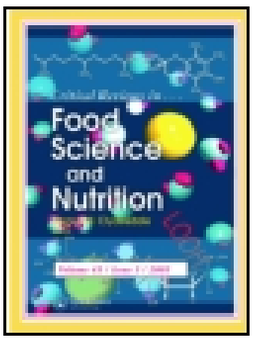

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REVIEW

Allergic reactions to hydrolysed wheat proteins: clinical aspects and molecular structures of the allergens involved

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ABSTRACT

Wheat gluten can be chemically or enzymatically hydrolysed to produce functional ingredients useful in food and cosmetics. However severe allergies to hydrolysed wheat proteins (HWP) have been described in Europe and Japan since the early 2000's. Triggering proteins and IgE epitopes were described both for French and Japanese cohorts and appeared remarkably similar leading to define a new wheat allergic entity. Deamidation induced by functionalisation generate neo-allergens responsible for this particular allergy. This article aims to review the processes leading to deamidation and the clinical features of the patients suffering from this allergy. Then the molecular determinants involved in HWP-allergy were exhaustively described and hypothesis regarding the sensitizing mechanism of HWP-allergy are discussed. Finally, current regulation and tools aiming at managing this risk associated with HWP are presented.

KEYWORDS

Allergy; gluten; gliadin; deamidation; IgE

Wheat is widely grown around the world in various climatic and agricultural conditions. Made of starch in a proportion of 80%, the wheat grain brings a significant part of the calories in many diets. In addition, its protein fraction (10 to 15% of the grain) comprises 80% proteins which form the gluten network after kneading. Gluten has unique viscoelastic properties that allow the preparation of breads, pasta, cakes, etc. This network consists of proteins, gliadins and glutenins, which have a remarkable amino acid composition since they contain from 16% to 30% proline and from 37% to 53% glutamine (Popineau and Denery-Papini 1996). These amino acids are present in repetitive motifs that constitute a large part of the sequences of these proteins. Depending on their sequence, there are several types of gliadins: alpha/beta-gliadins, gamma-gliadins and omega2 and omega5-gliadins. Omega-gliadins consist mainly of repetitive motifs. Glutenins are polymers that can reach molecular weights of several million Da; they are composed of low MW and high MW glutenin subunits linked by covalent bonds (intermolecular disulphide bridges). Non-covalent bonds also link gliadins with glutenins during the formation of the gluten network.

Like other foods such as milk or eggs, wheat flour is split into its main constituents for specific uses. Thus, starch is transformed into glucose syrups by the starch industry. Given the volumes of starch extracted, gluten has become an abundant protein source that is cheaper than animal protein (Day et al. 2006). Gluten can thus be used as an ingredient to enhance the baking quality of flour or to texture many products far beyond its traditional uses. However, its physical and chemical characteristics, which are its strength when it comes to baking or pasta making, represent a disadvantage when it concerns its incorporation in a wide range of products. Indeed, gluten proteins are insoluble in aqueous solutions and their propensity to aggregate and form a network limits their use.

Hydrolysed wheat proteins

Since the 1950s, several chemical or enzymatic processes have been described to increase the solubility of gluten and to give it new functional properties. Among them, deamidation of the many glutamines present in gluten proteins and/or proteolysis were frequently used. Deamidation by acid hydrolysis has been described as early as 1959 by Holme and Briggs (Holme and Briggs 1959). Gluten deamidated using this method has been proposed as a substitute for egg white in meringue desserts and cake icing (Mc Donald and Pence 1961) and as an emulsifier in dairy products (Wu, Nakai, and Powrie 1976). In addition to hydrolysing the amide function of glutamines, this treatment also causes a moderate hydrolysis of the peptide bonds of gluten proteins. Deamidation by alkaline hydrolysis has been proposed by Batey and Gras in 1981 (Batey and Gras 1981). In 2001, Kumagai et al. published a method of deamidation of gluten by cation exchange resin (Kumagai et al. 2007). The enzymatic methods described to modify gluten are for the most part based on the use of proteolytic enzymes. Considering enzymatic processes, pepsin hydrolysis has been first described by Draudt et al. since 1965 (Draudt et al. 1965). The use of trypsin, papain, bromelain or subtilisin has been subsequently described (Day et al. 2006). In the 1990s, the

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enzymatic deamidation of gluten was also proposed. Thus, Larré et al. reported the deamidation of gliadins with guinea pig liver transglutaminase which catalyses deamidation or cross-linking reactions depending on the availability of primary amines (Larré et al. 1993). The use of the glutaminase protein, which has the advantage of not creating intermolecular bonds or of hydrolysing gluten peptides, was suggested by Hamada et al. in 1994 to improve the functionality of food proteins (Hamada and Swanson 1994) and then applied to gluten (Yong, Yamaguchi, and Matsumura 2006). Since the 1990s at least, several of these treatments have been industrially applied. In particular chemical deamidation, which can be carried out under acidic or alkaline conditions, is an easy method to implement on an industrial scale.

