

Creating hypo-/nonallergenic wheat products using processing methods: Fact or fiction?

Haoran Gao¹ | Rick Jorgensen¹ | Rajsri Raghunath¹ | Siddharth Nagisetty¹ | Perry K. W. Ng² | Venu Gangur¹ 

¹ Food Allergy and Immunology Laboratory, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan, USA

² Cereal Science Laboratory, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan, USA

Correspondence

Venu Gangur, Food Allergy and Immunology Laboratory, Department of Food Science and Human Nutrition, Michigan State University, 469 Wilson Rd, East Lansing, MI 48823, USA.
Email: gangur@msu.edu

Abstract

Wheat allergy is a potentially life-threatening disease that affects millions of people around the world. Food processing has been shown to influence the allergenicity of wheat and other major foods. However, a comprehensive review evaluating whether or not food processing can be used to develop hypo-/nonallergenic wheat products is unavailable. There were three objectives for this study: (1) to critically evaluate the evidence on the effect of fermentation, thermal processing, and enzyme or acid hydrolysis on wheat allergenicity so as to identify the potential for and challenges of using these methods to produce hypo-/nonallergenic wheat products; (2) to identify the molecular effects of food processing needed to create such products; and (3) to map the concept questions for future research and development to produce hypo-/nonallergenic wheat products. We performed literature research using PubMed and Google Scholar databases with various combinations of keywords to generate the data to accomplish these objectives. We found that: (1) food processing significantly modulates wheat allergenicity; while some methods can reduce or even abolish the allergenicity, others can create mega allergens; and (2) fermentation and enzymatic hydrolysis hold the most potential to create novel hypo-/nonallergenic wheat products; however, preclinical validation and human clinical trials are currently lacking. We also identify five specific research concepts to advance the research to enable the creation of hypo-/nonallergenic wheat products for application in food, medical, and cosmetic industries.

KEYWORDS

Wheat Allergy, Food Processing Methods, Wheat Allergenicity, IgE, Hypersensitivity

1 | INTRODUCTION

Food allergy is one of the major health issues worldwide. It currently affects 10.8% of adults and 8% of children in the United States (Gupta et al., 2019; Sampson et al., 2018). In general, food allergy refers to the IgE antibody-mediated adverse immune reactions triggered by the ingestion of a specific food. Typical clinical symptoms associated with food allergy can vary from mild reac-

tions, such as hives, rashes, vomiting, diarrhea, and rhinoconjunctivitis, to serious life-threatening reactions, such as systemic anaphylaxis, and Baker's asthma (Sicherer & Sampson, 2018; Warren et al., 2020). The United States Food and Drug Administration (US FDA) has identified eight major foods that account for over 90% of allergic reactions: milk, eggs, fish, shellfish, tree nuts, peanuts, soybeans, and wheat; sesame has been recently added to this list as the ninth major food allergen in the United

States (Gangur & Acharya, 2021; US FDA, 2021). In addition, wheat is also regulated as a major allergenic food in Canada, the United Kingdom, Japan, Australia, New Zealand, and in all 28 European Union countries (Food Standards Australia & New Zealand, 2020; EFSA, 2004; FAO, 2020; Health Canada, 2018; Japan, 2019; United Kingdom, 2020; US FDA, 2021). The overall prevalence of wheat allergy is estimated to be 0.4%–3% in the United States and up to 0.9% at the global level (Cianferoni, 2016; Leonard & Vasagar, 2014; Venter et al., 2006). Thus, wheat allergy affects a significant proportion of the global human population.

Wheat is a staple food for most of the world's population. The global wheat production has been steadily increasing during the last decade. In addition, the supply and demand of wheat have fluctuated around 750 million tonnes/year, especially in the last 5 years (FAO, 2020). However, the per capita consumption of wheat flour has decreased in the United States by ~7.7% during the last two decades (from 143 pounds per person to 132 pounds per person; USDA, 2019). Reasons for this reduction in the United States are unknown. Potential reasons include avoidance of wheat products due to physician diagnosed health conditions (e.g., food allergy, celiac disease, nonceliac gluten sensitivity, etc.) or perceived health concerns without a formal diagnosis (Leonard et al., 2017; Pilolli et al., 2019). In Australia, 11% adults consciously avoid wheat in an attempt to alleviate fatigue and gastrointestinal symptoms (Golley et al., 2015). In the United Kingdom, motivating factors for wheat avoidance by people (without celiac disease and without wheat allergy) were: management of symptoms similar to inflammatory bowel disease, infertility, low mood, low energy, immune dysfunction, weight gain, and visual and auditory hallucinations (Harper & Bold, 2018). Thus, real, or perceived health issues linked to wheat pose a serious threat to the growth of global wheat food industry.

