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Evaluation of Protein Profiles, Bioactivity, Allergenicity and Toxicity of Peptides Generated After *in silico* Digestion of Common Wheat and Einkorn Wheat

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A R T I C L E I N F O	A B S T R A C T
	The belief that ancient wheat is more beneficial than common wheat has been growing trend in recent years among the consumers. The present study aims to compare bioactive peptide, allergen peptide and toxic peptide generation after gastrointestinal digestion of modern wheat (*Triticum aestivum*) and ancient wheat, einkorn (*Triticum monococcum* var. *monococcum*), using *in silico* tools. The primary sequences of both kinds of wheat were obtained from BIOPEP-UWM and UniProtKB/Swiss-Prot database. *In silico* digestion was applied using BIOPEP-UWM online tool. For the simulation of gastrointestinal digestion pepsin (pH 1.3) (EC 3.4.23.1), trypsin (EC 3.4.21.4), and chymotrypsin (EC 3.4.21.1) were selected and analyzed. Homology analysis was performed for each protein sequences using EMBOSS Needle program. Toxic and allergen peptides were predicted using ToxinPred online tool and Allergen FP v.1.0. The results showed that einkorn and common wheat proteins exhibited similar properties including high similarity rate (58.72-87.40%) indicating the percentage of matches between the two sequences and the identical bioactivities for peptides generated after digestion. Most of the bioactive peptides were dipeptides and the majority of them displayed more than one bioactivities including ACE inhibitory, DPP IV inhibitory or antioxidant activity, etc. Allergen peptides generated after *in silico* digestion were found to be similar for both kinds of wheat. *In silico* gastric digestion of einkorn and wheat caused toxic peptides production, but they were disappeared after *in silico* intestinal digestion. In conclusion, although there is a perception related to the Einkorn that is healthier than common wheat, *in silico* digestion of common wheat and einkorn did not support this perception.

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Introduction

Bioactive peptides have been defined as specific protein fragments with several health benefits and have a positive impact on body functions. They can release from food proteins after several processes such as enzymatic hydrolysis, fermentation, heat, and high-pressure treatments or during gastrointestinal digestion (Garg et al., 2018; Tu et al., 2018). In recent years, *in silico* approaches have been utilized to predict and design various bioactive peptides released from food-derived proteins (Darewicz et al., 2016; Kęska and Stadnik, 2016; Tulipano et al., 2015; Udenigwe, 2016). This approach combines statistical techniques, biology, math and computer science for analysis and evaluation of biological data (Valdés et al., 2017). *In silico* tools can be used with various purposes when studying about bioactive peptides (BAPs). For example, UnıProt, SwissProt and TrEMBL databases can provide sequences of food proteins while BIOPEP-UWM and PepBank databases can predict bioactive peptides (Garg et al., 2018). BIOPEP-UWM and ExPASy-

PeptideCutter online tools are useful to select specific enzymes and proteins. Among these online tools, BIOPEP-UWM offers different functions including databases of protein sequences, sensory peptides, bioactive peptides, etc. (Minkiewicz et al., 2019). This program enables for hydrolysis of food proteins with selected enzyme or enzyme combinations and comparison of the bioactivities of released peptides with known bioactive sequences found in the literature and/or different databases. BIOPEP-UWM has 3792 bioactive peptide sequences (accessed June 2019) with different activities including antibacterial, antioxidant, dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) and angiotensin-converting enzyme inhibitor (ACE-I). Also, BIOPEP-UWM can calculate the incidence of BAPs in a protein chain. Another program, PeptideRanker, evaluates the activity of released peptides by assigning scores between 0 and 1, to which, the score close to 1 corresponding to high activity. *In silico* tools also enable to predict allergenicity and toxicity of released

peptides. AlgPred, Allergen FP v.1.0 and/or AllerTop can predict allergenicity and ToxinPred can predict toxicity. Bioinformatics tools utilize two approaches, namely sequence similarity and motif combination to predict allergen proteins or peptides. AlgPred, APPEL, AllerTOP, AllergenFP are *in silico* tools working by using these approaches. Generally, AllergenFP is preferred since its higher sensitivity, accuracy, and specificity than others (Dimitrov et al., 2014).

Cereals, the main energy source of human nutrition, are grown in many regions around the world (Cavazos and Gonzalez de Mejia, 2013). The major wheat species grown throughout the World, *Triticum aestivum*, generally called as bread wheat or common wheat is a hexaploid species. *Triticum var. durum* is another species of wheat, which is mainly used for making pasta and known as pasta wheat or durum wheat. In addition to these species' other ancient wheat species, which are only grown in small areas, have been becoming a popular as they have been proposed to be rich sources of bioactive compounds and therefore suitable for producing a high value of food products with enhanced health benefits. The most popular one in Turkey is the diploid wheat einkorn (*Triticum monococcum* var. *monococcum*). Archeological findings show that wheat first occurred in parts of Turkey, Lebanon, Syria, the Levant, Israel, Egypt, and Ethiopia. Domesticated Einkorn wheat in Turkey dates back to 9000 BC (Cooper, 2015).

The identification study by DNA fingerprinting shows that the wild group of *Triticum monococcum boeoticum,* which is cultivated about 10000 years ago in Karacadağ Mountain (Turkey) can be a probable progenitor of diploid (2n=14) einkorn. The einkorn wheat is rich in protein, lipid, antioxidant compounds, and carotenoids, especially lutein and it can be a suitable choice for developing new functional foods because of its nutritional value (Hidalgo and Brandolini, 2014). The data available for ancient wheat species have been related to mostly on their dietary fiber contents (Brandolini and Hidalgo, 2011; Gebruers et al., 2008; Lùje et al., 2003), phytochemicals such as phenolics, carotenoids, and tocols (Grausgruber et al., 2004; Lachman et al., 2013; Shewry and Hey, 2015). Dietary fiber and major groups of phytochemicals in 5 lines of einkorn, emmer, and 161 wheat cultivars were investigated in the HEALTHGRAIN project, which is the largest study comparing ancient and modern wheat. Data showed that ancient wheat cultivars are generally lower in some components such as dietary fiber and higher in other components like polyphenols. Besides, the protein level of einkorn wheat was found to be higher than those of modern wheat in HEALTHGRAIN project (Shewry and Hey, 2015).

Till date, the best of our knowledge no studies about bioactive peptides produced by the consumption of einkorn wheat have been explored although they have been suggested as a healthier choice and a better nutritional profile than modern wheat. However, studies on the bioactive peptides' generation from different protein sources have been intensified recently due to their beneficial effects on health including antihypertensive, cholesterol-lowering, antioxidant, and anti-inflammatory effects (Tu et al., 2018; Udenigwe and Aluko, 2012).

This study aimed to compare bioactive peptide generation after gastrointestinal digestion of modern wheat (*Triticum aestivum*) and ancient wheat, einkorn (*Triticum*

monococcum var. *monococcum*), using *in silico* tools. In addition, allergenicity and toxicity of peptides generated from *in silico* digestion were predicted.

Methods

Protein Sequences of Wheat (Triticum aestivum) and Einkorn (Triticum Monococcum var. monococcum) Storage Proteins

Wheat proteins can be classified metabolic proteins like enzymes and non-metabolic proteins like storage proteins. Gliadin and glutenin, which are non-metabolic storage proteins, create 90% of the total protein content (Žilić et al., 2011). Therefore, these storage proteins were analyzed in the assays. The primary sequences of the wheat storage proteins (low molecular weight glutenin, ω-gliadin, γ-gliadin and α-gliadin) were obtained from BIOPEP-UWM database and high molecular weight glutenin was obtained from UniProtKB/Swiss-Prot and TrEMBL databases. The primary sequences of einkorn storage proteins (high molecular weight glutenin, low molecular weight glutenin, ω-gliadin, γ-gliadin, α-gliadin and avenin like protein) and their accession numbers were obtained from UniProtKB/Swiss-Prot and TrEMBL databases in the FASTA format.