Thus, depending on the type and/or intensity of the reaction to modify the gluten, the degrees of hydrolysis and/or deamidation rate can be extremely variable. As a result of this variability, there is a great diversity of modified glutens which have different uses in the food industry but also in the cosmetics industry. For example, certain modified glutens will thus be used for their emulsifying properties and will replace egg proteins (mayonnaise, sauces, and frostings); others, as sources of protein, will replace milk proteins (diets based on low-carb foods or for vegetarians). In cosmetics, they will be found in conditioners, creams or soaps to which they will give a creamy texture. These products have been labelled with several names: wheat proteins, wheat protein isolates, wheat hydrolysates or hydrolysed wheat proteins (HWP), which do not always reflect their characteristics: The term "**Isolate**" indicates that 80% of the total weight is protein but it gives no indication of the native or modified state of the proteins it is composed of; this state will depend on the process used for protein enrichment.

The terms "**wheat hydrolysates**" or "**hydrolysed wheat proteins**" are more precise since they indicate that the proteins have undergone a modification causing proteolysis. However, depending on the method used, chemical or enzymatic hydrolysis, and the intensity of the treatment, the size and the molecular structure of the constituents generated and the potential allergenicity of the modified proteins will be different. For food, Codex STD 163-1987, rev 1-2001 states that the naming in food applications should be vital wheat gluten, devitalised wheat gluten or solubilised wheat proteins. The term "functionalised wheat proteins" can also be used for wheat proteins having undifferentially undergone chemical or enzymatic modifications.

Clinical features of allergic reactions to hydrolysed wheat proteins

Several articles and case reports described allergic reactions due to HWP (Table 1). In 1988, a first case of occupational asthma to an alkaline derivative of gluten was reported in Canada (Lachance et al. 1988). The first cases of allergy involving products containing HWP were described in Europe between the years 2000 and 2006 (Lauriere et al. 2006; Pecquet et al. 2002; Sanchez-Perez, Sanz, and Garcia-Diez 2000; Varjonen, Petman, and Mäkinen-Kiljunen 2000). These were mainly cases of contact urticaria related to cosmetics. Laurière et al. described in particular a series of nine French patients, all wheat-tolerant, with contact urticaria to cosmetic products containing HWP and mentioned that six of the patients also reported urticaria or anaphylaxis when consuming foods containing HWPs (Lauriere et al. 2006). Contact urticaria can appear a few months after the application of the cosmetics for some patients. The cosmetics that caused the allergic reactions, moisturising products for the body or face, shampoos or conditioners or shower gel belonged to 11 different brands. The type of HWP present in these products was not known. In a case of occupational allergy with immediate reaction, when handling a cosmetic product, Tritisol HWP (Croda, United Kingdom) was unambiguously identified as the triggering product

Table 1. References describing symptoms of HWP-allergy and triggering ingredients. HWP, hydrolysed wheat proteins.

			Triggering products		
References	Number of patients	Symptoms	Cosmetic application	Food ingestion	Eliciting ingredient
Lachance et al. (1988)	1	Asthma	Occupational allergy		Wheat proteins treated with alkali
Sanchez-Perez, Sanz, and Garcia-Diez (2000)	1	Topical itchy erythematous, oedematous lesions	moisturizing cosmetic cream		HWP
Varjonen, Petman, and Mäkinen-Kiljunen (2000)	1	pruritic, erythematous, urticarial rash	moisturizing body cream		HWP
Leduc et al. (2003)	1	Anaphylaxis		Deli meats	GemTec
Lauriere et al. (2006)	6	Contact Urticaria	Shampoo, hair conditioner, shower gels		HWP
Denery-Papini et al. (2012)	15	Exercise Induced Anaphylaxis, Anaphylaxis, urticaria	3 on 15 patients reacted to cosmetics	Processed meat, Soup	HWP
Fukutomi et al. (2011); Chinuki and Morita (2012); Yagami et al. (2017)	> 2000	Contact urticaria Exercise Induced anaphylaxis	Soap	Wheat based product	GluPearl 19S Wheat
Christensen et al. (2017)	9	Anaphylaxis		Cake mix	Meripro 711 Wheat (3/9)
Delaunay et al. (2018)	1	contact urticaria, conjunctivitis, and dyspnoea	Occupational allergy		Tritisol

(Delaunay et al. 2018). In a skin test, this patient reacted positively to Tritisol and the cosmetic product containing 12% Tritisol and negatively to gluten; he also had IgE antibodies directed against deamidated gluten.

In France, Denery-Papini et al. (2012) described 15 cases of food allergy linked to the ingestion of products containing HWPs (processed meats, reconstituted meats, soups, industrial cakes) in wheat-tolerant patients. Three of these patients also had a reaction (contact urticaria) to cosmetics containing HWPs. These 15 patients had serious reactions to HWPs (Exercise-Induced Anaphylaxis (EIA), anaphylaxis, urticaria) and were sensitised to deamidated gluten. Six patients underwent an oral food challenge and had symptoms triggered by deamidated gluten (reactive doses between 0.44 g and 2 g). Five of them did not have a reaction to wheat flour (maximum doses tested between 30 and 70 g); the sixth patient also had a reaction during a challenge of unmodified wheat combined with exercise (27 g of wheat flour). The first of these cases of anaphylaxis was described in 2003 (Leduc et al. 2003). GemTec HWP (Manildra Group, Australia) incorporated into deli meats was responsible for the allergic reaction; sold under the name 'wheat isolate', it was characterised as a deamidated gluten. As a result of this allergic event, an extract corresponding to a deamidated gluten was proposed for the cutaneous diagnosis in 2006 (Battais et al. 2006). Between 2002 and 2014, the Allergy Vigilance Network (http://www.allergyvigilance.org/) identified 25 cases of serious allergic incidents related to HWPs.

