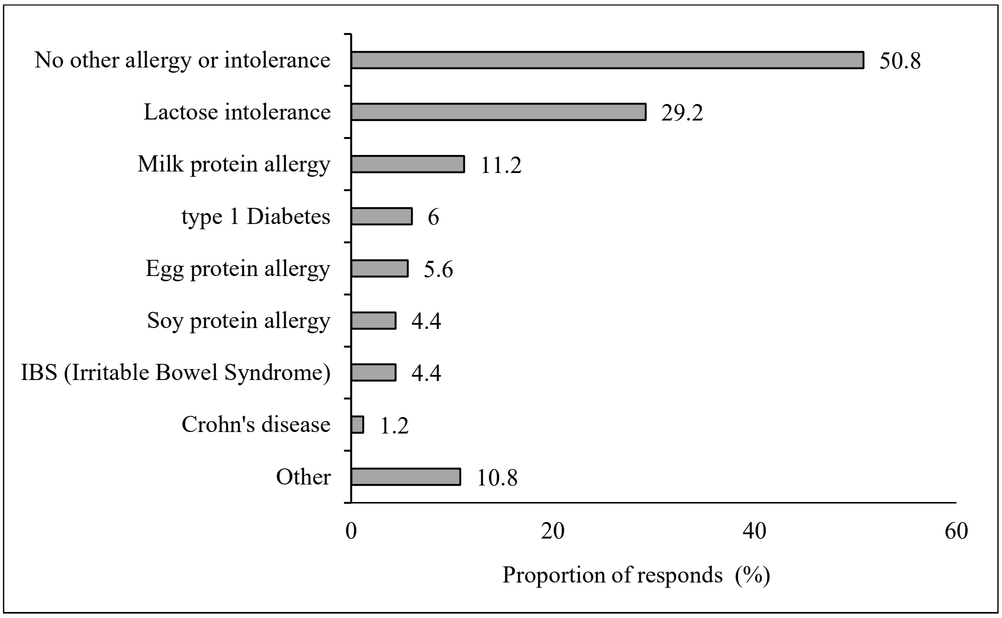

Figure 1. Percentage proportion of responders in terms of having other food consumption related health issue parallel with Coeliac disease

In general, based on the obtained data, man and woman independently from their age bake bread at home twice as often than buying convenient, ready to eat bread. The overlap between regular home bakers and regular buyers was 8%, which covers 15.7% of the regular home bakers and 27.4% of the regular buyers. 60% of this overlapping group reported no other food consumption related disorder, while in the 40% the most common issue was lactose intolerance and milk protein allergy. Those, who bakes bread at home more times a week buys convenient product once a week maximum, and the regular convenient product buyers bake bread at home maximum once in a week.

3.2. Farinograph measurements

Results of the DoE and Farinograph measurement are shown in Table 1. According to the results, sufficiently accurate linear model could not be fitted (Development time – R^2=0.16, Stability – R^2= 0.29, Softening – R^2=0.18). Based on this, non-linear model fitting was performed.

HPMC had a significant ($p<0.05$) effect on dough development time (DT), which is a result being in line with previous publication (SAHIN et al., 2020).

Table 1: Results of Farinograph measurements based on DoE plan

	Sample	**Flourmix** (%)	**HPMC** (%)	**Xanthan** (%)	**Development time** (min)	**Stability** (min)	**Softening** (FU)	**Consistency (**FU)	**Water absorption** (%)
White bread flour mix	1	100	0	0	17.1	0.1	23	300	70
	2	98.5	0	1.5	0.9	0.1	75	403	67.6
	3	97	0	3	19.2	0.7	28	459	64
	4	98.5	1.5	0	0.7	0	213	559	74.5
	5	97	1.5	1.5	3.5	0.1	130	419	68
	6	95.5	1.5	3	13	0	145	495	69.9
	7	97	3	0	0.6	0.1	228	614	77.9
	8	95.5	3	1.5	1.7	6.4	26	339	76
	9	94	3	3	5.3	0	220	524	70.6
Brown bread flour mix	10	100	0	0	19.1	0.9	35	419	68
	11	98.5	0	1.5	7.6	0.2	22	470	64.3
	12	97	0	3	18.8	1.2	25	526	65.7
	13	98.5	1.5	0	0.8	0	180	531	75.8
	14	97	1.5	1.5	0.7	0.1	142	435	68.4
	15	95.5	1.5	3	3.1	0.2	132	423	68.1
	16	97	3	0	0.6	0	175	581	77
	17	95.5	3	1.5	0.8	0	238	476	69.4
	18	94	3	3	11.9	0	151	476	69.4

Based on the result a good model in regard to development time could be applied in case of brown bread flour (R^2=0.85), while in case of white bread flour the result was R^2=0.71. Surface plot diagrams of the model showed the effect of pseudocereals presence in the brown bread flour mix, as less HPMC was needed for the optimal DT with the same amount of xanthan.

Reason for that could be the proteins from buckwheat and amaranth flour, which have a stronger structure with hydrocolloids and pea protein (MARIOTTI et al., 2009; TORBICA et al., 2010).