Sequences Alignment by EMBOSS Needle (EMBL-EBI) Analysis

Homology analysis was performed for each protein sequences of wheat and einkorn in FASTA format. Structural, functional and/or evolutionary relationships between two biological sequences (protein or nucleic acid) can be indicated by identify regions of similarity via pairwise sequence alignment. EMBOSS Needle was used for align these sequences. This program reads two input sequences and write their optimal global sequence alignment along their entire length to the file via Needleman-Wunsch alignment algorithm. The algorithm uses a dynamics programming method to ensure the alignment is optimum, by exploring all possible alignments and choosing the best conditions and a scoring matrix is read that contains values for every possible residue or nucleotide match (Needleman and Wunsch, 1970). Local or global pairwise alignments methods can be used with this tool and as a result of this analysis some information can be generated like scores, identity, similarity and gaps (Chojnacki et al., 2017). Proteins which have been similar amino acid sequences regions mean these proteins can be generated similar potential bioactive peptides (Vercruysse et al., 2009). This high similarity rate indicates that the peptides obtained *in silico* analysis applied to one of the proteins compared can be identified in the other protein. The similarity means the percentage of matches between the two sequences over the reported align region including any gaps in the length. Although the similarity is high, the same peptides may not be produced using the same enzymes. Therefore, each protein should be analyzed independently (Lafarga and Hayes, 2014). For the analysis, protein sequences of wheat and einkorn were entered into the program and the analysis was performed by selecting optimal parameters for protein align; Matrix (EBLOSUM62), Gap penalty (10.0) and Extend penalty (0.5).

Amino Acid Compositions Analysis

ProtParam tool which is an *in silico* analysis program that gives the amino acid compositions and some physicochemical properties of proteins or peptides was used to evaluate amino acid compositions of wheat and einkorn storage proteins.

In Silico Assessment

Proteolysis, bioactivities and frequency of occurrence of bioactive peptides

BIOPEP-UWM database hosted by Warmia and Mazury University, Olsztyn, Poland was used for simulated proteolysis of wheat and einkorn storage proteins (Minkiewicz et al., 2019). BIOPEP-UWM database was used for the analysis of wheat storage proteins. First, "enzyme action" tool was selected and in "protein database" section wheat protein sequences were chosen. For einkorn proteins, amino acid sequences of each protein copied from UniProtKB, were entered "for your sequence" section on BIOPEP-UWM tool. For the simulation of gastric digestion of proteins pepsin (EC 3.4.23.1) was selected on "enzyme action" tool. Afterward, for the simulation of gastrointestinal digestion pepsin (pH 1.3) (EC 3.4.23.1), trypsin (EC 3.4.21.4), and chymotrypsin (EC 3.4.21.1) were selected and analyzed. Theoretically, released peptides were obtained and they were submitted to "search for active fragments" and "calculate A_E, DHt, W, BE, V" section. After calculation of the bioactivities of released peptides, the classification was made according to the activities and the second classification was made according to their mono and multifunctional properties. A monofunctional peptide refers to a peptide that represents only a single bioactivity. However, the multifunctional peptide may exhibit more than one bioactivity such as ACE inhibitory, DPP-IV inhibitory and opioid activities. Some parameters including the theoretical degree of hydrolysis (DHt), the frequency of release of fragments with a given activity by selected enzymes (A_E) and the relative frequency of the release of fragments with a given activity by selected enzymes (W) and values were calculated after proteolysis using the following equations (Minkiewicz et al., 2019):

$$A_E = d/N$$
$$W = A_E/A$$

Where d is the number of fragments released by enzymes with a given activity from a given protein sequence, N is the total number of amino acid residues in a protein chain, and A is the frequency of bioactive fragments occurrence in protein sequence.

Toxicity and allergenicity prediction

The toxicity and the allergenicity of the peptides released after gastric and gastrointestinal proteolysis were predicted using the ToxinPred online tool and Allergen FP v.1.0 tool respectively. These tools were also used to predict the toxicity and allergenicity of the bioactive peptides.

Peptide ranking and bioactivity prediction

PeptideRanker online tool was used to determine activity score of bioactive peptides. The peptides with a score of more than 0.5 were considered as positive results in the present study.

Results and Discussions

Compositions and Homology of Selected Proteins

Protein profiles of wheat and einkorn and their sequences were obtained from BIOPEP-UWM and UniProt databases to determine peptide release and their activities (bioactivity, allergenicity, and toxicity) after *in silico* digestion. Five storage proteins of wheat and six storage proteins of einkorn were achieved. Table 1 shows protein sequences of einkorn (*Triticum monococcum)* and wheat (*Triticum aestivum*) obtained from UniProt and BIOPEP-UWM databases, respectively.

Figure 1 shows the theoretically calculated percentage of amino acids in wheat storage proteins. The predominant amino acid of all wheat storage proteins was glutamine. The highest percentage of glutamine was found in LMW glutenin (39.2%) whereas ɣ-gliadin (32.6) contained the least percentage of glutamine. In addition, wheat storage proteins are rich sources of serine (5.4-9.3%), valine (0.7-5.3%), proline (12.8-26.8%), phenylalanine (0.4-8.9%), leucine (4.6-8.6%), isoleucine (2.9-5.7%), and alanine (2.1-4.5%).

Similar to wheat storage proteins glutamine was the predominant amino acid in all of the storage proteins of einkorn. However, the highest percentage of glutamine (40.8%) was found in ω- gliadin (Figure 1). The glutamine percentage of avenin like protein was 25.4. The other amino acids of einkorn storage proteins were serine (4.2-8.6%), proline (7.7-27.3%) and aliphatic neutral amino acids like valine (2.3-5.2%), alanine (3.1-9%), isoleucine (0.6-5.8%), leucine (5.3-9.5%), and aromatic amino acids such as phenylalanine (0.4-7.5%). Pooja et al., 2017 and Szerszunowicz and Nalecz, 2018, reported the abundance of glutamine in different cereals' proteins. Similar to our results oat globulins predominantly contained glutamine and aliphatic amino acids (Szerszunowicz and Nalecz, 2018) and the predominant amino acid of rice bran globulin and prolamin was glutamine (Pooja et al., 2017).

Similarities and differences among the storage proteins of wheat and einkorn were shown in Table 2. Sequence similarity analysis compares similar regions between two protein sequences (Lafarga and Hayes, 2014) and the "identity" explains the percentage of matched amino acids in the total length of the aligned sequences. Greater values refer to the homology among the protein sequences (Altschul et al., 2005). The results of analyses showed that gliadin proteins and glutenin proteins of both kinds of cereals were similar ranges between 58.2 and 87.4%. Due to high similarities between the same proteins of wheat and einkorn, the release of peptides with the same sequences and activities after *in silico* digestion might be expected.

Allergenicity and Toxicity Prediction

Allergenicity of food proteins is one of the major health concerns associated with the consumption of new foods, supplements, etc. (Dimitrov et al., 2014; Rahaman et al., 2016). Generally, allergen proteins can resist to pepsin hydrolysis. Therefore, it is important to exert how gastrointestinal digestion can affect the release of allergen peptides. In addition, these proteins are generally thought to be resistant to proteolysis in the intestine, and some of them are partially heat-resistant.