Similar incidents occurred in Denmark (Christensen et al. 2017). Anaphylaxis was triggered in nine patients after consumption of products containing a HWP and in particular a cake mix that contained a small amount (< 1%) of an acid gluten hydrolysate called Meripro 711 (Tereos Syral, France). The nine Danish patients tolerated wheat, yet a diagnosis of wheat EIA was established later for three of them. These patients had specific IgE antibodies, positive skin tests and basophil activation tests to Meripro 711. An oral food challenge provoked a serious reaction in two patients at a very low dose of the cake mix and therefore was not performed in other patients. It should be noted that this publication reports only female cases, which raises questions regarding a potential dermal sensitisation with cosmetics.

Serious allergies to HWPs have been described in Japan since 2009 and have been the subject of an alert 2 years later following the reporting of more than 1,000 cases of wheat allergy in individuals, the majority of them women, most of whom had used a very popular soap, Cha no Shizuku® (Hiragun et al. 2013), 46 million units sold between March 2004 and September 2010 (Chinuki and Morita 2012; Fukutomi et al. 2011). In fact, in 2013, more than 1,900 patients were counted (Teshima 2014). The soap in question contained 0.3% of an acid gluten hydrolysate, Glupearl 19S (Katayama Chemical, Inc., Japan). The Japanese cases (Chinuki and Morita 2012) were characterised by a percutaneous and/or rhinoconjunctival sensitisation phase lasting for several months. Some of the patients developed contact urticaria and then an EIA when consuming wheat products. The time-frame reported between the use of the soap and the onset of the EIA to wheat varied between 1 month and 5 years. The other patients directly developed a systemic reaction to wheat ingestion, sometimes associated with moderate exercise. The predominant symptom was the angioedema of the eyelids; most patients reported anaphylaxis and some anaphylactic shock.

The cases described in Japan and Denmark were related to the use of a deamidated gluten, a consequence of its hydrolysis under acid conditions. Although the type of HWP present in the products consumed is not clearly identified for the 15 French patients, all were sensitised to deamidated gluten. The allergenic source therefore seems the same in these groups of patients. The main difference between the cases described in Europe and Japan is the tolerance to wheat; 14 of 15 French patients tolerated wheat (Denery-Papini et al. 2012); the 9 Danish patients tolerated wheat, however, a diagnosis of exercise-induced anaphylaxis (EIA) to wheat was established later for 3 of them (Christensen et al. 2017); in Japan most patients developed a food allergy to wheat (Yagami et al. 2017). The duration and frequency of the exposure, the sensitisation route and the type of product used (soap, presence of detergent) are probably at the origin of these differences.

In Japan, a national survey conducted on the basis of strict diagnostic criteria provides an assessment of 2,111 patients, of whom 2,025 women of an average age of 45 years (Yagami et al. 2017). The progress of these allergic responses could also be observed. After following-up 11 patients, Hiragun et al. indicated a decrease in the IgE concentrations measured for native gluten a few months after discontinuing the soap use (Hiragun et al. 2013). Some patients were then able to tolerate wheat again in small amounts and in the absence of co-factors. Others continued to have allergic reactions to wheat ingestion, even when measured levels of gluten-specific IgE were negative. However, they were still sensitised to deamidated gluten. In 2016, a retrospective study of 110 patients reported a remission rate of approximately 50% five years after discontinuing the soap use and reported a higher resolution rate for subjects younger than 30 years old (Hiragun et al. 2016).

Biochemical characteristics of deamidated glutens

The deamidation reaction

Proteins are formed by a linking of amino acids that gives them uniqueness and specific properties. The nature, number and arrangement of these amino acids will be at the origin of many of their physicochemical and structural properties. The protein skeleton corresponds to a sequence of amino acids bearing more or less reactive side chains. Two amino acids, asparagine and glutamine, carrying an amide side chain are particularly labile and can be deamidated. Deamidation of proteins involves the conversion of amide side chain fragments of asparagine and/or glutamine residues to carboxyl groups. Deamidation reactions are the most frequent non-enzymatic degradation reactions of amino acid residues. They occur on free asparagine and glutamine in solution as well as on peptides and proteins

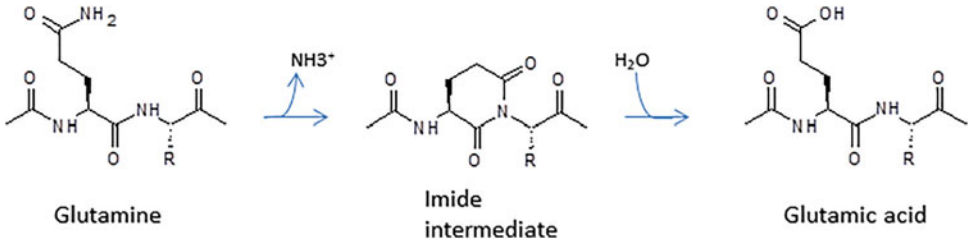

Figure 1. Conversion of glutamine to glutamic acid by deamidation.