Based on the result, a good fitting model could be found for dough stability (R^2=0.78) at 95% confidence level for the brown bread flour. For the white bread flour result was R^2=0.56. Similarly to the DT results, HPMC had a significant effect ($p<0.05$) in both cases, and the stability differed from each other with the same amount of hydrocolloids. Based on the surface plot diagrams, in case of brown bread flour the lowest HPMC dosage helped the stability regardless the xanthan amount. In contrast, xanthan effectively increased stability even in small amounts, its increasing amount showed no effect on stability. In case of white bread flour, the highest possible dosage of HPMC showed better stability with 1-2% dosage of xanthan. With that results the stability of the dough seemed to be with increased and better stability compared to brown bread flour.

Applying a model at 95% confidence level for the softening results showed good result with brown bread flour (R2=0.92), while less precise model was found for the white bread flour mix (R^2=0.62). Based on previous publications higher level of corn starch content was increasing the softening (TORBICA et al., 2010, WOJCIK et al., 2021). Based on the surface plot diagrams for brown bread flour higher level of HPMC addition increased the softening. On the other hand, the quantitative change of xanthan showed no effect on the softening. For reaching the desired level of softening the optimal HPMC addition was found to be 0-0.5% for both flour mixtures, regardless of the amount of xanthan.

During the Farinograph measurement the moisture content of the raw dough was determined as a fix parameter at 14%, which is important for industrial processability of the dough. Since the flour mixtures contained different level of hydrocolloids, which significantly affects the water absorption capacity. The models fitted to the measured points showed close correlation in case of brown bread flour (R^2=0.93) as well as for white bread flour (R^2=0.90). HPMC and xanthan both had a significant effect for both of the flour mixes.

3.3. Optimalization

Based on the R^2 and RMSEV values obtained during the measurement and validations, the final model was considered as appropriate (Figure 2). As a next step, using the final model equations a prediction modelling Excel macro was created. With the help of this prediction macro, two different gluten-free flour mixtures were created. Brown bread flour mix: 97% flour base mix, 2% HPMC and 1% xanthan gum with 68% water absorption. White bread flour mix:

97.1% flour base mix, 1.45% HPMC and 1.45% xanthan with 71% water absorption. Farinograph curves of the developed flour mixes are shown in Figure 3.

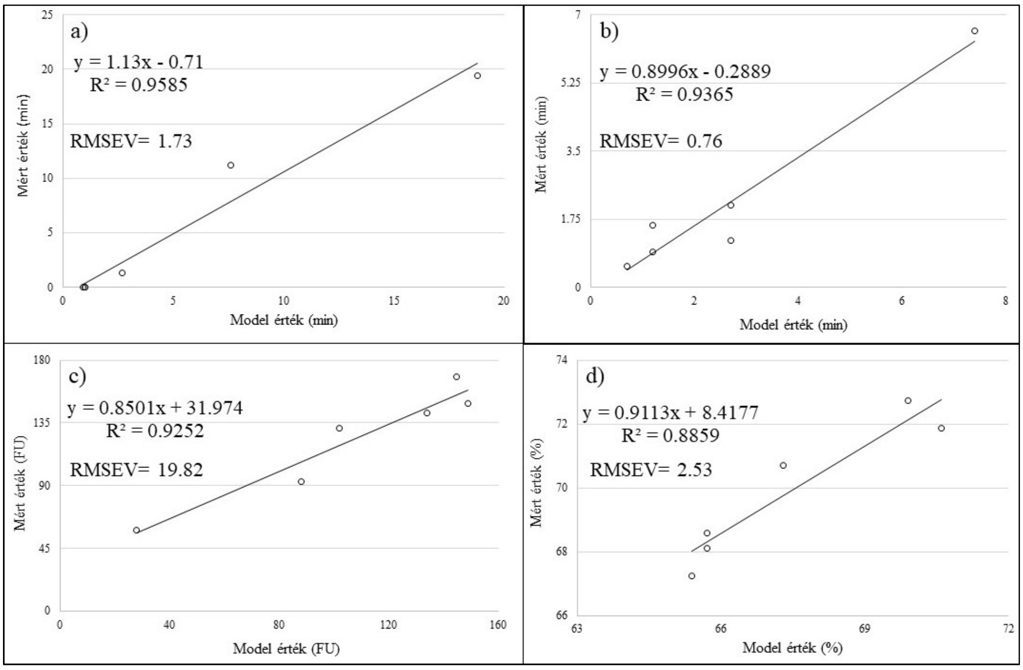

Figure 2. Results of validation measurements (a: Development time, RMSEV=1.73; b: Stability, RMSEV=0.76; c: Softening, RMSEV=19.82; d: Water absorption, RMSEV=2.53)

The farinograph curves of the newly developed flour mixes deviate from the other, commercially available gluten-free flour mixes, and in their parameters they approach better the properties of wheat flour.

