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Low molecular weight proteins of barley related to food allergy

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Abstract

As protein composition and modification are critical for malt and beer quality, proteomic approaches have the potential to improve malting and brewing processes, as well as to monitor and characterize important low molecular weight proteins related to food allergy. New product compositions and industrial processes create additional needs that require much greater technological development. The detection and quantification of allergenic proteins by mass spectrometry is promising and contributes to greater accuracy, thereby significantly improving consumer information. In the case of allergenic proteins, a wide range of isoforms, post-translational modifications and other structural changes during the technological process can increase or decrease their allergenicity. In this context, we focused on tracking barley proteins related to pathogens, a large proportion of which are allergy-related. These mainly include a group of protease/amylase inhibitors such as α-amylase/trypsin inhibitor CMa, CMb, CMe, α-amylase inhibitor BDAI-1. Similarly, a lipid transfer protein 1 has been identified as a major beer allergen.

Keywords: barley; allergy; protein; beer

1 Introduction

Studies on the influence of barley protein composition on the quality of malt and beer are extensive. Barley grains used for malting should, in principle, have a relatively lower protein content, as a higher protein content is thought to cause some negative effects, such as prolonging the malting process and degrading the quality of the malt and the quality of the beer (Dai et al., 2007). It has been shown that the effect of protein on the quality of malt and beer is complex due to the different types of protein (Robinson et al., 2007). The basic composition of barley proteins is represented by 4 main categories: glutenin, gliadin, albumin and globulin, which have different functions in malting and brewing beer. Some proteins are completely or partially unfolded by malt protease and transformed into other proteins during germination (Jones and Marinac, 2002). Degradation of malt storage proteins can affect many aspects of beer quality, including their clarity (Blšáková el al., 2022), foam stability, and haze formation (Niu et al., 2018; Wiesen et al., 2011). It is now more common to classify seed proteins into three groups: storage proteins, structural and metabolic proteins, and protective proteins (Bobalova and Strouhalova, 2024). The storage proteins of barley include proteins of the prolamin superfamily and storage globulins, which are present in the embryo, endosperm and aleurone layer. Enzymes such as β-amylases, α-amylases, peroxidases and lipoxygenases belong to the group of structural and metabolic proteins. The group of protective proteins includes pathogen-related proteins. As other cereals, barley contains protein inhibitors that can act against α-amylases and proteases of pathogens and pests.

2 Pathogen-related proteins

In general, pathogen-related proteins (PRs) are known to be an important group of barley proteins, a large propor-

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tion of which are related to allergies (Table 1). They represent approximately one third of the total protein content and play an essential role in pathogen attack on the plant (Benešová et al., 2011; Gorjanović, 2010). PRs affect the health of the beer consumer and the quality of beer in different ways. Among the most important roles are the function in the formation, stabilization and maintenance of foam, influencing the fermentation of brewer's yeasts, also affecting the colloidal turbidity of beer. The literature further suggests that they have antioxidant activity and may influence the breakdown of proteins and carbohydrates during malting and mashing (Carvalho et al., 2016).

The PR proteins were originally divided into five main categories (PR-1 to PR-5), (Van Loon, 1985), although more recent work has led to the identification of several additional classes of proteins. For example, 17 families of PR proteins have been named since 2014 (Sinha et al., 2014). Many of the protein families associated with pathogenesis also match human allergen families, although allergy may have nothing to do with the defence function of these proteins. Grouping these proteins according to their sequence features makes it possible to find potentially allergenic proteins from sequenced plant genomes, an area of study termed "allergenomics" (Di Girolamo et al., 2015).

2.1 Protease/amylase inhibitors

An important group of PR proteins that belong to PR-6 are protease/amylase inhibitors, such as α-amylase/trypsin inhibitor CMa, α-amylase/trypsin inhibitor CMb, α-amylase/trypsin inhibitor CMc, trypsin inhibitor CMe, α-amylase inhibitor BDAI-1 (Flodrová et al., 2014), serine protease inhibitors (serpins or protein Z4, Z7). An overview of the important amylase inhibitors is given in Table 2. The glycosylated members of the α-amylase inhibitor family (BMAI-1, BTAI-CMb) are known to be the most potent allergens related to Baker's disease (Sanchez-Monge et al., 1992). They are highly reactive both in vivo and in vitro. These major glycoprotein allergens carry a single asparagine-linked complex glycan that contains both β(1–2) xylose and α(1–3) fucose. The xylosyl residue and, to a lesser extent, the fucosyl residue are key IgE-binding epitopes and largely responsible for the allergenicity of these and unrelated proteins from plants.