(Juskowiak 2009). This reaction is particular because its reaction products are also genetically programmable amino acids that exist in a natural state. It is also known that deamidation can occur *in vivo* under physiological conditions; the carboxyl groups resulting from this reaction are thus considered post-translational modifications. In the 1970s, Robinson and Rudd hypothesised their role and suggested that hydrolysis of these amide side chains could serve as a biological clock in a number of physiological processes (Robinson and Rudd 1974), such as the ageing of the eye lens (Pande, Mokhor, and Pande 2015) or that of the Drosophila (Noah E Robinson and Robinson 2004b; Takemoto and Boyle 1998; Deverman et al. 2002). Under physiological conditions, deamidation of asparagines is at least 100 times faster than that of glutamines (Juskowiak 2009).

This reaction can also be obtained *in vitro* under various experimental conditions. It has been observed over a wide pH range with variable reaction rates depending on the conditions (Scotchler and Robinson 1974) and a lower reactivity around a pH of 6. It has also been shown that this reaction strongly depends on the steric environment of the Asp and Gln (Kossiakoff 1988) and on the nature of the neighbouring amino acids (Robinson et al. 2004).

In peptides and proteins, the mechanism of deamidation of asparagines and glutamines is complex and can pass through different intermediates which depend on the reaction conditions (pH, ionic strength, temperature) but also on the surrounding amino acid sequence and the conformation of the polypeptide chain (Robinson and Robinson 2004a). The main mechanism involves the cyclisation of the asparaginyl and glutaminyl residues which produces imide intermediates (succinimide and glutarimide, respectively) which will then form aspartic and glutamic residues carrying a carboxyl group (Fig. 1). When the pH is below 5, the deamidation rate increases to become very fast at a pH under 2 (Robinson and Robinson 2004a)

The amino acid composition of gluten proteins is singular due to their richness in amidated amino acids with an overrepresentation of glutamine (40% amino acids on average) compared to that of asparagine (2.9% amino acids) (Popineau and Denery-Papini 1996). This also explains why the deamidation reaction has a dramatic impact on gluten proteins, allowing the formation of molecules that are very rich in glutamic acid and therefore potentially highly negatively charged.

Industrially deamidated glutens (HWPs)

This part gives a few examples of deamidated glutens distributed as ingredients for the food or cosmetics industry.

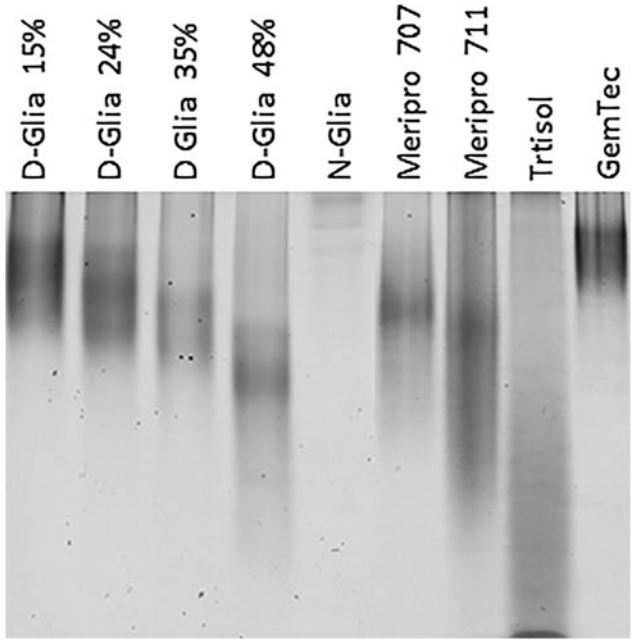

Figure 2. Analysis by native PAGE of deamidated gliadins (D-Glia) at various deamidation levels (15%, 24%, 35%, and 48%), native gliadins (N-Glia) and industrial HWP samples. Adapted with permission from Tranquet et al. 2015, Copyright © 2015 American Chemical Society.