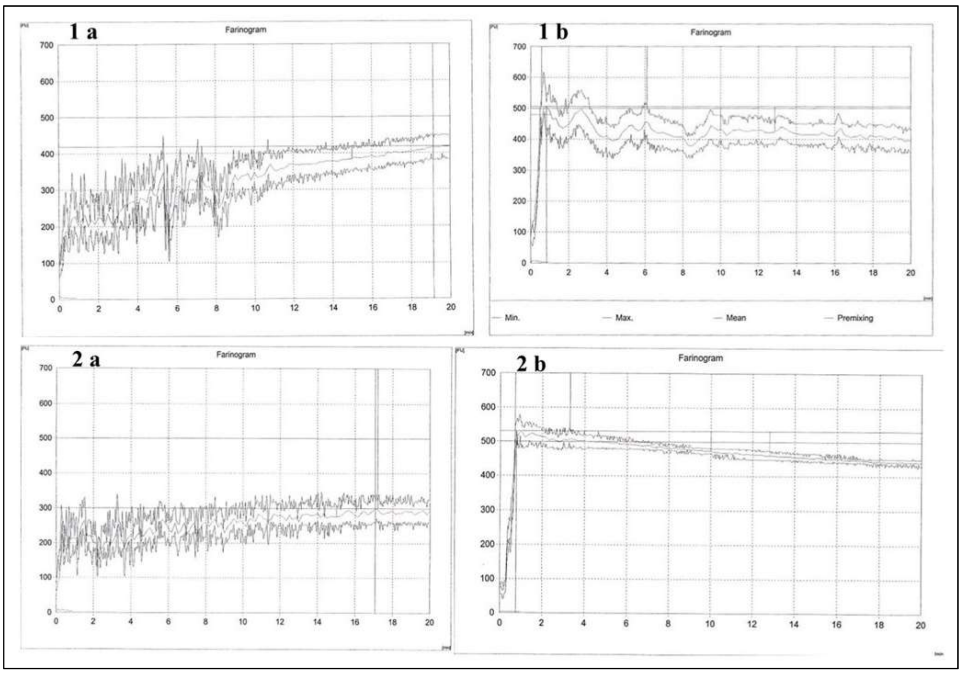

Figure 3. Farinograph curves of the developed flour mixes (1a: brown loaf mix base; 1b: brown bread flour mix with hydrocolloid; 2a: white loaf mix base; 2b: white bread mix with hydrocolloid)

Farinograph data of the developed mixtures shown in Figure 3 (with and without hydrocolloids) and wheat flour used as reference are shown in Table 2.

Table 2. Farinograph data of the developed flour mixtures and wheat flour

	Wheat flour	Brown bread flour mixture		White bread flour mixture	
		Without hydrocolloid	With hydrocolloid	Without hydrocolloid	With hydrocolloid
Consistency (FU)	494	419	506	300	531
Development time (min)	3,5	19,1	0,9	17,1	0,8
Stability (min)	5,8	0,9	5,5	0,1	2,6
Softening (FU)	53	35	74	23	57
Water absoprtion (%)	62,5	64,3	67,3	66,3	70,9

3.4. Texture Profile Analysis results

In line with the literature data, hardness values increased during the storage test. According to the ANOVA analysis ($p<0.05$) hardness of the gluten-free white and brown loaves were significantly lower compared to their wheat flour based counterparts. Storage test results revealed that the change of hardness values was slower and significantly ($p<0.05$) lower of the gluten-free loaves versus the wheat based ones (Figure 4). On the other hand, the gluten-free cob sample showed higher level of hardness from the 2nd day onwards, despite it was significantly lower on the first day compared to the wheat based product. The value between the hardness of those two peasant samples became significant ($p<0.05$) again on day 4. The change in the hardness of gluten free cob bread sample was faster and larger versus the wheat based peasant. From day 2, the hardness values of the gluten-free bread types did not differ significantly.

Among the related publications, a very significant decrease ($p <0.01$) in the cohesiveness of the samples was observed in the experiment of storing gluten-free breads from day 2 onwards (MOORE et al., 2004; PACIULLI et al., 2016). Gluten-free samples in this study also showed decrease in cohesion, but there was no significant ($p<0.05$) difference detected in case of brown loaf and peasant products between gluten-free and wheat based products. In case of white loaf gluten-free sample had significantly ($p<0.05$) higher cohesiveness on day 1 and 4. Gluten-free loaves did not differ significantly ($p<0.05$) from the wheat based peasant during the storage test, which is a remarkable difference compared to the previously published data (PACIULLI et al., 2016). There was no significant ($p<0.05$) difference measured between the gluten-free and wheat based brown loaves during the whole storage experiment.

The gluten-free loaf samples were described with significantly higher ($p<0.05$) value of springiness from day 2 onwards compared to their wheat-based counterparts. Despite the hardness of gluten-free cob increased, the springiness values showed improving results. This can be explained by the fact, that the initial viscosity of bread dough was different, softer compared to the loves. On the first day of the storage test, the peasant breads was softer, with the gluten-free sample being the most malleable.

On day 4, all gluten-free samples showed higher springiness values compared to their wheat-based counterparts. In case of loaves, the difference was significant ($p<0.05$) from day 2 onwards. According to the results, gluten-free bread samples in general had low hardness and high springiness values, therefore can be described as soft and melleable.

LDA results showed that all the gluten-free samples were categorized the same as wheat based cob (Figure 5). On day 1, the first discriminant variable (Function 1) described 87.5% of the variance, while on day 4, this value increased to 94.4%. This result clearly showed that the quality and texture profile attribute changes of the gluten-free samples during a 4 day long storage test were as good as the highest quality wheat based product's considered to be artisan. The result of the cross-validation showed that the established classification model was able to classify 78.57% based on the results of day 1, while according to the results of day 4 it was able to classify 71.43%.

The data proved that among hand-made gluten-free and wheat based products the initial and final quality was not different; therefore, the GF market was able to present a product, which lacks the inferior properties that were claimed before.

Wheat-based loaves samples were classified by the LDA test as different groups from the others during the whole storage test. Both products showed significantly worse results in hardness from the other samples through the whole study, and wheat-based brown loaf was significantly worse in springiness from the other samples from the 2nd day onwards. The significant difference of these two attributes led to show these samples as different product groups.