Table 1 *Brief overview of barley pathogen-related proteins*

PR	Mr (kDa)	Protein family	Protein	Allergen name	Allergen exposure	
PR-2	25–35	ß-1,3-glucanases				
PR-8	28	class III chitinases				
PR-3	25–35	class 1 chitinases				
PR-5	22-24	thaumatin-like proteins				
PR-4	13-19	chitinases				
PR-6	15-45	protease/α-amylase inhibitors	α-amylase inhibitor BMAI-1	Hor v 15, Hor v 15.0101	a major IgE-binding component of sera from bakers asthma patients	ingestion
			α-amylase, β-amylase	Hor v 16, Hor v 17		ingestion
			α-amylase inhibitor	Hor v 28		ingestion
			Serine protease inhibitor (Z-4)	Hor v 33		ingestion
			α-amylase inhibitor BDAI-1	Hor v BDAI		inhalation
			Trypsin inhibitor	Hor v BTI		inhalation
PR-13	14	thionin	α-hordothionin	Hor v 37		ingestion
PR-14	7–12	ns -LTPs	LTP1, LTP2	Hor v 7k-LTP, Hor v 14	anaphylaxis, hypersensitivity reaction	ingestion
PR-12	5	defensin				

Table 2 *Summary of important barley protease/amylase inhibitors*

Proteinase inhibitors PR-6			
Protein	Uniprot Accessions		Molecular weight (kDa)
Bowman-Birk type trypsin inhibitor (BBBI)	P12940	IBB_HORVU	13.82
chymotrypsin/subtilisin inhibitors CI-1A	P16062	ICIA_HORVU	8.88
chymotrypsin/subtilisin inhibitors CI-1B	P16063	ICIB_HORVU	8.96
chymotrypsin/subtilisin inhibitors CI-1C	P01054	ICIC_HORVU	8.26
chymotrypsin/subtilisin inhibitors CI-2A	P01053	ICI2_HORVU	9.38
chymotrypsin/subtilisin inhibitors CI-2B	P08626	ICI3_HORVU	8.30
α-amylase/trypsin inhibitors CMa	P28041	IAAA_HORVU	15.50
α-amylase/trypsin inhibitors CMb	P32936	IAAB_HORVU	16.53
α-amylase/trypsin inhibitors CMc	P34951	IAAC_HORVU	15.18
α-amylase/trypsin inhibitors CMd	P11643	IAAD_HORVU	18.53
α-amylase/trypsin inhibitors CMe	P01086	IAAE_HORVU	16.14
α-amylase inhibitor (BDAI-1)	P13691	IAA2_HORVU	16.43
α-amylase inhibitor (BMAI-1)	P16968	IAA1_HORVU	15.82
bifunctional α-amylase/subtilisin inhibitor (BASI)	P07596	IAAS_HORVU	22.16
serine protease inhibitor protein Z4	P06293	SPZ4_HORVU	43.28
serine protease inhibitor protein Z7	Q43492	BSZ7_HORVU	42.82

The chitinase family of proteins represents a pathogenesis-related proteins (mainly PR-4) that is also reported to play an important role in plant responses to abiotic stress. However, its role in the response to abiotic stress in barley is still unclear (Wan et al., 2024).

2.2 Nonspecific lipid transfer proteins

Another important group of PR proteins belonging to the PR-14 group are nonspecific lipid transfer proteins (ns-LTPs). These extracellular proteins are capable of mediating the transport of lipids and hydrophobic molecules. They are involved in plant adaptation to biotic and abiotic stress, recognition and inhibition of pathogenic bacteria and fungi, and assembly of defensive extracellular polymers. Their expression is regulated by pathogens, signaling molecules associated with plant defence, and abiotic and environmental stresses (Gorjanović et al., 2005). LTP proteins occur in two major forms: ns-LTP1 and ns-LTP2 with a molecular weight of 9 kDa and 7 kDa and a pI value of 8 (Chmelik et. al., 2009; Perrocheau et. al., 2006). Barley LTP1 (9 kDa) has been identified as a major beer allergen. LTP1 becomes a foam-forming form after during wort boiling and this improvement is also related to glycation during malting and acylation during mashing. Although modified forms of ns-LTP1 promote foam formation, their increased amount in beer could also be disadvantageous, as they may be responsible for the foaminess of malt (Perrocheau et al., 2006).