Four of these HWPs (Tritisol, GemTec, Meripro 711 and Glupearl) are responsible for some of the allergic reactions described in the previous chapter. GemTec, Meripro 711 and Glupearl HWPs were produced by acid hydrolysis. No information regarding the deamidation protocol for the Tritisol HWP is available. A few details are known about the conditions of production of Glupearl 19S, heating at 95 °C in hydrochloric acid for 40 min (Teshima 2014), and Meripro 711, pH below 2.5 and temperature above 80 °C (Kroghsbo et al. 2014). These products have been characterised biochemically to better understand their potential role in the allergy (Tranquet et al. 2015). Firstly, electrophoresis under native conditions makes it possible to visualise and to approximatively assess the deamidation of gluten proteins. Indeed, only these deamidated proteins are negatively charged and migrate under these conditions, unlike gliadins and native glutenins. Fig. 2 shows the migration in electrophoresis under native conditions of four industrial HWPs. The migration of these proteins is related to their deamidation rate and their rate of hydrolysis; the deamidation rate is known for the products Meripro 707 (25–30%) and Meripro 711 (60%). Thus, we can consider that the product GemTec is weakly deamidated, unlike Tritisol which in addition

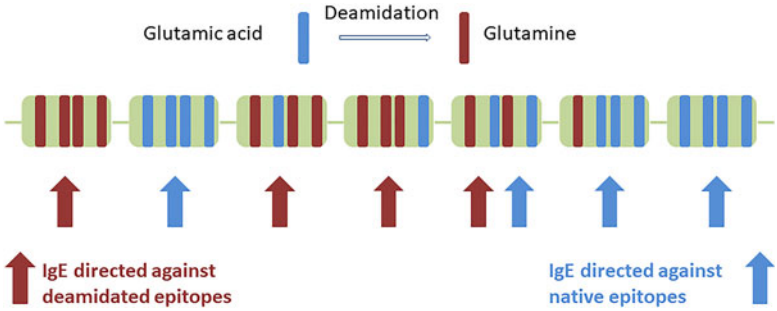

Figure 3. Schematic representation of the binding sites of IgE directed to native or deamidated epitopes in repeated sequences of prolamins (green blocks) deamidated by acid hydrolysis. Glutamine and glutamic acid are presented in blue and red respectively.

to a high level of deamidation also seems more hydrolysed than the two Meripro HWPs.

Recently, Tranquet et al. have published an ELISA method to detect and characterise deamidated glutens more precisely (Tranquet et al. 2015). The industrial deamidated glutens produced in Europe (Meripro 707, Meripro 711 and Tritisol), Australia (GemTec) and Japan (Glupearl 19S®) have been compared using this method and are distributed in three groups: Glupearl 19S, Tritisol and Meripro 711 are the most strongly deamidated (>60%), Meripro 707 is intermediate (25–30%) and GemTec has a deamidation rate of approximately 15%. Using monoclonal antibodies directed against the repetitive domains of the gliadins, Tranquet et al. have also shown that all these HWPs contain in varying proportions residual native epitopes (Tranquet et al. 2015; Tranquet et al. 2017) (Fig. 3). The molecular weight distribution, evaluated by steric exclusion chromatography for some HWPs (Meripro 707 and 711 data from the industry + Kroghsbo ref), suggests that they contain, just like native gluten, a majority of proteins migrating between 20 and 96 kDa. The analysis also reveals a decrease in the proportion of large polymers (exceeding 96 kDa) and an increase in the proportion of small polypeptides (under 20 kDa), which reflects proteolysis.

In summary, it appears that HWPs cover a range of heterogeneous products in terms of deamidation and polypeptide size. They are likely to contain in variable proportions a fraction of their proteins and/or a portion of the protein sequences which are not deamidated.

Molecular bases of reactions to HWPs

Protein fractions involved in patient sensitisation

Two teams in Japan described the IgE reactivity of wheat-allergic patients sensitised by the application of the Glupearl 19S® soap (Chinuki and Morita 2012; Fukutomi et al. 2011). Both teams demonstrated the presence of HWP-specific IgE antibodies associated with a lower recognition of wheat flour proteins in their native form (soluble proteins, gliadins and glutenins). The reactivity to native proteins is completely inhibited by the hydrolysate. The exploration of different industrial or laboratory-obtained HWPs suggests that IgE antibodies recognise HWPs produced by acid hydrolysis but not those obtained by enzymatic hydrolysis. The IgE of these patients recognised constituents comprised between 25 and 250 kDa in a series of glutens modified by different acid hydrolysis times, but they do not react with very highly modified glutens or with polypeptides less than 20 kDa resulted from proteolysis (Rika Nakamura et al. 2013). The same series of deamidated glutens is able to activate patients' basophils or a model of humanised rat basophils in the presence of patient IgE, unlike native gluten.

More recently, 10 HWPs marketed as ingredients in cosmetics have been analysed by Nakamura et al. (Nakamura et al. 2016), who showed that four of them induced positive skin prick test and ELISA responses in patients who became allergic after sensitisation with Glupearl. According to their data sheet, three of these products were obtained by enzymatic hydrolysis and had average MW of less than 3,500 Da while the fourth was obtained by alkaline hydrolysis. The latter was described with an average MW of 100,000 Da and its analysis by SDS-PAGE showed a very wide distribution of MWs between 250 kDa and 5 kDa. For this HWP, the basic pH treatment is certainly the cause of deamination and the wide range of molecular weights observed could be the result of heating under alkaline conditions which is known to cause not only partial hydrolysis, but also the formation of intra or intermolecular covalent bonds (Provansal, Cuq, and Cheftel 1975).