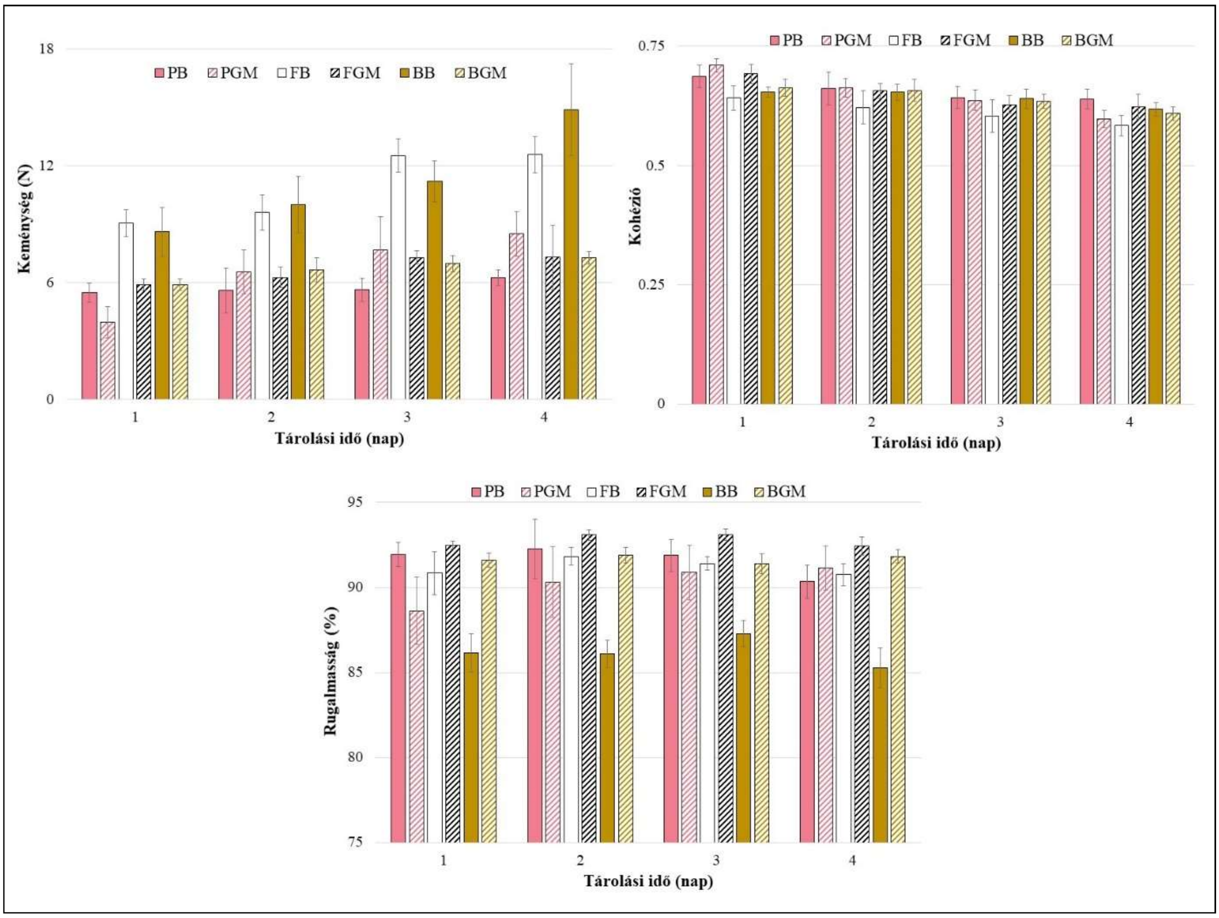

Figure 4. TPA results of the storage test (PB: wheat-based peasant bread; PGM: gluten-free peasant bread; FB: wheat-based loaf; FGM: gluten-free loaf; BB: wheat-based brown loaf; BGM: gluten-free brown loaf)

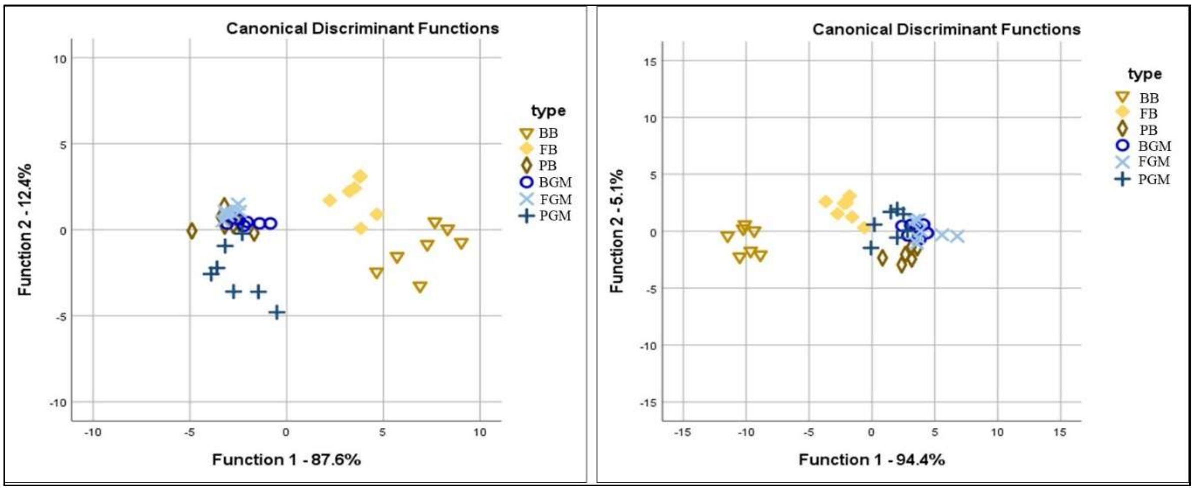

Figure 5. LDA results at day 1 (left) and day 4 (right) based on TPA results (PB: wheat-based peasant bread; PGM: gluten-free peasant bread; FB: wheat-based loaf; FGM: gluten-free loaf; BB: wheat-based brown loaf; BGM: gluten-free brown loaf)