Table 1 Storage proteins of einkorn and wheat and their sequences[c]

Einkorn Storage Protein (ID[a])	Amino Acid Residues
HMW glutenin (A0A0M4FY26)	809

MTKRLVLFAAVVVALVALTAAEGEASGQLQCERELQKHSLKACRQVVDQQLRDVSPECQPVVVGPVARQYEQQVVVPPKGGSFYPGETTPPQQLQQ
SILWGIPALLKRYYLSVTSPQQVSYYPGQASSQQSGQRQQEYYLTSPQQPEQWQQPGQGQSAYYPTSPQQPGQKQPRYYPTSPWQPGQLQQPTQGQQR
QQLGQGQQLRQGQQGQQSGQGQLRYYPTFSQQSGQLQQLAQGQQGQQPERGQQGQQSGQGQQLGQGQQGQQPGQKQQSGQGQQGYYPISPQQLG
QGQQSGQGQQSGQGQLSPQQPGQEQQPRQLQQPEQGQQGQEQPGQQGQQGPGQEGQQQPGQGQQGQQLGQGQPGYYPTSPQQSGQGQGPGYYPTSP
QQPGQLQQPAQGQQPGQEQQGQQPGQGQQGQQGQPGYYPTSPQQSGQGQQLGQWQQSGQGQPGHYPTSPLQPGQGQPGYYPTSPQQIGQGQQPGQL
QQPTQGQQGQQPGQGQQGQQPGQGQQGQQPGQGQPGYYPTSPQQSGQGQQPGQWQQPGQEQPGYHPTSSLQSGQGQQGYYPTSQQPGQGPQPGQ
WQQSGQGQQGYYPTSPQQSGQGQQPGQWLQPGQGQQGYYLTSPRQLGQGQQPRQWLQPRQGQQGYYPTSPQQQLGQGQQGYYPTSPQQPGQGQQ
GYDSPYHVSAEHQAASLKVAKAQQLAAQLPAMCRLEGGDALLASQ

Einkorn Storage Protein (ID[a])	Amino Acid Residues
LMW glutenin (B8XU50)	349

MKTFLVFALLALAAASAVAQISQQQQPPLFSQQQQPPFSQQQQPPFSQQQQSPFSQQQQQPPFSQQQQPPFSQQPPISQQQPQFLQQQQPPFSQQQQPPFS
QQQQPPYSQQQQPPFSQQQQPPFSQQQQPPFSQQQQQPPFTQQQQPPISQQQPPFSQQQQPPFSQQQQIPVIHPSVLQQLNPCKVFLQQQCIPVAMQQC
LARSQMLQQSICHVMQQQCCQQLRQIPEQSRHESIRAIVYSIILQQQQQQQQQQQGSIIQYQQQQPQQLGQCVSQPQQQLQQQLGQQPQQQQLTHGAF
LQPHQIAQLEVMTSIALRNLARMCSVNVALYETTTSVPLGVGIGIGVY

Einkorn Storage Protein (ID[a])	Amino Acid Residues
ω-gliadin (D6QY51)	348

ARQLNPSNKELQSPQQSFFHQQQPFPTQQSYPQQPYPQQPYSSQQPFPTIQQQFSQQPQQPFPQPQQPIPLQPQQPFPQQSQQPFPQPQQPFSWQPQQPSPL
QPQQPFPQPQLPLPQQPEQIIPQQSQQPFPLQAQQPFPQPQQPFPHQPQQQFPQPQQPTPLQPQLPFPQQPQQPFPQPQQQFPWQPQQPFSQPQQPIPQQPQQQ
VFPLQPQQPFPQQLQQPFPQPQQPQLFPQPEQPFPQQSQQLLPLQPQQPFSQQPQQSQQSFPQPQPRQPQQPSILQPQQPLPQQPQQPHQSFIQPQQPLPQQA
EQIISKQPQQPSPQQPHQPQQPYLQQQQQPSGSSVIGIGGQ

Einkorn Storage Protein (ID[a])	Amino Acid Residues
γ-gliadin (B8XU38)	311

MKTLLILTIIAVALTTTTANIQVNPSSQVQWPQQQQPFPQLQQPFSQQPQQIFPQPQQTFPHQPQQAFPQPQQTFPHQPQQQFPQPQQPQQPFPQQPQQQFP
QPQQPQQPFPQPQQPQLPFLQQPQQPFPQPQQPFPQLQQPQQPLAQPQQPQQPFPQQQQPLIQPYLQQQMNPCKNCLLQQCNPVSLVSSLVSMILPR
SDCQVMRQQCCQQLAQIPQQLQCAAIHGVVHSIIMQQEQQQQQQGIQILRPLFQLVQGQGIIQPLTTAQLEVIRSLVLGNSPKHVHVFVPTECSTTKGTFAS
IVADIGGQ

Einkorn Storage Protein (ID[a])	Amino Acid Residues
α-gliadin (B8XU27)	309

MKTFLIISLLAIVATTATTAVRVPVPQLQPQNPSLQQPQEQVPLVQQQQFPGQQQTFPPQQPYPQPQPFPAQQPYPQPQLFPQPQPFPPQLPYPQPQPFPPQ
QPYPQPQTQHLQPQQPISQQQAQQQQQQQQQQQQQQQQQQQQILQQILQQQQLIPCRDVIVLQQHNIAHESSQVLQQSSYQVLQQLCCQQLRQIPEQS
RCQAINNVVHAIILHQQQQQQQGQHQPSSQVSYQQPQQQYPSGQGSFQPSQQNPQAQGFVQPQHLPRLEEISNLALQTLPAMCNVYIPPYCSTTLRHLASS
VPTEKRKL

Einkorn Storage Protein (ID[a])	Amino Acid Residues
Avenin like protein (A7XUQ1)	284

MKVFILALLALAATTAIAQLETTCSQGFAQSQQQQQPAQRQLLEQKKLCAAFLQQQCSPLRMPFLQTQVEQLSSCQIVQYQCCQQLAQIPVPTRCHAIH
NVEEAINQQQSQQQLQEVQQQAQHKTMRMLLENLSLMCNIYVPLQCQQQQQLGQQQQQQLQVQLTPCTTFLQPQCSPVTVPFPQIPADQPTSCQNVQ
HQCCPQLSQIPEQFRCQAIHNVAEAIRQQQPQQQCQGMYQPQQPAQLESIRMSLQALRSMCSIYIPVQCPTPTGYKIPSVATYTGGAC