There are at least eight human clinical conditions that have been associated with wheat: (1) celiac disease; (2) nonceliac gluten/wheat sensitivity; (3) food protein-induced enterocolitis syndrome; (4) eosinophilic esophagitis; (5) wheat food allergy (including vomiting, diarrhea, hives, systemic anaphylaxis, atopic dermatitis, rhino-conjunctivitis, and oral allergy syndrome); (6) wheat-dependent exercise-induced anaphylaxis; (7) contact urticaria; and (8) Baker's asthma (Cabanillas, 2020; Juhász et al., 2018; Quirce et al., 2016; Ricci et al., 2019). All these conditions are mediated by the overactivation of the immune system. Although the celiac disease is an autoimmune reaction triggered by gluten, the nonceliac gluten sensitivity is thought to be due to the activation of innate immune system by unknown components of wheat (Cabanillas, 2020). Mechanisms of food protein-induced enterocolitis syndrome and eosinophilic esophagitis are

incompletely understood. Only the last four diseases are mediated by the IgE antibodies. Both systemic anaphylaxis and Baker's asthma can be potentially deadly, and therefore require emergency medical treatment (Cabanillas, 2020; Cianferoni et al., 2013; Jin et al., 2019; Quirce et al., 2016; Ricci et al., 2019). As the scope of our study was to determine the impact of food processing on IgE antibody-mediated adverse reactions to wheat, we excluded the first four conditions from our analysis.

Wheat allergies are generally mediated by IgE antibodies produced against wheat proteins. Despite possessing high nutritional value and high palatability, the allergenic proteins present in wheat can trigger serious allergic reactions in genetically susceptible consumers (Carter & Frischmeyer-Guerrero, 2018; Cianferoni, 2016). Wheat contains 10%–18% total protein on a dry weight basis. Based on their solubility, the proteins are classified into four families as follows: albumins (water/saline-soluble, 10%–12% of the total protein), globulins (saline-soluble, 5%–8% of the total protein), gliadins (alcohol-soluble, 30%–40% of the total protein), and glutenins (acid-soluble, 45%–50% of the total protein; Jin et al., 2019). The nongluten proteins (albumins and globulins) are the structural and metabolic proteins. They serve as nutrient reserves for germinating the embryo and protecting it from insects and pathogens before germination (Dupont & Altenbach, 2003). The gluten proteins (gliadins and glutenins, also known as prolamins) are the seed storage proteins. Gliadins are monomeric proteins that interact through hydrogen bonds and contain mostly intramolecular disulfide bonds. Glutenins are polymeric proteins linked by inter- and intramolecular disulfide bonds. Gliadins may be cross-linked to the glutenin network through intermolecular disulfide bonds as well.

All four families of wheat proteins together contain at least 107 allergenic proteins (www.Allergome.org). All the three wheat genomes (A, B, and D) encode for the wheat allergens. Recently chromosomal locations of 41 allergen encoding genes have been mapped, and IgE epitopes are well characterized for many, but not all of these allergens (Juhász et al., 2018). Major wheat allergens that are well characterized include: ω -1, 2, 5 gliadin, $\alpha/\beta/\gamma$ -gliadins, the high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS), β -amylase, α -amylase/trypsin/subtilisin inhibitor proteins, lipid transfer protein, chitinase, glyceraldehyde-3 phosphate dehydrogenase, triosephosphate isomerase, peroxidase, glutathione S-transferase, globulin-3, serpins, and α -purothionin (Cianferoni, 2016; Juhász et al., 2018). For detailed information on wheat allergens, and their IgE epitopes, readers are referred to these excellent articles (Denery-Papini et al., 2011; Matsuo et al., 2015; Monaco et al., 2021; Pahr et al., 2013).

TABLE 1 Commonly consumed wheat food products are either thermally processed or fermented plus thermally processed

Wheat food product	Processing method	
	Fermentation	Thermal processing
Cookies	<i>Saccharomyces cerevisiae</i>	Baking (177°C, 8–12 min)
Crackers	<i>Saccharomyces cerevisiae</i>	Baking (232°C, 12–15 min)
Pizza	<i>Saccharomyces cerevisiae</i> ; <i>Lactobacillus sanfranciscensis</i>	Baking (232°C, 15–20 min)
Bread	<i>Saccharomyces cerevisiae</i>	Baking (232°C, 20–25 min)
Sourdough bread	<i>Saccharomyces cerevisiae</i> ; <i>Saccharomyces delbrueckii</i>	Baking (218°C, 60 min)
	<i>Lactobacillus brevis</i> ; <i>Lactobacillus amylophilus</i>	Baking (218°C, 60 min)
Cornbread	No	Baking (203°C, 20–34 min)
Breakfast cereal	No	Baking and extrusion
Naan	<i>Saccharomyces cerevisiae</i>	Baking (260°C, 4–5 min)
Pretzel	<i>Saccharomyces cerevisiae</i>	Baking (218°C, 10 min)
Graham cracker	No	Baking (180°C, 10–12 min)
Pie crust	No	Baking (177°C, 45–50 min)
Waffle	No	Baking (360°C, 2–3 min)
Cake	No	Baking (175°C, 30–40 min)
Cake doughnut	No	Baking (218°C, 12–14 min)
	No	Frying (190°C, 3 min)
Yeast doughnut	<i>Saccharomyces cerevisiae</i>	Baking (180°C, 13 min)
<i>Saccharomyces cerevisiae</i>	Frying (191°C, 2 min)	
Roti/chapati	No	Frying (8–9 min)
French toast	No	Frying (4–6 min)
Flour tortilla	No	Frying (1–1.5 min)
Beer	<i>Saccharomyces cerevisiae</i>	Pasteurization (98°C)
Pasta	No	Extrusion (51°C)
	No	Boiling (100°C, 10 min)
	No	Drying (12%–13% moisture)
Soy sauce	<i>Aspergillus oryzae/sojae</i> ; <i>Pediococcus halophilus</i> ; <i>Zygosaccharomyces rouxii</i>	Roasted (150°C, 30–45 s)