3.5. Moisture content changes of bread samples during storage

Moisture content of the measured bread samples are shown in Table 3. Based on the results, gluten-free samples always had higher level of moisture independently which bread type was measured and compared with the wheat-based ones, or where the sample was taken for the measurement.

Table 3. Moisture content of the bread samples (1: middle of the slice; 2: in between the middle and the crust; 3: near to the crust; PB: wheat-based peasant bread; PGM: gluten-free peasant bread; FB: wheat-based loaf; FGM: gluten-free loaf; BB: wheat-based brown loaf; BGM: gluten-free brown loaf)

Sample	Location of sampling	1. day	2. day	3. day	4. day
	1	48,74	47,55	44,29	43,91
PGM	2	47,54	46,57	44,18	43,82
	3	47,21	45,41	43,82	43,12
	1	49,09	47,69	45,91	45,09
BGM	2	48,57	47,17	44,96	44,32
	3	48,09	46,92	43,06	42,75
	1	48,98	48,9	47,95	47,31
FGM	2	48,91	48,14	47,2	46,68
	3	48,42	47,93	46,87	45,85
	1	39,82	39,1	37,96	35,98
PB	2	39,26	38,19	37,23	34,88
	3	35,37	34,02	32,43	31,53
	1	42,41	42,04	41,43	40,78
BB	2	42,57	41,83	40,93	38,93
	3	38,18	37,42	36,22	35,47
	1	38,58	38,23	37,68	36,83
FB	2	37,83	37,53	36,67	35,35
	3	34,19	33,89	33,05	32,76

3.6. Hyperspectral imaging results

The results of principal component analysis (PCA) on the NIR spectra of breads stored at room temperature were determined as: 1364, 1379, 1384, 1413, 1437, 1480, 1514, 1528, 1533 nm.

The result of LDA model based on the complete pre-treated data set for bread type classification are shown in Figure 6. It can be observed that along the first discriminant variable, the sample points representing wheat-based and gluten-free bread types are clearly distinctive. Along the second discriminant variable, certain bread types showed greater overlap, but showed a distinct trend in the following order: brown, white, and peasant bread.

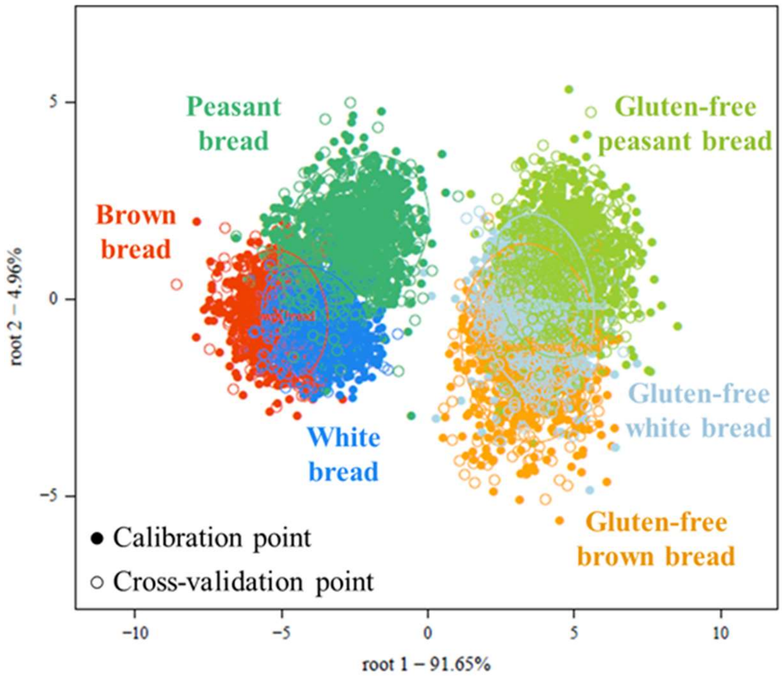

Figure 6. LDA results based on bread type and moisture content (N=8390, NrPC=30)

Table 4 summarizes the accuracy with which the LDA performed on the complete data set differentiated each bread type. The mean correct classification during calibration and validation was 76.84 and 76.54%. The most accurate classification was in case of white and cob bread types.

Table 5 summarizes the accuracy with which the differences occurred during storage of the different types of bread could be distinguished based on the NIR spectra. A similarity in the LDA results was that the best classification models were typically obtained by applying smoothing or second derivative spectrum pre-treatments after smoothing. The average correct classification for the six bread types was between 78-85% for model building and 75-84% for validation.

The LDA model and its results built on the entire pre-treated data set by storage time classification is illustrated in Figure 7. Based on the figure, it can be observed that in the space bounded by the first and second discriminant variables, the sample points belonging to each storage day significantly overlap, there is no clear grouping trend. The mean correct classification during calibration and validation was 60.41 and 60.28%.