Wheat Storage Protein (ID[a])	Amino Acid Residues
HMW glutenin(P08489)	838

MAKRLVLFVAVVVALVALTVAEGEASEQLQCERELQELQERELKACQQVMDQQLRDISPECHPVVVSPVAGQYEQQIVVPKGGSFYPGETTPPQQLQ
QRIFWGIPALLKRYYPSVTSPQQVSYYPGQASPQRPGQGQQPGQGQQSGQGQQGYYPTSPQQPGQWQQPEQGQPGYYPTSPQQPGQLQQPAQGQQPG
QGQQGRQPGQGQGPGYYPTSSQLQPGQLQQPAQGQQGQQPGQGQQGQQPGQGQQPGQGQQGQQPGQGQQPGQGQQGQQLGQGQQGYYPTSLQQSG
QGQPGYYPTSLQQLGQGQSGYYPTSPQQPGQGQQPGQLQQPAQGQQPEQGQQGQQPGQGQQPGQGQGPGYYLTSPQQSGQGQQPGQGQQPGQGQGYYP
TSSQQPTQSQQPGQGQQGQQGQVGQGQAQQPGQGQQPGQGQPGYYPTSPLQSGQGQPGYYLTSPQQSGQGQQPGQLQQSAQGQKGQQPGQGQQPGQ
GQQGQQPGQGQQPGQGQGPGYYPTSPQQSGQGQQPGQWQQPGQGQPGYYPTSPLQPGQGQPGYDPTSPQQPGQGQQPGQLQQPAQGQQGQQLA
QGQQGQQPAQVQQGQQPAQGQQGQQLGQGQQGQQPGQGQQPAQGQQGQQPGQGQQGQQPGQGQQPGQGQPWYYPTSPQESGQGQQPGQWQQP
GQWQQPGQGQPGYYLTSPLQLGQGQQGYYPTSLQQPGQGQQPGQWQQSGQGQHGYYPTSPQLSGQGQRPGQWLQPGQGQQGYYPTSPQQSGQGQ
QLGQWLQPGQGQQGYYPTSLQQTGQGQQSGQGQQGYYSSYHVSVEHQAASLKVAKAQQLAAQLPAMCRLEGGDALSASQ

Wheat Storage Protein (ID[b])	Amino Acid Residues
LMW glutenin (1329)	388

MKTFLVFALLALAAASAVAQISQQQQPPPFSQQQQPPFSQQQQPPFSQQQQSPFSQQQQQPPFSQQQQPPFSQQPPLISQQQQLPFSQQQQPQFSQQQQPPY
SQQQQPPYSQQQQPPFSQQQQPPFSQQQQPSFSQQQQQPPFTQQQQPPFSQQSPISQQQQQQQQQQQQQPFTQQQQPPFSQQPPISQQQQPPFSQQQQPPFS
QQQQIPVIHPSVLQQLNPCKVFLQQQCIPVAMQRCLARSQMLQQSICHVMQRQCCQQLRQIPEQSRHESIRAIIYSIILQQQQQQQQQQQQQQQQGSIIQY
QQQPPQQLGQCVSQPQQQLQQQLGQQPQQQQLAHGTFLQPHQIAQLEVMTSIALRTLPRMCSVNVPLYETTTSVPLGVGIGVGVY

Wheat Storage Protein (ID[b])	Amino Acid Residues
ω-gliadin (1418)	280

MKTFLIFVLLAMAMKIATAARELNPSNKELQSPQQSFSYQQQPFPQQPYPQQPYPSQQPYPSQQPFPTPQQQFPEQSQQPFTQPQQPTPIQPQQPFPQQPQ
QPQQPFPQPQQPFPWQPQQPFPQTQQSFPLQPQQPFPQQPQQPFPQPQLPFPQQSEQIIPQQLQQPFPLQPQQPFPQQPQQPFPQPQQPIPVQPQQSFPQQSQ
QSQQPFAQPQQLFPELQQPIPQQPQQPFPLQPQQPFPQQPQQPFPQQPQQSFPQQPQQPYPQQQPYGSSLTSIGGQ

Wheat Storage Protein (ID[b])	Amino Acid Residues
γ-gliadin (1317)	282

MNIQVDPSSQVPWPQQQPFPQPHQPFSQQPQQTFPQPQQTFPHQPQQQFSQPQQPQQQFIQPQQPFPQQPQQTYPQRPQQPFPQTQQPQQPFPQSQQPQQ
PFPQPQQQFPQPQQPQQSFPQQQPSLIQQSLQQQLNPCKNFLLQQCKPVSLVSSLWSMILPRSDCQVMRQQCCQQLAQIPQQLQCAAIHSIVHSIIMQQEQ
QEQRQGVQILVPLSQQQQVGQGTLVQGQGIIQPQQPAQLEVIRSLVLQTLATMCNVYVPPYCSTIRAPFASIVAGIGGQYR

Wheat Storage Protein (ID[b])	Amino Acid Residues
α-gliadin (1305)	313

MKTFLILALVATTATTAVRVPVPQLQPKNPSQQQPQEQVPLVQQQQFPGQQQQFPPQQPYPQPQPFPSQQPYLQLQPFPQPQPFLPQLPYPQPQSFPPQQP
YPQQRPKYLQPQQPISQQQAQQQQQQQQQQQQQQQQQQILQQILQQQLIPCRDVVLQQHNIAHASSQVLQQSTYQLLQQLCCQQLLQIPEQSRCQAIHN
VVHAIIMHQQEQQQQLQQQQQQQLQQQQQQQQQQQQPSSQVSFQQPQQQYPSSQVSFQPSQLNPQAQGSVQPQQLPQFAEIRNLALQTLPAMCNVYIP
PHCSTTIAPFGIFGTN

Table 2 Homology analysis of wheat and einkorn proteins

Protein name and ID		Identity (%)	Similarity (%)[c]	Gaps (%)	Scores
Wheat[a]	Einkorn[b]				
α-gliadin (1305)	α-gliadin (B8XU27)	76.8	81.0	9.8	1251.0
γ-gliadin (1317)	γ-gliadin (B8XU38)	68.5	71.7	15.6	1059.0
ω-gliadin (1418)	ω-gliadin (D6QY51)	54.1	58.2	29.3	966.0
HMW glutenin (P08489)	HMW glutenin (A0A0M4FY26)	71.5	73.2	15.8	3136.0
LMW glutenin (1329)	LMW glutenin (B8XU50)	85.3	87.4	10.1	1663.5