3 Allergenic proteins

The majority of food allergens of plant origin are proteins with biological functions involved in the storage, structure and defence of plants. The presence of heavy metals, air pollution, pesticides and other factors can increase the allergenicity of these proteins. Similarly, a broad range of isoforms, post-translational modifications and other structural changes during food processing can increase or decrease allergenicity (López-Pedrouso et al., 2020).

Although the main food allergens are water-soluble proteins, the alcohol-soluble fraction of prolamins (hordeins) from barley proteins is also capable of activating allergic disease. Hordeins are the most abundant proteins, accounting for approximately 40–50% of the total protein fraction of mature barley (Flodrova et al., 2012), and are considered to be triggers of celiac disease (CD) and food allergy. Immunological studies are known to have revealed potential peptide sequences in barley that may stimulate CD-associated T-cells (Hardy et al., 2015).

3.1 Allergenic proteins analysis

In order to obtain quality barley, the above-mentioned protein groups need to be monitored and identified and, with food allergies being a worldwide food problem, it is clear that detection and quantification of allergens is necessary for proper food labelling.

It has been reported in the literature that gluten proteins can be modified during processing, leading to changes in protein structure (Juhász et al., 2020). For this reason, it can be difficult to calculate the gluten content, which poses a health risk for people with CD. Determination of allergenic components is often performed using enzyme-linked immunosorbent assays (ELISA). This method is sensitive, but on the other hand has the disadvantage that multiple allergens cannot be detected simultaneously (Popping, 2013). Therefore, mass spectrometry-based proteomics is used, which allows high-throughput multiplex analyses (Ahsan et al., 2016; Bahmani et al., 2021). For food allergens, 1D and 2-DE immunoblot technologies are widely used, followed by MALDI-TOF/TOF and more recently LC-MS/MS has become useful (Lexhaller et al., 2019).

Several allergenic proteins were characterized by gel electrophoresis and mass spectrometry. Some authors have also used a combination of LC and mass spectrometry to investigate the structure of barley proteins. Quite often MALDI-MS with linear mode is applied (Bobalova et al., 2008). It has the advantage of being a rapid and inexpensive analysis. A brief analytical strategy is described in Figure 1.

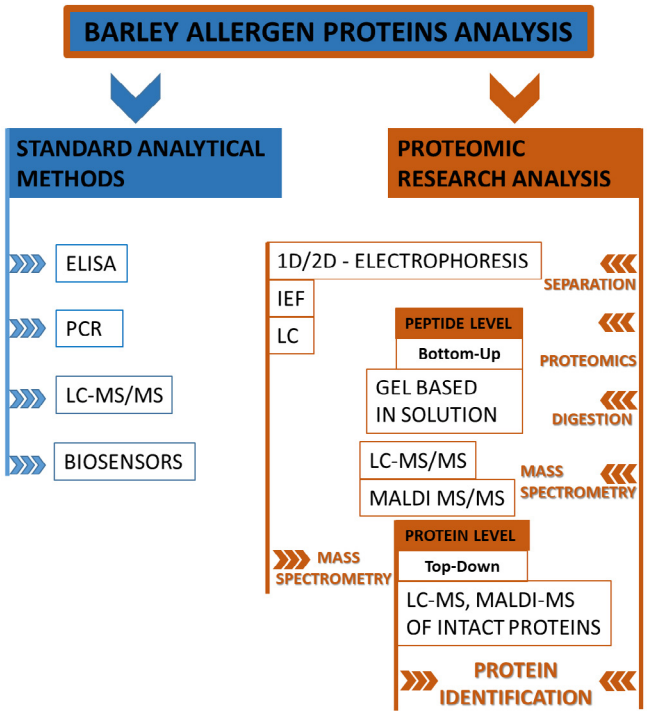

Figure 1 *Schematic of the analytical strategies used for barley allergen protein analysis*

Since most of the known allergens present in barley are low molecular weight (LMW) proteins, Flodrova et al. (2014) focused on their qualitative and quantitative monitoring. They identified various LMW proteins (Table 3), most of which were proven allergens belonging to the amylase/trypsin inhibitor family. Subsequently, they determined and compared the quantitative profiles of these LMW proteins in pairs: barley and malt, using the iTRAQ method, which is based on covalent labelling of the N-terminus and amino side chains of peptides from protein digestion with different mass tags. The iTRAQ data provided information on the quantitative changes of the proteins in the processed cereal materials. Comparison of barley grain with malt (Figure 2) showed that the amount of most LMW proteins in malt is reduced compared to the amount in barley grains. Despite this, all proteins remained in the malt in detectable amounts. The most intense decrease was observed for LTP1. It accounted for more than 65%. These results are consistent with the findings of Perrocheau et al. (2005) and support the theory that the amount of protease/α-amylase inhibitors decreases during barley germination.