In general, the inherent factors affecting the allergenic potential of food proteins include the protein's structure, stability to gut digestion process, and the glycosylation patterns (Huby et al., 2000). Wheat foods are typically consumed only after processing as a means of preservation and making foods edible (Mills & Mackie, 2008). Most food processing procedures are able to modify the structure of wheat proteins or introduce new structures, which may affect the way proteins are released, broken down during digestion and presented to the immune system (Chizoba Ekezie et al., 2018; Huby et al., 2000; Jiménez-Saiz et al., 2015; Jin et al., 2019; Lepski & Brockmeyer, 2013; Mills et al., 2009). Therefore, deciphering how food processing may affect wheat allergenicity requires elucidation of changes to the structure and properties of wheat proteins at the molecular level. Hence, the impact of food processing on allergenic properties of wheat is a primary challenge that must be addressed to enable the possibility of creating novel hypo-/nonallergenic wheat products.

The common types of wheat processing include fermentation followed by thermal treatment, thermal treatment with or without pressure, and enzyme or acid hydrolysis (Tables 1–5; EFSA, 2014; Mills & Mackie, 2008; Thomas et al., 2007). Hydrolyzed wheat protein (HWP) and hydrolyzed gluten protein are produced at industrial scales for use as ingredients in skin and healthcare products (Table 2; Tranquet et al., 2020).

An up-to-date comprehensive review on the effects of processing on wheat allergenicity is unavailable. There were three objectives for this study: (1) to critically evaluate the evidence on the effect of fermentation, thermal processing, and enzyme or acid hydrolysis on wheat allergenicity so as to identify the potential for and challenges of using these methods to produce hypo-/nonallergenic wheat products; (2) to identify the molecular effects of food processing needed to create such products; and (3) to map the concept questions for future research and development to produce hypo-/nonallergenic wheat products.

TABLE 2 Both humans and animals are exposed to wheat allergens via skincare and cosmetic products: A summary of commercial products containing wheat proteins

Product	Wheat content	Type of product	Examples
Human	Hydrolyzed wheat protein	Shampoo	The Naked Object, Rituals. TRESemmé, Garnier
		Nail polish	Esika
		Primer	IT Cosmetics
		Eyeliner-mascara	N/A
		Moisturizer	Glow Recipe, Marcelle
		Soap	Jolly Jabón, Canmepris
		Hand sanitizer	Sally Hansen, Essential Organics, Kosho
		Hand/nail care	Botaneco Garden, Kypwell Essentials
		Exfoliating towelettes	Alba Botanica
		Hair gel/mask/wax	TREsemmé, Briogeo, Watson's
		Face/neck care	Erin's Faces, Lancome Paris, Omved Therapies
		Eye cream	Lancome Paris, Revlon, Erin's Faces
		Hair conditioner	Miracle 9, L'Oreal Paris
		Antiseptic	Tea Tree Therapy
		Baby diaper rash cream	Mom & World
Veterinary	Wheat germ/bran extract	Lipstick	Estee Lauder
	Hydrolyzed wheat gluten	Shower gel	Korres Natural World
	Wheat protein extract	Conditioner	Tiger Brands
		Hair oil	Conatural
		Shampoo and conditioner	Buddy Wash
	Hydrolyzed wheat protein	Shampoo	Furminator
		Conditioner	TropiClean

To accomplish these objectives, we conducted a literature search without date limits using the PubMed and Google Scholar databases. Various combinations of keywords (IgE, wheat, processing, allergy, allergenicity, immunogenicity, antigenicity, food allergy, anaphylaxis, adverse reaction, and hypersensitivity) were used. Articles in English were retrieved and analyzed to produce summary tables. As scope of this work was limited to only IgE-mediated diseases caused by wheat, articles on non-IgE mediated wheat disorders (celiac disease, non-celiac gluten/wheat sensitivity, eosinophilic enterocolitis, and food protein-induced enterocolitis) were excluded. Our findings and interpretations are presented.

2 | IMPACT OF FOOD PROCESSING ON WHEAT ALLERGENICITY: EVIDENCE FROM THE LITERATURE

Relative to the research reports on the effects of food processing on peanut allergens, the processing effects on

wheat allergens have been modestly studied (Blanc et al., 2011; Meng et al., 2019; Zhang et al., 2019). Wheat food products are made using two general types of processing methods to create commonly consumed foods (Table 1): (1) products made by microbial fermentation followed by thermal processing, and (2) products made by thermal processing with or without pressure and with or without drying (boiling, baking, extrusion, and frying). Wheat is also used as an industrial ingredient in a number of skin health and cosmetic products (Table 2). These products contain HWP or HWG that are produced by hydrolyzing the wheat protein using acids or enzymes (Tranquet et al., 2020). Furthermore, upon consumption, wheat proteins undergo enzymatic digestion in the gut that also can influence their allergenicity. Therefore, wheat allergenicity testing involves not only the effect of wheat processing methods, but also the susceptibility of processed products to enzymatic digestion, to simulate gut digestion as discussed below.