[a]Wheat Protein sequences from BIOPEP-UWM, [b]Einkorn Protein sequences from UniProtKB, [c]Sequence similarity analysis was made by EMBOSS Needle (EMBL-EBI), Parameters: Matrix (EBLOSUM62), Gap_penalty (10.0), Extend_penalty (0.5), The Identity: is the percentage of identical matches between the two sequences over the reported aligned region (including any gaps in the length), The Similarity: is the percentage of matches between the two sequences over the reported aligned region (including any gaps in the length).
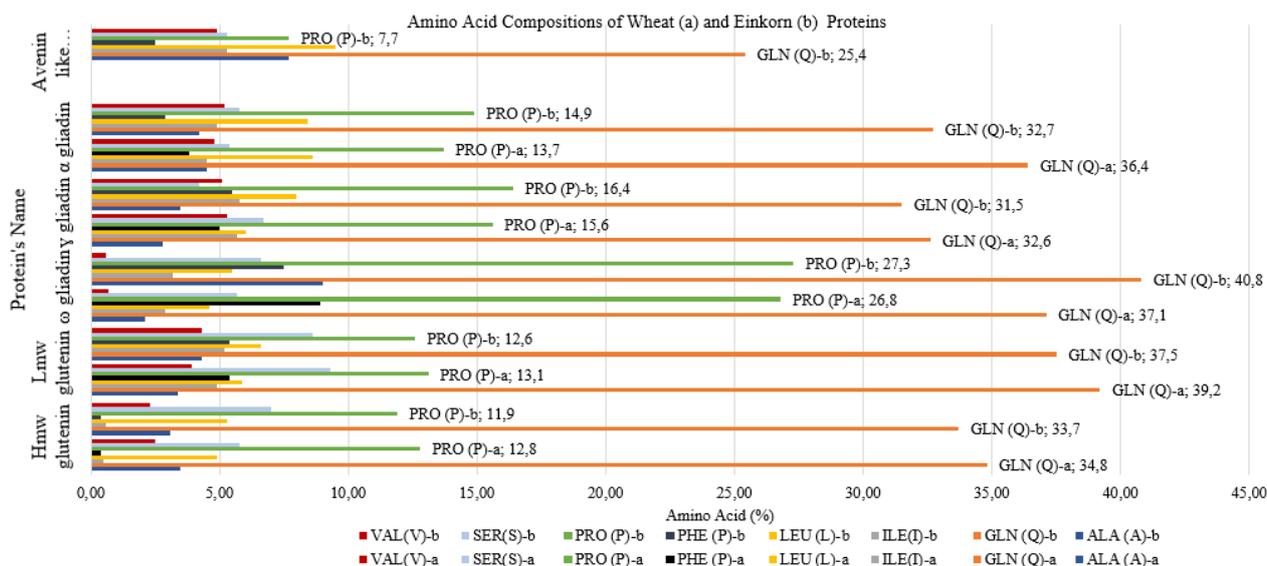
Figure 1 Theoretically calculated percentage of amino acids in the primary structure of wheat and einkorn storage proteins.
(a) The percentage of other calculated amino acids for complete wheat protein sequences: E(1-7,5%),C(0-3,17%), G(1,1-5,6%), T(2,3-3,2%), Y(1,4-4,3%), R(0,4-2,5) N(0-2,2),H(0-1,9),K(0,5-1,1),M (0,4-1,8 %), D(0-0,7),W<1% (b) The percentage of other calculated amino acids for complete einkorn protein sequences: E(0-3,5%), C(0,5-6,7%), G(1,1-16,8%), T(0,9-5,6%), Y(0,3-5,9%), R,N,H,K,M (0-3 %) , D,W<1%
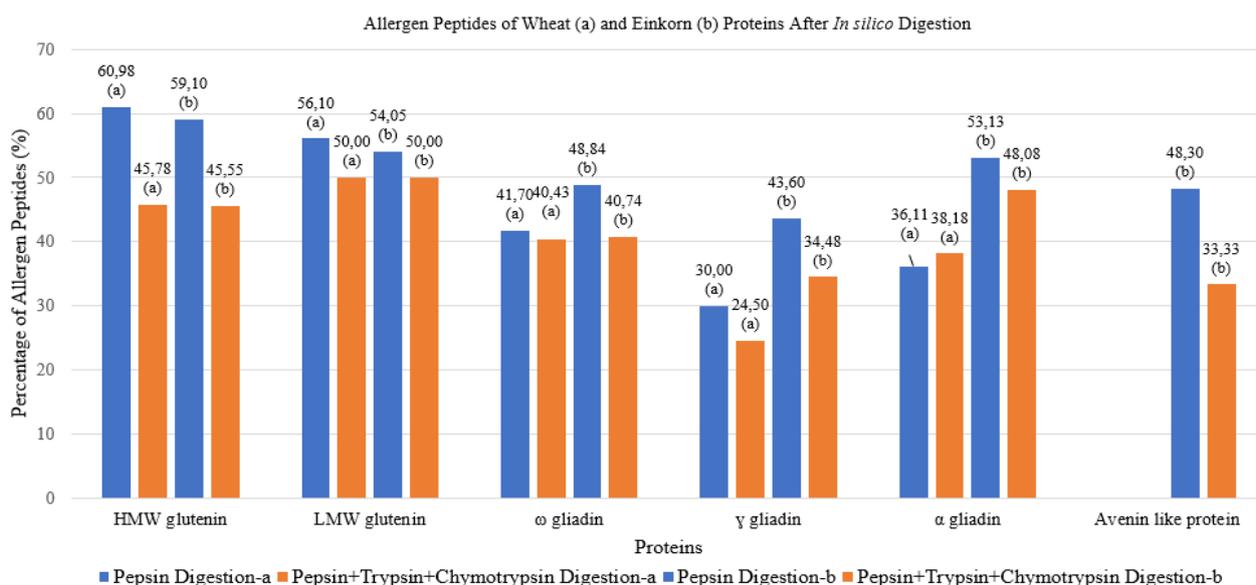
Figure 2 Percentage of allergen peptides after wheat and einkorn protein digestion

Allergenicity predictions of all released peptides after *in silico* digestion showed that the HMW glutenin protein of wheat contains more allergen peptide (60.98%) than other wheat and einkorn proteins after pepsin digestion (Figure 2). However, the occurrence of allergen peptides decreased to 45.78% after intestinal digestion. Allergome database, which has been designed to supply information about allergenic molecules and sources, listed 101 completely or partially sequenced experimentally discovered *Triticum aestivum* allergens. Five of them were belonging to glutenin

(HMW glutenin subunit and LMW glutenin subunit) and gliadin (α-gliadin, β-gliadin, γ-gliadin and ω-5-gliadin) proteins. Among the einkorn proteins, the release of allergen peptides after pepsin digestion of HMW glutenin was higher than those of other proteins. The important finding was that the increase in the release of allergen peptides after gastrointestinal digestion (gastric + intestinal) of α-gliadin than that of gastric digestion. On the contrary, the release of allergen peptides from other proteins decreased after gastrointestinal digestion regarding gastric digestion. Alergome database listed 4 completely or partially sequenced experimentally discovered *Triticum monococcum* allergens. None of them were belonging to storage proteins of *Triticum monococcum.*

To the best of our knowledge, studies related to allergenicity prediction of peptides using *in silico* tools are scarce. Bioactive properties of oat protein hydrolysate using AllerTOP and ACE inhibitory activity of rice bran proteins using both AllerTop and AllergenFP programs were reported (Bleakley et al., 2017; Pooja et al., 2017).

Examination of toxicity of all peptides after gastric and gastrointestinal digestion showed that peptides with PRSDCQVMRQQCCQLL sequence released after gastric digestion of einkorn γ-gliadin and PAMCNVYIPPYCSTTL sequence released after gastric digestion of einkorn α-gliadin could have toxic effects. Two peptides sequences, SSCQIVQYQCCQQL and PQIPADQPTSCQNVQHQCCPQL, which were released following gastric digestion of einkorn avenin like protein were found to be toxic and allergen. However, intestinal digestion of einkorn γ-gliadin, α-gliadin and avenin like protein caused the disappearance of toxic peptides. In the study of Šuligoj et al., 2013 toxicity of einkorn wheat was found to be lower than that of modern wheat. However, researchers stated that the consumption of einkorn wheat could be harmful for celiac patients so einkorn wheat consumption by celiac patients was not recommended (Šuligoj et al., 2013). Toxic peptide sequences were determined following gastric digestion of common wheat LMW glutenin, α-gliadin, and γ- gliadin. These sequences were (QQSICHVMRQRQCCQQL), (PAMCNVYIPPHCSTTIAPF) and (PRSDCQVMRQQCCQQL-ATMCNVYVPPYCSTIRAPF) for LMW glutenin, α-gliadin, and γ-gliadin, respectively. Similar to einkorn proteins these sequences disappeared after gastrointestinal digestion.

In the case of bioactive peptides, no toxicity and allergenicity were observed for both kinds of wheat after gastric and gastrointestinal digestion.

Bioactivity Profiles of Digested Proteins

In silico gastric and gastrointestinal digestion of modern wheat and einkorn wheat showed that DPP IV inhibitory, ACE inhibitory, antioxidative, stimulating vasoactive substance release, glucose uptake stimulating, antiamnestic, celiac toxic, renin inhibitory, and DPP III inhibitory peptides were released (Table 3).