Common points are to be noted between the Japanese patients and the French cases described by Laurière et al. in 2006 (Lauriere et al. 2006). The nine patients described by Laurière et al. are all women who had used cosmetics containing HWPs to which they reacted in a skin test but whose production method is not known. Skin tests performed on five of these patients indicated that patients had a reaction only to wheat proteins modified by acid or alkaline hydrolysis but not to native wheat proteins.

Tranquet et al. complemented these characterisations by showing an impact of the deamidation rate of the involved industrial HWPs in Europe and Japan on the interaction with the IgE antibodies of allergic patients: the antigenicity and the activation potential of allergy effector cells (basophils) are proportional to the intensity of deamidation of HWPs (Tranquet et al. 2017).

Allergens and epitopes recognised by IgE antibodies of patients allergic to HWPs

In the French cases related to the ingestion of foods containing HWPs, Denery-Papini et al. explored the IgE

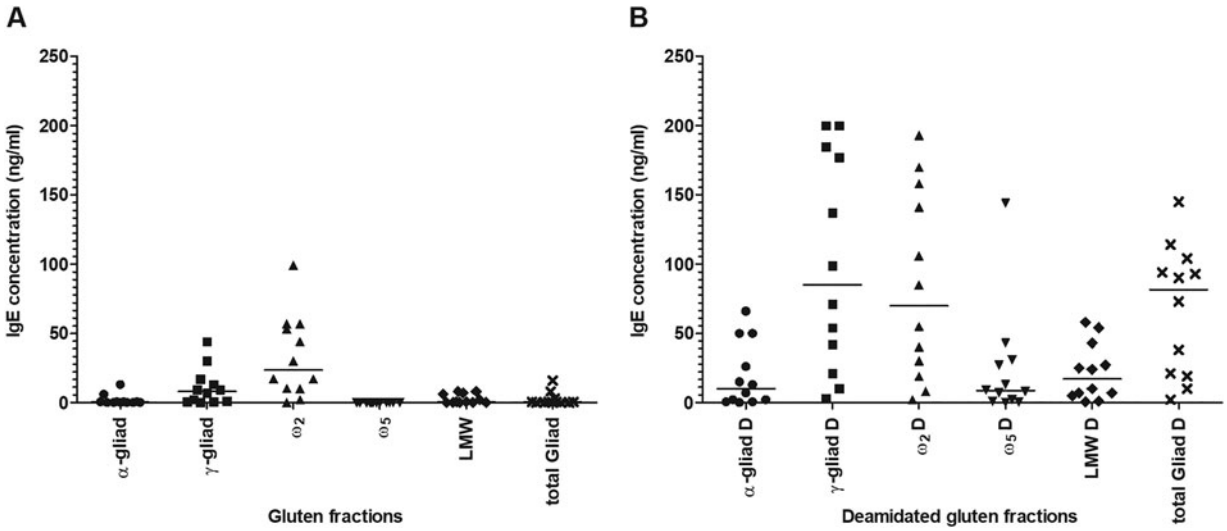

Figure 4. IgE reactivity profiles in ELISA of sera from patients allergic to deamidated gluten. Concentrations of specific IgE towards native (A) and deamidated (B) gluten fractions are expressed in ng/ml. Bars stand for median concentrations. α-gliad, γ-gliad, ω2, ω5 and total gliad: α, γ, ω2, ω5 and total gliadins; LMW: low molecular weight glutenin subunits – α-gliad D, γ-gliad D, ω2D, ω5 D and total gliad D: deamidated α, γ, ω2, ω5 and total gliadins; LMW D: deamidated LMW glutenin subunits. Adapted with permission from Denery-Papini et al. (2012), Copyright © 2012, John Wiley and Sons.

reactivity at the molecular level using purified gliadins or glutenins in native or deamidated form (Denery-Papini et al. 2012). Most patients had IgE antibodies for certain native proteins, in particular gamma- and omega2-gliadins, but not IgE specific for omega-5-gliadins or soluble flour proteins (Fig. 4). For all patients, the intensity of the IgE response increased significantly when the proteins were deamidated, in particular to deamidated gamma fractions, omega2-gliadins and total gliadins. These deamidated fractions are also capable of inducing the degranulation of a model of humanised basophils sensitised with the serums of these patients. Based on the native sequences of the two most recognised allergens (gamma and omega2-gliadins), the linear epitope mapping technique identified a native sequence, the octapeptide QPQQPFPQ, as a consensus epitope. Remarkably, this motif is repeated at least eight times on the sequences of these two gliadins. The intensity of the IgE response increases strongly when several glutamine positions are modified to glutamic acid; the deamidated epitope best recognised by the IgE antibodies of patients corresponded to the peptide QPEEPFPE. The studies of Denery-Papini et al. and Nakamura et al. demonstrated that the deamidation of gluten generates neo-epitopes responsible for the allergy to HWPs (Denery-Papini et al. 2012; Nakamura et al. 2013).

In the case of patients who have reacted to HWPs both dermally and orally (Laurière et al. 2007), IgE antibodies also recognise omega-2-gliadins and some gamma-gliadins but not omega-5-gliadins.