Table 4. The accuracy with which the LDA performed on the complete data set differentiated each bread type (1: wheat based brown loaf; 2: wheat-based white loaf; 3: gluten-free brown loaf; 4: gluten-free white loaf; 5: gluten-free cob; 6: wheat-based cob)

	Bread type	1	2	3	4	5	6	Mean correct classification
Calibration	1	79,15	7,48	0	0	0	5,72	**76,84%**
	2	19,67	90,25	0	0	0	7,9	
	3	0	0	65,03	22,21	5,69	0,07	
	4	0	0	29,15	60,96	14,94	0	
	5	0	0	5,82	16,69	79,37	0	
	6	1,18	2,26	0	0,15	0	86,31	
Validation	1	79,26	7,76	0	0	0	5,72	**76,54%**
	2	19,67	89,84	0	0	0	7,86	
	3	0	0	64,86	22,94	6,02	0,07	
	4	0	0	29,36	60,12	15,17	0	
	5	0	0	5,79	16,8	78,81	0	
	6	1,07	2,4	0	0,15	0	86,35	

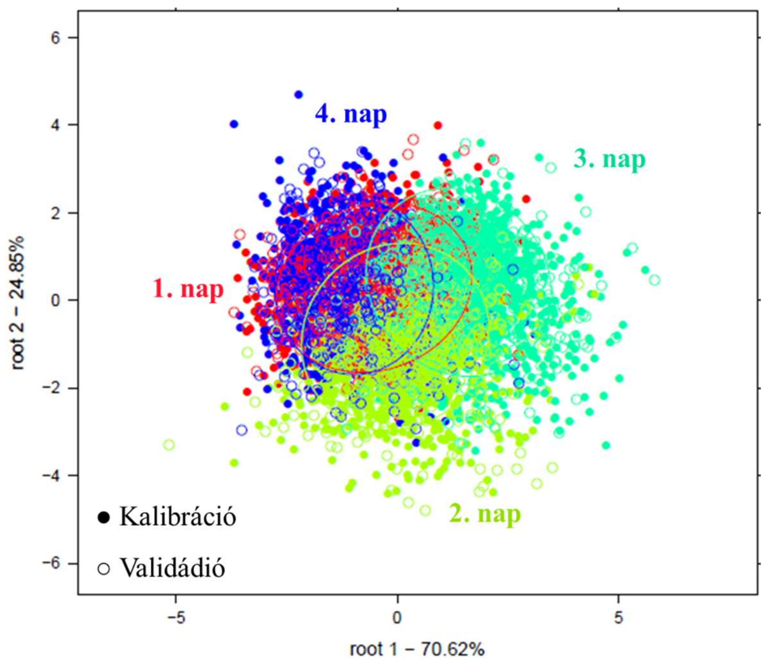

Figure 7. LDA model and its results built on the entire pre-treated data set by storage time

Table 5. Classification of bread samples based on their moisture content (PB: wheat-based cob; PGM: gluten-free cob; FB: wheat-based loaf; FGM: gluten-free loaf; BB: wheat-based brown loaf; BGM: gluten-free brown loaf)

Bread type	Spectrum pre-treatment	Latent variables	Average correct classification (%)	
			Calibration	Validation
BB	sgol-2-13-2	30	78,18	75,79
BGM	sgol-3-9-0	25	80,32	79,68
FB	sgol-2-13-2	29	82,23	81,58
FGM	sgol-3-9-0	30	82,61	81,28
PB	sgol-3-9-0	30	85,54	84,27
PGM	sgol-2-13-2	29	82,89	81,91

3.7. Sensory evaluation

Using profile analysis method, trained panellists associated flavours, aromas and linked terms to the samples which were previously not present on the bread aroma wheel. As new elements corn taste, suffocating aftertaste due to dry texture. Among smell attributes empty fragrance, and in case of pseudocereal present walnut and sawdust attribute appeared. Among the existing properties of the bread aroma wheel, the most common elements were sour-fermented taste, yeasty smell and flavour, overly sweet or salty taste, cheese like aroma, hard, crumbling and rubbery texture.

Taking all examined properties into account, based on the ANOVA and LDA analysis (Figure 8.) the group of gluten-free diet followers evaluated the gluten-free samples differently than members of the other two groups.

Higher level of overlap was detected between the trained participants and the group of non-diet followers, who consumer wheat-based breads. Based on the scores given during the sensory assessment, 89.11% of the responders were placed in their original group, which was 88.67% after cross-validation. Background reason can be that the difference among wheat-based breads is smaller than among gluten-free products. In contrast, gluten-free products show greater variation in their composition and manufacturing technology (ROMAN et al., 2019).