Bioactive peptides having DPP IV inhibitory activity and glucose uptake stimulating effect peptide (VL) from einkorn wheat HMW glutenin after pepsin hydrolysis and bioactive peptides having DPP IV inhibitory activity (QL) and VL from modern wheat HMW glutenin were observed. However, the activity scores of VL and QL were 0.126 and 0.292 respectively, which indicated low activity. Digestion

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of wheat HMW glutenin with gastrointestinal enzymes caused the generation of five bioactive peptides. Some of them have ACE inhibitory activity (IF) and DPP IV and ACE inhibitory activity (GY) with high activity scores (0.949 and 0.741 respectively) (Table 3). In the case of einkorn HMW glutenin variety of bioactive peptides was high and among these peptides, dipeptides QW (0.928) with DPP IV inhibitory activity and GY (0.741) with DPP IV and ACE inhibitory activities displayed higher activity scores. This difference in bioactive peptide formation could be explained with the differences in the structure of einkorn and wheat HMW glutenin (as shown in Table 2). Two bioactive peptides with identical sequences were obtained after pepsin hydrolysis of LMW glutenin proteins of both kinds of wheat. In addition, dipeptide PF (0.993) released after pepsin hydrolysis of wheat LMW glutenin. However, most of the bioactive peptides occurred after gastrointestinal digestion of both wheat LMW glutenin proteins have same bioactive peptides. The nonidentical bioactive peptides found in modern wheat were TL, PF, PR, AH and VPL and found in einkorn wheat was TH. The number of peptides having different activities was higher in modern wheat than those of einkorn wheat after gastric and gastrointestinal digestion of their ω-gliadin proteins. The similar tendency was obtained for γ- gliadin proteins of two kinds of wheats. However, celiac toxic pentadecapeptide sequence (VQGQGIIQPQQPAQL) was determined in wheat γ-gliadin after gastric and gastrointestinal digestion. *In silico* digestion of einkorn α-gliadin generated five bioactive peptides while wheat α-gliadin generated six bioactive peptides. Similar to wheat γ-gliadin, *in silico* digestion of wheat α-gliadin caused the release of celiac toxic dodecapeptide (PSQQQPQEQVPL). *In silico* gastrointestinal digestion of avenin like protein caused the release of nine dipeptides having DPP IV inhibitory, ACE inhibitory and DPP III inhibitory, antioxidative, glucose uptake stimulating activities and one tripeptide having DPP IV inhibitory, stimulating vasoactive substance release and antiamnestic activities.

In silico bioactivity analysis showed that most of the released peptides displayed DPP IV and ACE inhibitory activities. DPP IV is a serine protease that mediates the inactivation of the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which are incretin hormones that stimulate insulin secretion. DPP IV inhibitor or gliptins enhance incretin levels, inhibit glucagon release, increase insulin secretions, and decrease blood glucose levels (Bleakley et al., 2017). For these reasons, they have been considered good functional agents to treat diabetes mellitus type 2 (Bleakley et al., 2017; Hatanaka et al., 2012; Pratley, 2008; Udenigwe et al., 2013). Angiotensin-I-converting enzyme (ACE-I, EC 3.4.15.1.) has a crucial role in hypertension control, type-2 diabetes, and other metabolic disorders (Li et al., 2016; Zheng et al., 2017). Inhibition of ACE-I can cause regulation of blood pressure so ACE-I inhibitors can be effective for the treatment of hypertension (Ondetti and Cushman, 1982). *In silico* gastrointestinal digestion showed that bioactivities of the peptides released from modern wheat and einkorn were identical. However, hydrolysis degree of proteins, frequency of release of fragments from intact proteins and relative frequency of release of fragments from intact proteins were different (Table 4).

Table 3. Einkorn and wheat proteins derived bioactive peptides and their PeptideRanker scores

Einkorn	Type of peptide[c]	Bioactive peptides after pepsin digestion[b]	Bioactive peptides after pepsin,trypsin and chymotrypsin digestion[b]
Protein (Mw,Da)[a] -(pI)[a]		Sequences	Sequences (Peptide Ranker Score)
HMW glutenin (87954.30) - (8.08)	mono	-	QK[2](0.062), EL[3](0.073), SL[1](0.330), QL[1](0.292), QW[1](0.928), QY[1](0.233), TK[1](0.033)
	bi	VL[1,5](0.126)	GY[1,2](0.741), VL[1,5](0.126)
	tri	-	-
LMW glutenin (40007.93) - (8.70)	mono	AL[1] (0.438)	AR[2](0.394), AL[1] (0.438), TH[1](0.076), VM[1](0.329)
	bi	VF[1,2](0.815)	VF[1,2](0.815),
	tri	-	-
	tetra		TF[1,2,8,9](0.827)
ω-gliadin (40492.95)-(7.14)	mono		AR[2](0.394),EL[3](0.073), QL[1](0.292), SW[1](0.934)
	Bi	PL[1,2] (0.811), PF[1,9] (0.993)	PL[1,2](0.811), PH[1,2](0.541), PW[1,3](0.993), PF[1,9] (0.993)
	tri	-	-
	tetra	-	-
γ-gliadin (35360.43) - (8.49)	mono	QL[1](0.292)	SL[1](0.330), QL[1](0.292),TL[1](0.144), VH[1](0.070)
	bi	VL[1,5](0.126), PF[1,9](0.993)	VF[1,2](0.815), PL[1,2](0.811), PH[1,2](0.541),VL[1,5](0.126), PR[2,9](0.788), PF[1,9] (0.993)
	tri	IL[1,2,5](0.393)	IL[1,2,5](0.393)
	tetra		-
α-gliadin (35478.95) - (8.57)	mono	RHL[3](0.410), AL[1](0.438)	AL[1] (0.438), PY[1](0.737)
	bi	-	PR[2,9](0.788)
	tri	-	
	tetra	-	TF[1,2,8,9](0.827), VY[1,2,3,9](0.100)
Avenin like protein (32035.86) - (7.81)	mono	AL[1](0.438), SL(0.330)	AL[1] (0.438), SL[1](0.330), QL[1](0.292),TM[1](0.356), SM[9](0.628)
	bi	-	IY[2,3](0.315), VF[1,2](0.815), PF[1,9] (0.993),
	tri	IL[1,2,5](0.393)	IL[1,2,5](0.393),VPL[1,4,6]
Wheat	Type of peptide[c]	Bioactive peptides after pepsin digestion[b]	Bioactive peptides after pepsin,trypsin and chymotrypsin digestion[b]
Protein (Mw,Da)[a] -(pI)[a]		Sequences	Sequences (Peptide Ranker Score)
HMW glutenin (89173.42)-(5.83)	Mono	QL[1](0.292),	EL[3](0.073), QL[1](0.292), IF[2](0.949),
	bi	VL[1,5](0.126)	VL[1,5](0.126), GY[1,2](0.741),
	tri	-	-
LMW glutenin (44729.97) - (9.04)	mono	AL[1] (0.438),	AR[2](0.394), AL[1] (0.438), TL[1](0.144),VM[1](0.329)
	bi	VF[1,2](0.815) PF[1,9] (0.993),	VF[1,2](0.815), PF[1,9] (0.993), PR[2,9](0.788),
	tri		VPL[1,4,6](0,368), AH[1,2,3](0.210)
	tetra		TF[1,2,8,9](0.827)
ω-gliadin (32553.35) - (5.06)	mono	IF[2](0.949),PEL[3](0.235), PF[1,9] (0.993),VL[1,5](0.126), PL[1,2](0.811),	IF[2](0.949), EL[3](0.073), PEL[3](0.235), PL[1,2](0.811), SY[1,2] (0.262), PW[1,3](0.993), VL[1,5](0.126), PF[1,9] (0.993)
	bi		
	tri		-
	tetra		TF[1,2,8,9](0.827)
γ-gliadin (32327.64) - (8.71)	mono	VQGQGIIQPQQPAQL[7]	PQR[2](0.488),PR[2](0.788), SL[1](0.330), SM[9](0.628), VQGQGIIQPQQPAQL[7](0.232)
	bi	VL[1,5](0.126)	PH[1,2](0.541),VL[1,5](0.126)
	tri	VPL[1,4,6](0,368)	IL[1,2,5](0.393), VPL[1,4,6](0,368)
	tetra		VY[1,2,3,9](0.100)
α-gliadin (36144.67) - (8.58)	mono	AL[1] (0.438),QL[1](0.292)	IY[2](0.315), AL[1](0.438), PSQQQPQEVPL[7](0.332), PK[1](0.326) PY[1](0.737) QL[1](0.292)
	bi	-	-
	tri	IL[1,2,5](0.393)	IL[1,2,5](0.393)
	tetra		TF[1,2,8,9](0.827), VY[1,2,3,9](0.100)