In their turn, Japanese researchers characterised the involved molecular allergens in dermally-sensitised patients allergic to wheat (Yokooji et al. 2013, 2015) and found results very similar to those seen in the French patients; using recombinant gliadins and glutenins, they noticed that patients were frequently sensitised to native alpha, gamma and omega2-gliadins but rarely and/or weakly to omega-5-gliadins. Among the recombinant proteins, only gamma-gliadins were capable of inducing the release of histamine in a basophil-activation test. Using synthetic peptides corresponding to native or deamidated sequences of a gamma-gliadin, they identified an identical consensus epitope QPQQPFPQ and the deamidated epitope PEEPFP, which is included in the epitope previously described by Denery-Papini et al. (2012). The concordance of the French and Japanese studies in the identification of the epitopes involved in the HWP allergy highlights the similarities that exist between these two sets of patients. The identification of these motifs led Yokooji et al. to evaluate their use for the development of a method of serological diagnosis of patients allergic to HWPs.

The profile of allergen and epitope recognised by patients allergic to deamidated gluten in the French and Japanese studies is remarkable by its homogeneity: two classes of gliadins were strongly recognised by IgE antibodies: gamma- and omega-2-gliadins, especially when they were deamidated and very similar consensus epitopes were identified. This profile differs from the profiles observed in conventional wheat allergies (with or without exercise) for which more heterogeneous sensitisation profiles were observed. Beyond the dominance of ω5 in EIA cases (Nilsson et al. 2015; Ebisawa et al. 2012; Altenbach et al. 2015; Matsuo et al. 2008), several other gluten proteins are recognised (Hiroaki Matsuo et al. 2005; Hofmann et al. 2012), as well as proteins of the albumin/globulin fraction (Battais et al. 2005; Mäkelä et al. 2014). It should be noted, however, that patients with conventional wheat allergies may have positive ELISA or skin test responses to diagnostic extracts of deamidated gluten without clinical significance. These responses are generally of weaker intensity than those observed for native wheat proteins and can be explained by the low presence of glutamic acid in native molecules and/or by the persistence of native epitopes in these HWPs.

Hypotheses regarding the sensitisation mechanisms

The studies of European and Japanese patient groups therefore highlight a new allergic entity related to sensitisation by

deamidated gluten proteins. In all cases, patients had serious symptoms. These patients had IgE antibodies to deamidated gluten and most of them also had IgE antibodies to native gluten proteins, but in all cases the responses (specific IgE concentrations or activation of basophils) measured for deamidated glutens were higher than those measured for native protein fractions, in contrast to the responses observed in conventional wheat food allergies.

On the other hand, despite a very high similarity in the allergens and epitopes recognised by their IgE antibodies, it should be noted that most of the French patients described tolerate wheat-based products whereas Japanese patients had food allergies to wheat, in particular EIAs. The various studies carried out in these patients sensitised to deamidated gluten and allergic or not to wheat thus raise a series of questions:

- Why do people who tolerate conventional wheat-based products become allergic to deamidated gluten?
- Why do Japanese patients also become allergic to wheat?
- Is the allergenic potential of deamidated gluten superior to that of wheat given the large number of Japanese cases and the severity of the symptoms observed in both Europe and Japan?

Studies conducted on animal models (mice and rats) can provide some answers. In 2012, Gourbeyre et al. sensitised mice intraperitoneally and noticed that, compared to native gliadins, gliadins deamidated by acid hydrolysis induced a higher production of IgE antibodies to deamidated gliadins but also to native gliadins (Gourbeyre et al. 2012). In 2018, Castan et al. confirmed this last point by stating that sensitisation with deamidated gliadins induced in mice a stronger allergic reaction than sensitisation with native gliadins, with in particular earlier IgE production and immune response (Castan et al. 2018). These studies illustrated both the high sensitising potential of deamidated wheat proteins and the fact that the persistence of native sequences in these products also generate IgE antibodies capable of recognising native proteins. In 2014, Kroghsbo compared the allergenic potential of native, deamidated glutens and an enzymatic hydrolysate intraperitoneally or orally (Kroghsbo et al. 2014). Native gluten and the enzymatic hydrolysate had similar antigenicity characterized by similar epitope patterns in contrast to deamidated gluten which also induced antibodies against different neo-epitopes.

Skin sensitisation to HWPs had also been studied. First in a mouse model with deamidated gluten (Glupearl 19S) by Adachi et al. (Adachi et al. 2012). In this model, it has been shown that Glupearl 19S was more effective than native gluten in inducing the production of specific IgE antibodies to deamidated gluten proteins but also to native proteins. The observed symptoms were also stronger with the deamidated gluten regardless if the animals had been sensitised with deamidated gluten or native gluten. In 2015, Matsunaga et al. showed in a model of passive cutaneous anaphylaxis (PCA) in hamsters that deamidated gluten (Glupearl 19S) had a higher sensitising potential compared to native gluten (Matsunaga et al. 2015).