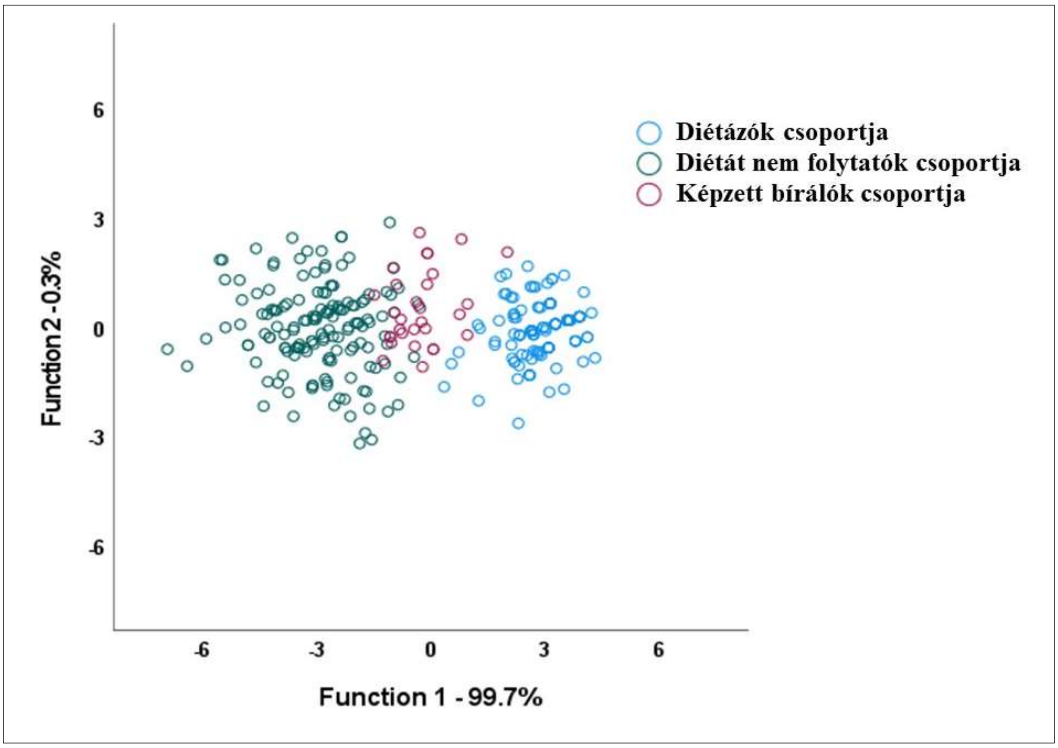

Figure 8. LDA analysis of gluten-free samples by the 3 different groups (n=110) based on sensory attributes (n=17)

In general, the properties that most determine the likeability of the gluten-free samples during sensory evaluation are: softness by touch and chewing, porosity, aroma and flavour intensity and crumbliness (PAGLIARINI et al., 2010; ALENCAR et al., 2017). Based on the ANOVA results, among properties examined by touching or smelling (porosity, softness by touch, smell) the group of trained participants and the non-diet followers gave significantly ($p< 0.05$) lower, while with porosity significantly ($p<0.05$) higher points compared to group of diet-followers. In case of smell intensity, there was no significant ($p<0.05$) difference detected between trained panellists and diet-followers. In terms of taste properties (intensity, softness during chewing, crumbliness) both the trained participants and the non-diet followers gave significantly ($p<0.05$) different (typically lower) scores compared to diet followers. In case of white and brown loaves lower taste intensity and softness, while higher perception of crumbling properties were detected in those groups compared to group of diet-followers. This is in contrast to previously published data (LAUREATI et al., 2012), where no significant difference was found between the dieting and non-dieting groups when tasting gluten-free breads.

Based on the LDA analysis of the 6 most important properties of gluten-free and wheat-based bread samples, taken on non-diet followers results showed an overlap between gluten-free loaves and wheat-based peasant sample (Figure 9). In case of trained panellists overlap was detected between gluten-free brown loaf and wheat-based peasant bread. There was no overlap between gluten-free and wheat-based loaves among trained panellists and non-diet followers. Overall, the ratio of correctly classified items was 84%, and 82.33% after cross-validation in the non-diet follower group. There was no overlap between the diet-followers and the other two groups, reinforcing the previously showed results, that diet followers rated all the different bread types differently.

As a final outcome, gluten-free products received higher rank for liking from trained panellists and non-diet followers over their wheat-based counterparts. Gluten-free samples were judged to be more uniform in porosity, more intense to softness by touch and by chewing, and by lower intensity in smell and crumbliness. Exception to this were the intensity of the gluten-free peasant bread sample's taste intensity, which was judged to be more salty compared to its wheat-based counterpart, and the sweeter taste profile of the gluten-free white loaf sample.