[1]DPP IV inhibitor; [2]ACE inhibitor; [3]Antioxidative; [4] Stımulatıng vasoactıve substance release; [5]Glucose uptake stimulating; [6]Antiamnestic, [7]Celiac toxic ,[8]renin inhibitor, [9]DPP III Inhibitor, [a]Molecular weight and isoelectric points data is obtained from ProtParam Program [b]BIOPEP-UWM Database, c The classification in the peptide type column indicates the number of bioactive properties of the peptides. mono: the peptide only has one bioactivity. (e.g. ACE inhibitor). bi: the peptide has two bioactivities. (e.g. ACE and DPP IV inhibitor). tri: the peptide has three bioactivities. tetra: the peptide has four bioactivities.

Table 4. DHt, A_E and W values of einkorn and wheat derived bioactive peptides

Einkorn Proteins

After Pepsin Digestion				After pepsin+trypsin+chymotrypsin digetion			
Activity	DHt%	AE	W	Activity	DHt%	AE	W
HMW glutenin							
GUSP	5.693	0.0012	0.1622	ACE inhibitor	17.0762	0.0025	0.0046
DPP IV inhibitor		0.0012	0.0017	Antioxidative		0.0012	0.0263
				GUSP		0.0012	0.1622
				DPP IV inhibitor		0.0099	0.0142
LMW glutenin							
ACE Inhibitor	12.0690	0.0029	0.0101	ACE inhibitor	19,5402	0.0143	0.0499
DPP IV inhibitor		0.0086	0.0114	DPP IV inhibitor		0.0201	0.0266
				Hypotensive		0.0029	0.1443
				DPP III inhibitor		0.0029	0.0337
ω-gliadin							
ACE inhibitor	12,9683	0.0115	0.0288	ACE inhibitor	17,5793	0.0172	0.0431
DPP IV inhibitor		0.0144	0.0163	Antioxidative		0.0057	0.2836
DPP III inhibitor		0.0029	0.0481	DPP IV inhibitor		0.0259	0.0294
				DPP III inhibitor		0.0029	0.0481
γ-gliadin							
ACE inhibitor	13,5484	0.0032	0.0088	ACE inhibitor	22,2581	0.0225	0.0619
DPP IV inhibitor		0.0129	0.0159	DPP IV inhibitor		0.0386	0.0474
GUSP		0.0064	0.1245	GUSP		0.0096	0.1868
DPP III inhibitor		0.0032	0.0474	DPP III inhibitor		0.0064	0.0948
α-gliadin							
Antioxidative	11,0390	0.0032	0.0760	ACE inhibitor		0.0097	0.0263
DPP IV inhibitor		0.0032	0.0041	DPP IV inhibitor		0.0129	0.0163
				Antioxidative	22,7273	0.0032	0.0760
				Hypotensive		0.0032	0.1649
				DPP III inhibitor		0.0097	0.1499
Avenin like protein							
ACE inhibitor	12,0141	0.0035	0.0116	ACE inhibitor		0.0106	0.0350
DPP IV inhibitor		0.0141	0.0209	DPP IV inhibitor		0.0352	0.0521
GUSP		0.0035	0.1423	Antioxidative		0.0035	0.1659
				Stimulating	25,4417	0.0070	0.2846
				DPP III inhibitor		0.0070	0.0864
				Antiamnestic		0.0035	1.0000

Wheat Proteins

After Pepsin Digestion				After pepsin+trypsin+chymotrypsin digetion			
Activity	DHt%	AE	W	Activity	DHt%	AE	W
HMW glutenin							
Stimulating	5.2443	0.0012	0.1690	ACE inhibitor		0.0024	0.0043
DPP IV inhibitor		0.0024	0.0034	Antioxidative		0.0024	0.0518
				Stimulating	14,6254	0.0012	0.1690
				DPP IV inhibitor		0.0036	0.0051
LMW glutenin							
ACE inhibitor	11.3695	0.0026	0.0097	ACE inhibitor		0.0155	0.0578
DPP IV inhibitor		0.0103	0.0136	DPP IV inhibitor		0.0258	0.0342
DPP III inhibitor		0.0026	0.0315	DPP III inhibitor		0.0077	0.0933
				Antioxidative	18.6047	0.0026	0.1121
				Antiamnestic		0.0026	0.5000
				Stimulating		0.0026	0.1008
				Hypotensive		0.0026	0.1008
ω-gliadin							
ACE inhibitor	13.6201	0.0143	0.0348	ACE inhibitor		0.0214	0.0521
DPP IV inhibitor		0.0179	0.0202	Antioxidative		0.0143	0.5000
Antioxidative		0.0036	0.1259	DPP IV inhibitor	19,3548	0.0286	0.0323
Stimulating		0.0036	0.2011	Stimulating		0.0036	0.2011
DPP III inhibitor		0.0036	0.0420	Hypotensive		0.0036	0.1440
				DPP III inhibitor		0.0071	0.0828
γ-gliadin							
Antiamnestic	11.0320	0.0035	1.000	ACE inhibitor		0.0177	0.0504
DPP IV inhibitor		0.0071	0.0088	DPP IV inhibitor		0.0213	0.0265
Stimulating		0.0071	0.1431	Stimulating		0.0106	0.2137
Celiac toxic		0.0035	0.0759	DPP III inhibitor	20.6406	0.0106	0.1495
				Antioxidative		0.0035	0.0759
				Celiac toxic		0.0035	0.0759
				Antiamnestic		0.0035	1.0000
α-gliadin							
ACE inhibitor	12.5000	0.0032	0.0771	ACE inhibitor		0.0096	0.0281
DPP IV inhibitor		0.0128	0.0156	DPP IV inhibitor		0.0288	0.0352
Stimulating		0.0032	0.0094	Antioxidative		0.0032	0.1003
				Celiac toxic	22,4359	0.0032	0.0358
				Stimulating		0.0032	0.0771
				Hypotensive		0.0032	0.1250
				DPP III inhibitor		0.0064	0.1179

GUSP: Glucose uptake stimulating peptide;

Table 5. Sequences of possible bioactive peptides derived einkorn and wheat proteins according to PeptideRanker