To explain why Japanese patients responded to the ingestion of conventional non-HWP-containing cereal products, Nakamura et al., suggested that in vivo modification during digestion and/or absorption of native wheat proteins could generate neo-epitopes similar to those present in acid gluten hydrolysates causing patient sensitisation (Nakamura et al. 2013). The authors analysed the action of different enzymes: pepsin, pancreatin and tissue transglutaminase and suggested that the latter enzyme generates, by partial deamidation of the gluten (in vitro experiments), epitopes that could be the cause of cross reactions with the HWP. Gluten proteins are in fact transglutaminase substrates which, depending on the medium conditions, can catalyse deamidation or polymerisation reactions of these proteins. On the other hand, the authors did not explain how the conditions promoting deamidation of gluten peptides by transglutaminase could be fulfilled in vivo in these patients as in the case of the celiac disease. This hypothesis does not explain why European patients are only very rarely allergic to native wheat proteins.

The consumption or the skin application of a product containing epitopes different from those present in wheat flour, neo-epitopes induced by deamidation against which an oral tolerance response has not been established, would therefore explain the appearance of this new allergic entity in people previously tolerant to wheat. For the Japanese patients and some European patients, the generation of an allergic response may have been facilitated by the dermal penetration, which is less tolerogenic than the oral route (Chinthrajah et al. 2016), and by the application of a more hydrophilic product than gluten or the presence of detergents as in soap. The duration and chronicity of sensitisation may also explain why Japanese patients also responded to native wheat proteins.

Managing the risk of allergies to deamidated HWPs

Functionalisation of wheat proteins by deamidation is likely to generate neo-epitopes that are important to detect, both to diagnose patients and to control products that may contain them (food or cosmetics).

In fact, the native wheat extracts proposed for skin tests and for specific IgE assays are ineffective for the diagnosis and laboratory exploration of these patients allergic to deamidated gluten. The characterisation of modified gluten, involved in the first French case described by Leduc et al., carried out in our group, resulted in the proposal in 2006 of a deamidated gluten extract for the diagnosis of patients allergic to deamidated gluten (Battais et al. 2006). For the same purpose, Nakamura et al. proposed in 2014 to use Glupearl 19S for Japanese patients (Nakamura et al. 2014).

In terms of food and cosmetic labelling, the management of this specific risk is just as delicate. In fact, on the one hand, the labelling regulations do not allow the identification of products containing deamidated gluten and, on the other hand, the analytical methods dedicated to the detection of gluten are strongly impacted by deamidation (Kanerva et al. 2011; Tranquet et al. 2015). The identification of a major epitope of deamidated gluten (Denery-

Papini et al. 2012) allowed thus the development of a specific detection method applicable to foods and cosmetics (Tranquet et al. 2015). Similarly, a chimeric IgE antibody mimicking the IgE reactivity of patients allergic to HWPs is now proposed as a tool to evaluate in vitro the allergenicity of functionalised glutens (Tranquet et al. 2017). In Europe, glutens deamidated by acid treatment were withdrawn from the food market by some manufacturers in 2011. The industry association "Starch Europe" interviewed in January 2018 said that seven members representing gluten producers in Europe no longer produce deamidated glutens for human consumption, but market HWPs obtained by enzymatic hydrolysis. No cases of allergy to deamidated gluten have been identified by the allergy vigilance network since 2014. In the case of cosmetic products, the Scientific Committee for Consumer Safety of the European Commission (SCCS) recommended in 2014 (SCCS/1534/14) that they are used in cosmetics after extensive proteolysis generating polypeptides/peptides whose average size will be less than 3.5 kDa. Yet a recent publication in EFSA journal indicated that peptides of 9 amino acids (about 1 Kda) could be sufficient to cross-link IgE on effector cells (Naegeli et al. 2017). Recent results published by Nakamura et al. (Nakamura et al. 2016), however, show that HWPs for cosmetics with average molecular weights below this limit induced a reaction at the prick test in patients sensitised to Glupearl 19S. In view of these results, this 3.5 kDa limit could be reconsidered.

In conclusion, the example of functionalisations made to gluten highlights the importance of developing tools and strategies for testing new ingredients, such as those under discussion in the COST ImpARAS network. Whatever the process leading to the deamidation of gluten proteins this modification impacts the allergenic potential of gluten. Deamidated gluten proteins seem to have a higher sensitisation capacity than native ones and are the cause of IgE antibodies directed against epitopes different from those involved in conventional wheat food allergies. The induction of an immune response by these new epitopes on wheat proteins is probably facilitated by the lack of an active tolerance to these sequences. In addition, sensitization by a less tolerogenic route, such as skin, appears to be an aggravating factor. The IgE antibodies of the HWP-allergic patients mainly bind to deamidated gluten proteins at the level of the sequences repeated about eight times and containing a consensus epitope. The presence of several copies of the same epitope on these allergens could lead to a very effective activation of allergy effector cells and may explain the severity of the symptoms described in all the studies.

More generally, the valorisation of by-products in order to increase the sustainability of productions or the increase of the added value of existing ingredients can be achieved through protein modifications. It can be assumed that modifications leading to noticeable changes in the proteins sequences will have more impact in terms of neo-allergenicity. Currently, there are no consensual tools for a priori risk assessment for such products or ingredients. Because of the complexity of allergic diseases, only approaches combining accurate product characterization, in vitro studies and animal models appear to be able to adequately address this need.

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