TABLE 3 Effect of fermentation on wheat allergenicity and immunogenicity

Authors	Wheat variety	Method of fermentation	Method of allergenicity testing	Results	Comments
Di Cagno et al. (2002)	Unspecified	Fermentation using LAB: (<i>Lactobacillus alimentarius</i> 15 M, <i>Lactobacillus brevis</i> 14G, <i>Lactobacillus sanfranciscensis</i> 7A, and <i>Lactobacillus hilgardii</i> 51B) at dosage of 5×10^7 CFU/g of wheat flour	Sourdoughs were used for extracting proteins (albumin, globulins, gliadins, and glutenins); 2D electrophoresis; and agglutination test	LAB-fermented dough showed reduced quantity of albumins/globulins (almost all hydrolyzed by $\geq 50\%$) and to some extent of gliadins; glutenins were not affected; it also reduced agglutination activity of gliadins	Allergen quantity was reduced; gliadin toxicity was reduced; however, IgE reactivity was not investigated
De Angelis et al. (2007)	Type O wheat flour (<i>Triticum aestivum</i> , AABBDD), variety Svevo, cultivated in the south of Italy	Doughs were fermented with <i>Saccharomyces cerevisiae</i> with or without VSL#3 treatment (<i>Streptococcus thermophilus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgarius</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium longum</i> , and <i>Bifidobacterium infantis</i>)	Sourdoughs were used for extracting albumins, globulins, and gliadins, and proteins tested for allergenicity by SDS-PAGE, 2D electrophoresis, and IgE western blotting	Yeast fermentation caused minor reductions in gliadins, albumins, and globulins (14 kDa reduced mostly; small reductions in 45 to 97 kDa); VSL#3 caused marked loss of IgE reactivity of gliadins, albumins, and globulins	Hypoallergenic gliadins, albumins, and globulins may be produced using this method; glutenins were not studied
Leszczyńska et al. (2009)	Wheat flour (Type 500, genome unspecified) from “Kruszzynek” mill	Wheat flour was fermented using LAB from six homofermentative (<i>Lactobacillus plantarum</i>) and five heterofermentative (three <i>Lactobacillus brevis</i> and two <i>Lactobacillus sanfranciscensis</i>) strains with <i>Saccharomyces cerevisiae</i> ; 2×10^7 CFU/g of dough	Gliadins extracted from fermented dough were tested for immunogenicity in an indirect IgG ELISA	Fermentation using LAB strains mixed with yeast in a 1:1 ratio showed the highest capacity to reduce the IgG reactivity of wheat flour	Allergenicity of gliadins was not investigated

(Continues)

TABLE 3 (Continued)

Authors	Wheat variety	Method of fermentation	Method of allergenicity testing	Results	Comments
Stefańska et al. (2016)	Commercial wheat flour of the 550 types (genome unspecified)	Wheat flour was fermented using LAB: CM4 (<i>Lactobacillus curvatus</i> 750(13), <i>Pediococcus acidilactici</i> EKO26, <i>Pediococcus pentosaceus</i> 1850 (3) and <i>Lactobacillus coryniformis</i> pA), CM5 (<i>Lactobacillus coryniformis</i> pA, <i>Weissella cibaria</i> EKO31, <i>Pediococcus pentosaceus</i> EKO23, <i>Lactobacillus plantarum</i> KKP 593/p), and CM10 (<i>Lactobacillus helveticus</i> Lh10, <i>Lactobacillus plantarum</i> W37/54, <i>Pediococcus pentosaceus</i> 1850(3))	Albumins/globulins, gliadins, and glutenins were extracted from dough and tested for allergenicity by IgE western blotting	Reduced allergenicity of albumins, globulins (13 proteins: 22–103 kDa), and gliadins (seven proteins: 24–39 kDa)	This method reduced allergenicity of albumins, globulins, and gliadins; glutenins were not studied
Kobayashi et al. (2004)	Unspecified	Japanese soy sauce fermentation protocol (Higashimaru Shoyu Co. Ltd.) using wheat, soybean, mold, lactic acid bacteria, and yeast	Pooled sera from five wheat-allergic children as IgE source; western blot, ELISA, inhibition ELISA	Progressive and total destruction of gluten and nongluten allergens to undetectable levels	Fermentation also reduced allergenicity of soybean (Magishi et al., 2017); authors suggested that soy sauce may be tolerated by wheat and soy-allergic patients

Abbreviations: CFU, colony forming units; 2D, two-dimensional; LAB, lactic acid bacteria; VSL#3, an experimental preparation of LAB cocktail; W, Weissella.