Einkorn proteins					
After pepsin digestion			After pepsin+trypsin+ chymotrypsin digestion		
Sequence	Activity	Allergenicity	Sequence	Activity	Allergenicity
HMW glutenin					
PAMCRL	0.898	P.A.	GQW	0.935	P.N.A.
WGIPAL	0.885	P.A.	GGSF	0.908	P.A.
RYYPTF	0.825	P.A.	CR	0.865	P.N.A.
			PTF	0.850	P.N.A.
			PAM	0.835	P.A.
LMW glutenin					
GAF	0.955	P.N.A.	GAF	0.955	P.N.A.
α-gliadin					
PQPQPF	0.920	P.A.	PQPQPF	0.920	P.A.
PYPQPQPF	0.898	P.N.A.	PAM	0.835	P.A.
PPQQPYPQPQPF	0.822	P.A.	IPCR	0.830	P.N.A.
PRL	0.806	P.N.A.	IPPY	0.809	P.N.A.
γ-gliadin					
QQPF	0.874	P.A.	QQPF	0.874	P.A.
QQQMNPCKNCL	0.806	P.N.A.	CL	0.880	P.N.A.
			GTF	0.849	P.N.A.
ω-gliadin					
PWQPQQPF	0.915	P.N.A.	PQPQPF	0.920	P.A.
QQPF	0.874	P.A.	QQPF	0.874	P.A.
PQPQQPF	0.838	P.A.	PQPQQPF	0.838	P.A.
QPQQPF	0.812	P.N.A.	QPQQPF	0.812	P.N.A.
Avenin like protein					
RMPF	0.977	P.A.	CAAF	0.862	P.N.A.
CAAF	0.862	P.N.A.	QCCPQL	0.830	P.A.
RSMCSIYIPVQCPTP TGYKIPSVATYTGGC	0.809	P.A.			
Wheat proteins					
After pepsin digestion			After pepsin+trypsin+ chymotrypsin digestion		
Sequence	Activity	Allergenicity	Sequence	Activity	Allergenicity
HMW glutenin					
GQWL	0.920	P.N.A.	GGSF	0.908	P.A.
PAMCRL	0.898	P.A	GQW	0.935	P.N.A
WGIPAL	0.885	P.A.	PGQW	0.929	P.N.A.
			CR	0.865	P.N.A.
			PAM	0.835	P.A.
LMW glutenin					
QQQCIPVAMQRCL	0.845	P.N.A.	CL	0.880	P.N.A.
			GTF	0.849	P.N.A.
α-gliadin					
QPF	0.955	P.N.A.	QPF	0.955	P.N.A.
PQF	0.935	P.A.	GIF	0.952	P.N.A.
PQPQPF	0.920	P.A.	PQF	0.935	P.A.
PPQQPYPQPQPF	0.822	P.A.	PPQQPY	0.920	P.N.A.
			PQPQPF	0.920	P.A.
			PAM	0.835	P.A.
			IPCR	0.830	P.N.A.
γ-gliadin					
WSMIL	0.916	P.N.A.	QPF	0.955	P.N.A.
PQPHQPF	0.865	P.A.	PQQPF	0.879	P.N.A.
NPCKNF	0.817	P.N.A.			
ω-gliadin					
PWQPQQPF	0.915	P.N.A.	QPF	0.955	P.N.A.
QQPF	0.874	P.A.	PQPQQPF	0.838	P.A.
PQPQQPF	0.838	P.A.	QPQQPF	0.812	P.N.A.
QPQQPF	0.812	P.N.A.	PSQQPF	0.800	P.A.

P.A. MEANS "Probable Allergen" and P.N.A. means "Probable Non Allergen" according to AllergenFP.

Hydrolysis degree of all proteins after pepsin hydrolysis was lower than those of pepsin+trypsin+chymotrypsin hydrolysis. This value for HMW glutenin of wheat and einkorn obtained from pepsin hydrolysis was 5.244% and 5.693% respectively. The highest DHt after pepsin hydrolysis was obtained for einkorn γ-gliadin (13.548%) and wheat ω-gliadin (13.620%). After gastrointestinal digestion DHt values were 22,436% and 25.442% for wheat α-gliadin and einkorn avenin like protein, respectively. The highest and the lowest A_E values after pepsin hydrolysis were obtained for wheat ω-gliadin as 0.0179 and einkorn HMW glutenin as 0.0012, respectively (Table 4). Gastrointestinal digestion caused more bioactive peptides generation than gastric digestion. The highest DHt, A_E and W values among proteins of wheat and einkorn were obtained for einkorn avenin like protein as 25.442%, wheat α-gliadin as 0.288 and wheat γ-gliadin as 1.000 respectively (Table 4).

Therefore, consumption of these wheat varieties can help to the regulation of both glucose levels and hypertension.

Possible Bioactive Peptides

PeptideRanker scores of all peptides released by digestion of wheat and einkorn proteins were determined and those greater than 0.5 were considered as possible bioactive peptides (PBPs). Table 5 shows sequences of bioactive peptides whose PeptideRanker scores greater than 0.8.

The number of PBP produce after gastric and gastrointestinal digestion of einkorn proteins was higher than that of wheat proteins. Gastric digestion caused the release of 74 PBPs and 40 PBPs from einkorn and wheat proteins, respectively. These values for gastrointestinal digestion were 86 PBPs for einkorn proteins and 49 PBPs for common wheat proteins. The most possible bioactive peptide generation among the einkorn proteins was obtained for ω-gliadin (20 for gastric digestion and 24 for gastrointestinal digestion). This was followed by ɣ- gliadin (15 for gastric, 18 for gastrointestinal digestion). Einkorn HMW glutenin generated the least number of PBPs for each digestion step. In the case of wheat proteins, α-gliadin produced more PBPs for both digestion steps and ω-gliadin (12 for gastric and 14 for gastrointestinal digestion) followed it. The least number of PBPs was observed in HMW glutenin.

One of the most important findings among these results was the detection of peptides having possible allergenicity according to AllergenFP program. Results showed that approximately 57% of PBPs (42 out of 74) generated after gastric digestion of einkorn proteins might exert allergenicity. This value was 50% (20 out of 40) for common wheat proteins after gastric digestion. Gastrointestinal digestion did not cause any change in the probability of allergenicity of peptides. For einkorn proteins, 41 possible allergen peptides out of 86 PBPs were determined whereas this number was 23 out of 49 for common wheat proteins after gastrointestinal digestion. In addition, gastric digestion caused possible toxic peptide generation for both wheat proteins. Two of them were originated from einkorn avenin like protein (SSCQIVQYQCCQQL score:0.669; PQIPADQPTSCQNVQHQCCPQL score: 0.531) and the other was originated from wheat α-gliadin (PAMCNVYIPPHCSTTIAPF score:0.661). However, they disappeared after intestinal digestion.

Conclusion

Wheat is a common ingredient of many food products including bread and bakery goods. However, misperception about the common wheat consumption and health disorders including obesity, diabetes mellitus, etc. causes people to consume different ancient species of wheat instead of common wheat. The result of the presented study showed that einkorn and common wheat proteins exhibited similar properties including high similarity rate and the identical bioactivities (ACE inhibitory, DPP IV inhibitory, antioxidative, stimulating vasoactive substance release, glucose uptake stimulating, antiamnestic, celiac toxic, renin inhibitory, DPP III inhibitory) of peptides generated after digestion. Most of the bioactive peptides were dipeptides and the majority of them displayed more than one

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bioactivities including ACE inhibitory, DPP IV inhibitory or antioxidant activity. PeptideRanker scores showed that both kinds of wheat generated PBPs. However, it should be stated that their bioactivities must be examined using both *in vitro* and *in vivo* methods. Allergenicity prediction displayed that release of allergen peptides was similar for both kinds of wheat.

In conclusion, although there is a perception related to the einkorn that is healthier than common wheat, *in silico* digestion results did not support this perception in terms of protein profiles and peptide production. However, these results need to be supported by *in vitro* and *in vivo* studies.

Acknowledgments

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