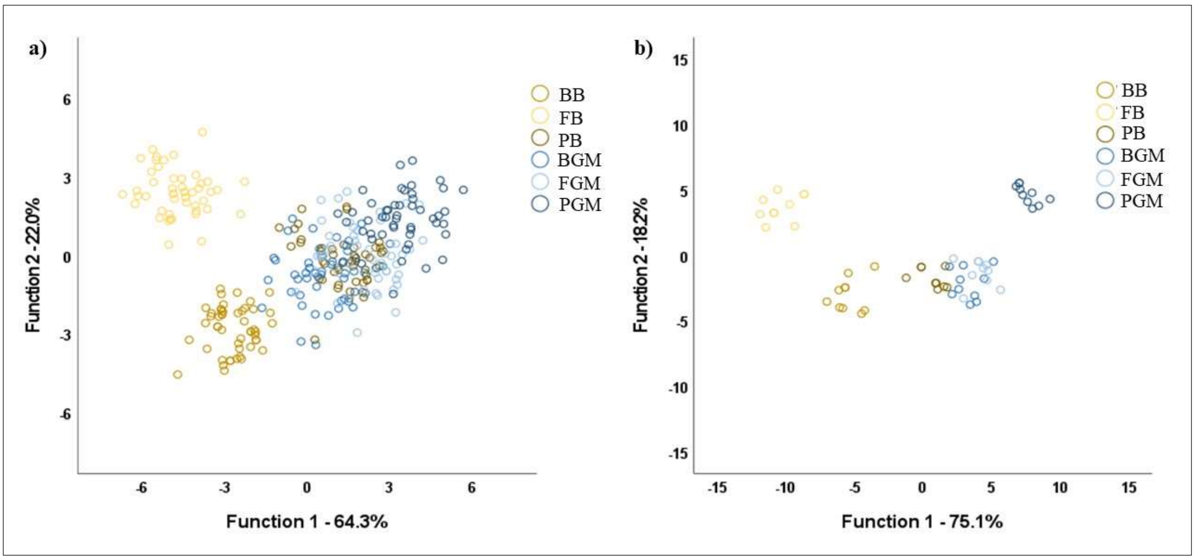

Figure 9. Result of LDA analysis based on six key organoleptic characteristics of gluten-free and wheat flour-based bread samples (a: non diet followers; b: trained panellists; PB: wheat-based cob; PGM: gluten-free cob; FB: wheat-based loaf; FGM: gluten-free loaf; BB: wheat-based brown loaf; BGM: gluten-free brown loaf)

4. NEW SCIENTIFIC RESULTS

1. The distribution of gluten-free bread consumption habits in Hungary by age group and bread type, as well as the other dietary needs of people following gluten-free diet were determined. With the primary research it turned out that 49.2% of dieters should not just follow a gluten-free diet. Based on the survey results, range of raw materials were determined that can be used for product development, considering consumer needs.
2. I determined by farinograph measurements the effects of pea protein, amaranth flour, buckwheat flour, and their variable mixture on dough development time, stability,

softening and water absorption in a corn starch-based system in the presence of hydrocolloid and fibre.

Effects of hydrocolloid addition (HPMC and xanthan) in varying proportions on the dough development time, stability, softening and water absorption were determined using TAC bread flour mixtures for white and brown bread. Significant effect of hydrocolloids and the presence of buckwheat and amaranth proteins on all analysed parameters were proved.

3. Based on the farinograph measurements model equations were developed on dough development time, stability, softening and water absorption using TAC bread flours for white and brown bread.
4. With the developed prediction tool new gluten-free flour mixtures were created, whose farinograph curve differed from the commercially available samples' and were closer of the wheat flour curve parameters.
5. With the texture profile analysis (TPA) method it was proved that the newly developed gluten-free breads performed significantly better ($p<0.05$) or at the same level compared to their wheat-based counterparts in terms of hardness, springiness and cohesiveness during a 4 day long storage test.
6. Hyperspectral imaging (HSI) method was successfully applied to monitor the change in moisture content during the aging of bread in wheat flour and gluten-free samples. PCA method was able to determine the wavelengths that best describe the moisture content in bread products. Based on the moisture content gluten-free and wheat-based products could be separated with LDA analysis.
7. During the sensory evaluation of the examined samples significant difference ($p <0.05$) was found between the different groups of reviewers (gluten-free diet followers, non-diet followers, trained panellist. Missing attributes on the bread-aroma wheel were successfully determined, which can be used for better description of gluten-free bread products in the future. Properties that were most responsible for significant differences between the panellist groups (verified by ANOVA test) were identified (softness by touch and chewing, porosity, smell and taste intensity, crumbliness).

5. PUBLICATIONS

<u>Publications in Scientific journals:</u>

- **Marcell Tóth**, Tímea Kaszab, Anikó Lambertné Meretei, (2022). Texture profile analysis and sensory evaluation of commercially available gluten-free bread sample, *European Food Research and Technology*, 248, 1447-1455. DOI: 10.1007/s00217-021-03944-2 IF: 3.0
- **Marcell Tóth**, Tímea Kaszab, Anikó Lambert-Meretei: Case study of commercially available gluten-free bread products (2022): Texture changes during storage and sensory analysis. *Progress in Agricultural Engineering Sciences*, DOI: 10.1556/446.2022.00039
- **Marcell Tóth**, Gyula Vatai, András Koris (2020). Gluten-free bread from ingredients and nutrition point of view: A mini-review. *Emirates Journal of Food and Agriculture*, 32(9), 634-639. DOI: 10.9755/ejfa.2020.v32.i9.2145, IF: 1.04
- **Marcell Tóth**, Gyula Vatai, András Koris (2020). Consumers' acceptance, satisfaction in consuming Gluten-free bread: A market survey approach. *International Journal of Celiac Disease*, 8, 44-49. DOI: 10.12691/ijcd-8-2-1

Conference Full paper:

- Flora Vitális, ***Marcell Tóth***, Ferenc Firtha, Anikó Lambert-Meretei, Tímea Kaszab (2021): Hyperspectral imaging for moisture content detection in gluten-free bread samples – First experimental approach. *Proceeding of Lippay-Ormos-Vas Scientific meeting*, pp. 787-800. ISBN: 9789632699882

- ***Marcell Tóth***, András Koris, Anikó Lambert-Meretei, Tímea Kaszab (2021): Rheological study on commercially available gluten-free flours. *Proceeding of Lippay-Ormos-Vas Scientific meeting*, pp. 716-729. ISBN: 9789632699882

- ***Marcell Tóth***, Tímea Kaszab, Anikó Lambert-Meretei (2021): Texture profile analysis and porosity measurement of commercially available gluten-free bread samples. *Proceeding of Lippay-Ormos-Vas Scientific meeting*, pp. 704-715. ISBN: 9789632699882