TABLE 4 Effect of thermal processing on wheat allergenicity

Authors	Wheat variety	Processing conditions	Method of allergenicity testing	Results	Comments
Simonato et al. (2001)	Common wheat flour (AABBDD) used to prepare round bread loaves in a bakery	Baking at 220°C for 30 min	HCl-extracted proteins from unheated dough and baked bread crumb and crust were examined using SDS-PAGE and IgE western blot	In dough: HMW-, S-poor, and S-rich prolamins (gliadins and glutenins), α -amylase inhibitors, and 16 kDa allergen were IgE reactive. In bread crumb: lower amount of all above allergens; 16 kDa allergen disappeared In bread crust: new IgE-reactive large protein aggregates formed; 16 kDa allergen was not detectable	Dough contained albumins, globulins (including α -amylase inhibitors and 16 kDa allergen), and prolamins (gliadins and glutenins) allergens; baking reduced all these allergens in the crumb; baking created new large allergens in the crust
Scibilia et al. (2006)	50% durum (AABB) and 50% tender (genome unspecified)	Cooked wheat (conditions unspecified)	Meals containing raw or cooked wheat were used for double-blind placebo-controlled food challenge testing	Cooked and raw wheat products exhibited no significant differences in allergenicity upon oral challenge.	Cooking did not change oral allergenicity of wheat; processing conditions (e.g., time and temperature) of these meals were not specified.
Pastorello et al. (2007)	50% of common (genome AABBDD) and 50% durum (AABB)	Boiled wheat flour in water and cooled immediately	Albumins, globulins, gliadins, and glutenins extracted from raw and cooked wheat flour were tested in IgE western blot.	Albumin/globulins (including 12–16 kDa α -amylase/trypsin inhibitor), gliadin and glutenin allergenicity did not change by boiling; 9 kDa LTP lost allergenicity in some cases but not others	Boiling did not change allergenicity of albumins, globulins, gliadins, and glutenins; boiling had inconsistent effect on LTP

(Continues)

TABLE 4 (Continued)

Authors	Wheat variety	Processing conditions	Method of allergenicity testing	Results	Comments
De Zorzi et al. (2007)	Durum wheat (genome AABB)	Pasta processing: 6 g of flour + 1.8 ml water were mixed to form dough, and dried at various temperature: 20°C, 60°C, 85°C, 110°C, and 180°C to reach 10% moisture; Dried samples were then cooked in salted boiling water for 10 min.	Acetic acid-soluble proteins were extracted from dried and boiled MPSs, and subjected to SDS-PAGE, IgE western blot and dot blot	In 20°C–110°C pasta: HMW-GS, LMW-GS, and $\alpha/\beta/\gamma$ -gliadins retained their IgE reactivity while LMW-albumins were abolished In 180°C pasta: HMW protein aggregates formed	Pasta drying at lower temperatures did not change allergenicity of globulins, gliadins, and glutenins; abolished allergenicity of LMW-albumins; high-temperature drying created large-sized allergens
De Gregorio et al. (2009)	Unspecified commercial products; wheat bran and flour: <i>Triticum aestivum</i> , cultivar Astral (AABBDD)	Baking (commercial products used; conditions unspecified); pastas were boiled for 10 min for dried and 2 min for fresh ones	SSWPs extracted from French bread, whole meal bread, white tin loaf bread, whole meal tin loaf bread, toasted bread, whole meal toast bread, white pasta, whole meal pasta, fresh pasta, biscuit, pizza, baby cereal food, breakfast cereals, wheat flour and bran and raw/cooked pasta were tested for allergenicity using IgE western blot, ELISA, and skin prick test	Breakfast cereals did not provide appreciable IgE reactivity; raw flour and bran, biscuit and nonboiled pasta showed 5–10 major allergens from 5 to 100 kDa; boiled pastas showed few allergens (2–5), 20–50 kDa; much lower allergens were observed in pizza, baby cereal food, and breakfast cereal; two main allergens: 20 and 32 kDa in breads; no allergens found in toasted breads	SSWPs from commercial breads and cooked pastas exhibited 50% lower IgE reactivities compared to raw flour; uncooked pastas and biscuits had considerable allergenicity; gliadins and glutenins were not studied
Lupi et al. (2019)	<i>Triticum aestivum</i> , cultivar Recital (AABBDD)	Boiling: 8 mg of purified gliadins with 4 ml of water, heated at 100°C for 20 min	Total gliadins and α -gliadins were extracted, purified by RP-HPLC, and tested using IgE dot-blot and RBL-SX38 cell degranulation	IgE reactivity of total and α -gliadins were abolished; boiled gliadins did not degranulate RBL-SX38 cells	Boiling abolished allergenicity of purified gliadins

Abbreviations: HMW-GS, high molecular weight glutenin subunit; LMW, low molecular weight; LTP, lipid transfer protein; MPS, model pasta sample; RBL-SX38, rat basophilic leukemia cell line; RP-HPLC, reversed-phase high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