In contrast, e.g., endochitinase 1 or chitinase were detected in the malt sample by polyacrylamide gel separation, in-gel digestion and MALDI-TOF/TOF. These proteins are either not present in ungerminated grain and start to appear during malting or are present in very small and undetectable amounts in the grain sample (Benkovska el al., 2011). Some authors also purified BDAI-1 from a commercial beer sample by ammonium sulphate precipitation, anion exchange chromatography and metal ion affinity chromatography (Iimure et al., 2015).

4 Conclusion

Food allergy is a serious health problem that is occurring more and more frequently all over the world. For this reason, it is essential to establish accurate and sensitive detection and analytical methods. Mass spectrometry is known to have high specificity and sensitivity and represents a promising non-immunoassay method for the evaluation and quantification of allergenic proteins. In this article, an overview of current research in the field of mass spectrometry-based allergen detection is presented. The work focuses on the most important allergenic proteins of barley from the group of α-amylase/trypsin inhibitors, namely BMAI-1, BDAI-1, CMe, CMb and LTP1. The quantitative changes of these proteins during the

Table 3 *Summary of selected LMW proteins of barley*

Protein	Uniprot Accession	Allergome code; gene name	Molecular weight (kDa)
α -amylase inhibitor (BMAI-1)	P16968 · IAA1_HORVU	418; Hor v 15, 3328; Hor v 15.0101	15.82
α -amylase/trypsin inhibitor CMe	P01086· IAAE_HORVU	8779; Hor v BTI	16.14
α -amylase/trypsin inhibitor CMb	P32936 · IAAB_HORVU	418; Hor v 15	16.53
α -amylase inhibitor (BDAI-1)	P13691 · IAA2_HORVU	877; Hor v BDAI	16.43
non-specific lipid transfer protein 1 (LTP1)	Q9SES6_HORVU	950; Hor v 14	9.69

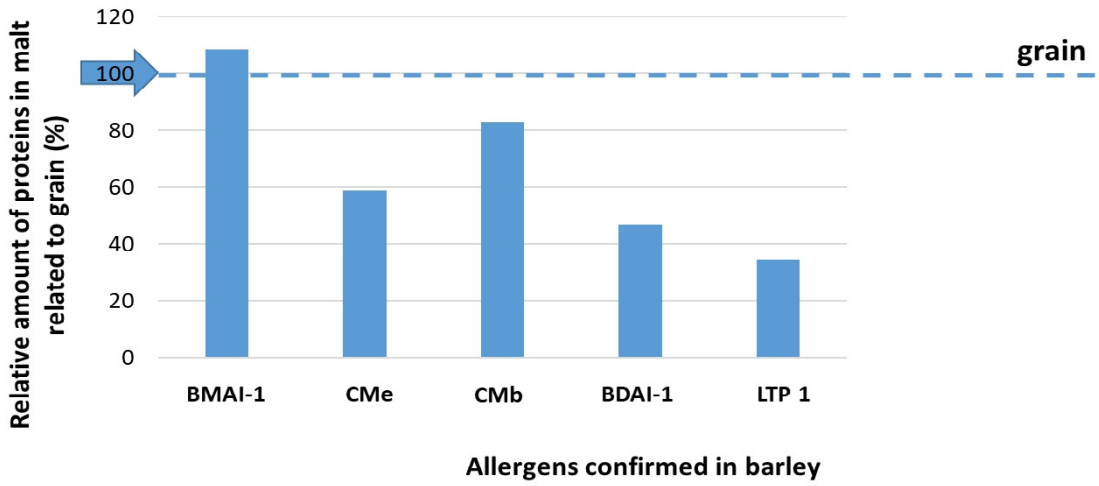

Figure 2 *Percentage of individual LMW of malted protein relative to the original amount in the barley grains (set to 100%)*

malting process can be monitored using the iTRAQ method. Comparison of barley grain with malt showed that the amount of the monitored proteins in malt is reduced compared to the amount in barley grain.

In conclusion, the detection and quantification of allergenic proteins using mass spectrometry-based proteomics is promising and would contribute to the advancement of barley allergen research as well as improve consumer awareness.

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