TABLE 5 Effect of enzymatic hydrolysis on wheat allergenicity

Authors	Wheat variety	Enzymes	Method of allergenicity testing	Results	Comments
Simonato et al. (2001)	Common wheat flour (AABBDD) used to prepare round bread loaves in a bakery	Pepsin and pancreatin	Bread digested with 0.2 N HCl + pepsin followed by pancreatin; supernatant tested; SDS-PAGE and IgE western blot	In dough: digestion abolished HMW prolamins and LMW allergens including α -amylase inhibitors; except 16 kDa unknown allergen In bread crumb: digestions abolished HMW prolamins; LMW glutenins were resistant In bread crust: HMW prolamins/LMW glutenins formed large aggregates that were resistant; 16 kDa allergen was abolished	Prolamins in dough were more susceptible to digestion; however, allergens in bread crumb and crust were relatively resistant due to formation of large aggregates that retained allergenicity; α -amylase inhibitors were destroyed
(Yamamoto et al., 2004; and Tanabe et al. 2008)	Commercial soft flour (genome unspecified) used to prepare hypoallergenic cupcake	Actinase-treated flour used in making hypoallergenic cupcake	IgE western blot, ELISA, and oral provocation test in nine atopic dermatitis patients allergic to wheat	Degraded IgE epitope (Gln-Gln-Pro) on glutenin implicated in atopic dermatitis; seven out of nine patients did not react to eating cupcake made from hypoallergenic wheat flour	Authors also demonstrated that other enzymes (bromelain, cellulase, and collagenase) reduced allergenicity of wheat glutenin epitope as well (Tanabe et al., 1996; Watanabe et al., 2000). However, only actinase-treated wheat product was tested in humans
De Angelis et al. (2007)	Type O wheat flour (<i>Triticum aestivum</i> , AABBDD), variety Svevo, cultivated in south Italy	Pepsin and pancreatin	Bread digested with enzymes, albumins, globulins, and gliadins were tested; SDS-PAGE, 2D electrophoresis, and IgE western blotting	In Baker's yeast bread: digestion with pepsin and pancreatin reduced IgE-binding capacity of proteins in the range from 31 to 45 kDa (including α - and ω -prolamins); IgE-binding proteins at 14, 60, and 97 kDa were not affected; further hydrolysis with VSL#3 abolished all remaining allergens mentioned above In VSL#3 bread: Digestion eliminated almost all IgE-binding proteins	Digestion of Baker's yeast bread reduced allergenicity of albumins, globulins, and gliadins; digestion of VSL#3 bread almost completely abolished allergenicity of all three proteins; glutenins were not studied
De Zorzi et al. (2007)	Durum wheat (AABB)	Pepsin and pancreatin	Digestion of MPSS; acetic acid-soluble proteins extracted and tested; SDS-PAGE, IgE western blot, and dot blot	MPSS dried at 20°C–110°C and digested: no detectable IgE-reactive gliadins, glutenins, and albumins/globulins MPSS dried at 180°C and digested: high molecular weight protein Maillard-type protein aggregates and one 42 kDa protein retained allergenicity after digestion	Low-temperature pasta allergens were destroyed by digestion; high-temperature pasta allergens were resistant to digestion; therefore, hypoallergenic pastas can be made using lower drying temperatures; higher drying-temperature pastas were hyperallergenic

(Continues)

TABLE 5 (Continued)

Authors	Wheat variety	Enzymes	Method of allergenicity testing	Results	Comments
De Gregorio et al. (2009)	Unspecified commercial products; wheat bran and flour: <i>Triticum aestivum</i> , cultivar Astral (AABBDD)	Pepsin, trypsin, and chymotrypsin	Digestion of commercial bread and wheat flour; SSWPs extracted and tested for allergenicity; SDS-PAGE and IgE inhibition ELISA	Digestion significantly reduced the IgE-binding capacity of SSWPs from bread (1.6-fold) and raw flour (threefold)	Effect of digestion on allergenicity of gluteins was not investigated; digestion of commercial breads did not abolish but reduced allergenicity significantly
Li et al. (2016)	Commercial wheat flour (genome unspecified)	Papain, flavorzyme, trypsin, α -chymotrypsin, pepsin, and alcalase	Wheat flour was hydrolyzed; SE-HPLC purified gliadins were tested for IgE reactivity using competitive inhibition ELISA	Nondigestive proteases were more effective than digestive proteases in inhibiting IgE-binding capacity of gliadins; pepsin showed the least ability in reducing IgE reactivity; alcalase and papain were the most effective in reducing IgE binding	Sequential use of alcalase–papain treatment yielded the lowest content of gliadin and lowest allergenicity; other protein fractions were not investigated
Lupi et al. (2019)	<i>Triticum aestivum</i> , cultivar Recital (genome AABBDD)	Pepsin	HPLC-purified gliadins were boiled and digested with pepsin; allergenicity was tested using IgE dot blot and RBL-SX38 cell degranulation	Boiling abolished IgE binding; pepsin digestion of boiled gliadin induced a few new IgE epitopes, which were unable to induce degranulation	Pepsin digestion of boiled gliadins created new nonfunctional IgE epitopes

Abbreviations: HMW, high molecular weight; LMW, low molecular weight; MPS, model pasta sample; SSWPs, salt-soluble wheat proteins; VSL#3, an experimental preparation of lactic acid bacteria cocktail.

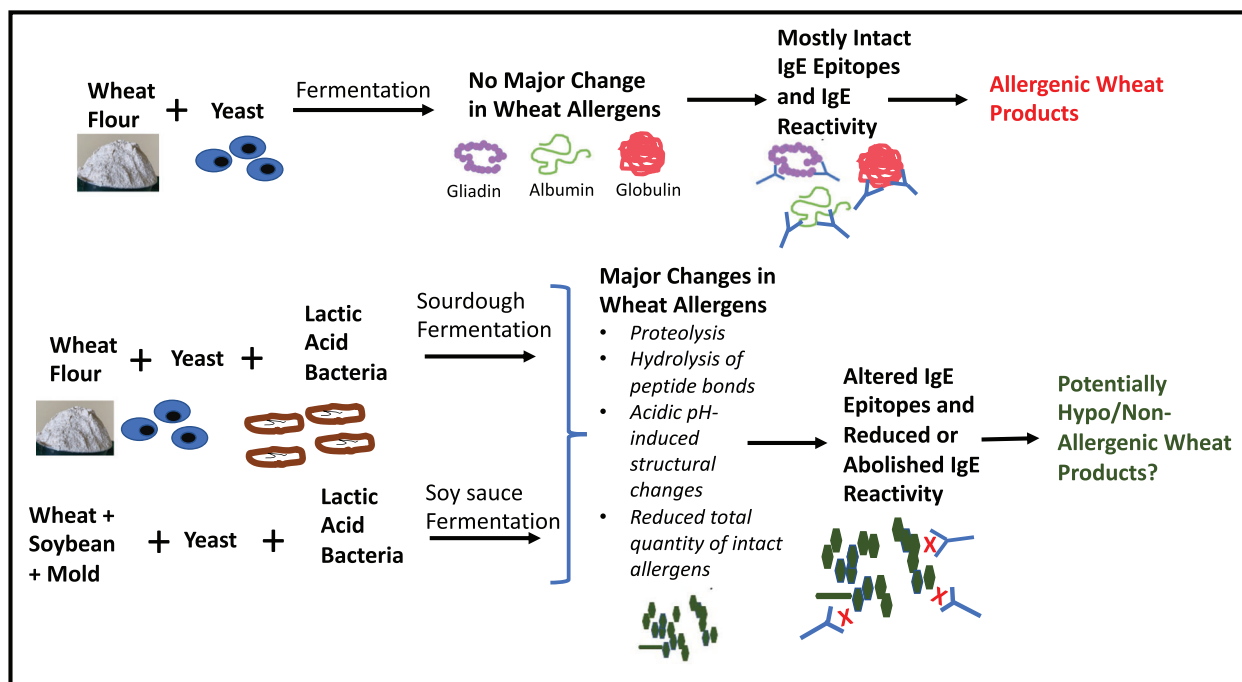


FIGURE 1 Impact of fermentation on allergenicity of wheat

Note: Top of panel: Gliadin allergens present in wheat flour upon traditional fermentation with Baker's yeast (*Saccharomyces cerevisiae*) do not show major changes in their allergenic structures. Consequently, IgE epitopes remain largely intact, and react with IgE antibodies from wheat-allergic subjects. Baking and gastric digestion can reduce their allergenicity to some extent (see Tables 5 and 6). However, yeast fermented–baked wheat products in general retain substantial allergenicity in vitro and in vivo and therefore must be avoided by wheat-allergic subjects. Bottom of panels: Wheat flour upon sourdough fermentation with a cocktail of selected strains of lactic acid bacteria show major loss of IgE-binding epitopes on albumins, globulins, and gliadins. Baking and simulated gastric digestion further reduce the allergenicity of such products to almost negligible levels in vitro (see Tables 5 and 6). Similarly, during Japanese soy sauce fermentation using microbes (mold plus yeast plus lactic acid bacteria), IgE epitopes on both gluten and nongluten allergens are completely destroyed to undetectable levels. Therefore, such products may be potentially hypoallergenic, which remains to be established by preclinical and clinical testing (see details in the text).

2.1 | Wheat fermentation can reduce or even eliminate wheat's allergenicity

Effects of conventional bread fermentation with yeast, sourdough bread fermentation with lactic acid bacteria (LAB), and soy sauce fermentation using mold, yeast, and LAB on the wheat allergenicity have been reported as discussed below (Table 3; Figure 1).

Di Cagno et al. (2002) were the first to investigate the effect of fermentation on wheat immunogenicity. They determined the effects of sourdough fermentation using selected LAB strains. Fifty-five strains of LAB were pre-screened for their proteolytic activity using a digest of albumin and globulin as substrates. The best proteolytic strains were then used in wheat fermentation. Protein fractions (albumins, globulins, gliadins, and glutenins) were extracted from fermented doughs and subjected to two-dimensional electrophoresis to evaluate proteolysis. Results suggested that albumins, globulins, and gliadins were degraded by 50%, whereas glutenins remained unaf-

ected. Furthermore, gliadins exhibited significantly lower toxicity as measured by an agglutination test. This study showed for the first time that the selected LAB can reduce the total quantity of immunogenic and allergenic proteins. The strength of this study was that they were the first to report the effects of sourdough fermentation on the integrity and toxicity of wheat proteins including allergens. However, they neither directly tested the effect of fermentation on IgE antibody reactivity in vitro nor conducted preclinical/clinical testing of their products.

De Angelis et al. (2007) investigated the effects of conventional yeast fermentation versus LAB fermentation on the allergenicity of wheat. They used a cocktail of nine LAB (*Streptococcus thermophilus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum*, and *Bifidobacterium infantis*), noted here as VSL#3, for sourdough fermentation. They found that yeast fermentation caused a minor reduction in the IgE reactivity of gliadins, albumins, and globulins in the

dough compared to the wheat flour. In contrast, fermentation with the VSL#3 caused marked loss of IgE reactivity of albumins, globulins, and gliadins. Further, the allergenicity of the bread prepared from this sourdough was also reduced and subsequent gut enzyme digestion almost completely abolished the allergenicity of the sourdough bread as discussed in later sections (Table 5). These results were highly encouraging. A major strength of this study is that they provided the first evidence of potential hypoallergenicity of LAB fermented sourdough bread. Limitations of this study were that they did not test the effect of LAB fermentation on allergenicity of glutenins and they also did not conduct any in vivo testing of their product in animal models or in wheat allergic humans.

Leszczyńska et al. (2009) tested the immunogenicity of sourdoughs prepared from yeast plus LAB fermentation. Wheat flour was fermented with six homofermentative (*L. plantarum*) and five heterofermentative (three *Lactobacillus brevis* and two *Lactobacillus sanfranciscensis*) strains along with the yeast (*Saccharomyces cerevisiae*). They used human anti-gliadin antibody (serum) and conducted an indirect IgG ELISA to test the immunoreactivity of gliadins extracted from the fermented sourdough. Lowest immunoreactivity of gliadins was observed when wheat flour was fermented using mixed LAB strains plus yeast at the ratio of 1:1, showing that there was cooperation between the two microbes in reducing the immunogenicity of gliadins. A major strength of this study is that it showed that the combination of yeast plus LAB is better than using LAB alone to reduce the immunogenicity of gliadins. Limitations of their study are that they neither tested the IgE antibody reactivity nor disease elicitation by these products in wheat allergic patients (Table 3).

Stefańska et al. (2016) studied the effect of LAB fermentation on wheat allergenicity. Commercial wheat flour was fermented using the following mixed strains of LAB that had shown the highest proteolytic activities in a prior experiment: CM4 [*Lactobacillus curvatus* 750(13), *Pediococcus acidilactici* EKO26, *Pediococcus pentosaceus* 1850(3) and *Lactobacillus coryniformis* pA], CM5 [*L. coryniformis* pA, *Weissella cibaria* EKO31, *P. pentosaceus* EKO23, *L. plantarum* KKP 593/p], and CM10 [*Lactobacillus helveticus* Lh10, *L. plantarum* W37/54, *P. pentosaceus* 1850(3)]. Albumins, globulins, and gliadins were extracted from sourdough, and tested for their IgE reactivity using a western blot method. They found that the LAB fermentation significantly reduced the IgE reactivity of albumins, globulins as well as gliadins. However, because LAB did not hydrolyze the glutenins and therefore, they did not study its IgE reactivity. A major strength of this study is that they further confirmed the previous report (De Angelis et al., 2007) that LAB fermentation does reduce the allergenicity of wheat, and extended the concept by using different types of LAB.

Two limitations of their study are that they did not present any data on the allergenicity of glutenins, and they did not test the allergenicity of this product in vivo in animal models or in wheat allergic humans.

Soy sauce is a very popular oriental product now consumed worldwide. It has been used as a traditional seasoning in Japan and other East Asian countries for centuries. In general, it is produced using soybean and wheat as food ingredients (at the 1:1 ratio), and mold, yeast, and LAB as microbes for fermentation process (Table 1). Kobayashi et al. (2004) elegantly studied the fate of salt-insoluble (gluten) and salt-soluble (nongluten) wheat allergens during the various steps involved in the production of a Japanese soy sauce (Higashimaru Shoyu Co. Ltd. Tatsuno, Hyogo, Japan). They used pooled serum from five children (who had anaphylactic reactions to wheat) as source of anti-wheat IgE antibodies (with levels of 83 to >100 U/ml) in western blot, direct ELISA, and inhibitory ELISA to track wheat allergens. The wheat allergic children had IgE antibodies against multiple allergens in both salt-soluble and salt-insoluble wheat protein fractions. They found that during the *koji* stage (mold cultivation and enzyme production), salt-insoluble (gluten) allergens became salt-soluble and during the *moromi* stage (fermentation with yeast and LAB), all salt-soluble allergens were completely degraded by microbial proteolytic enzymes. They then screened 10 commercial soy sauce products of six different kinds (Koikuchi, raw-koikuchi, usukuchi, raw-usukuchi, raw-saishikomi, and shiro) for wheat allergens, and found none. Based on these findings, they concluded that “no wheat allergen is contained in the soy sauce” products they studied suggesting that these products might be hypo-/nonallergenic (Table 3). It is unclear at present whether or not wheat allergic humans can tolerate these products without allergic reactions as there are no preclinical/clinical studies testing them in animal models or in wheat allergic humans. Nevertheless, this study raises the possibility that soy sauce produced by other countries/companies may also be free from detectable wheat allergens; this remains to be evaluated. These findings suggest that potentially hypo-/nonallergenic wheat products, such as this one, may be created using the combination of mold, yeast, and LAB in fermentation.

In summary, these studies together show that: (1) the conventional yeast fermentation of wheat flour does not markedly reduce its allergenicity; (2) LAB fermentation of wheat flour can reduce wheat allergenicity; (3) LABs differ in their ability to reduce wheat allergenicity, and therefore, large-scale screening of LAB strains would be necessary to identify the most suitable ones for producing hypo-/nonallergenic wheat proteins and products; (4) the combination of yeast plus LAB offers an improved approach to create hypoallergenic wheat bread products although

allergenicity must be directly tested yet; and (5) Japanese soy sauce may be nonallergenic as it contains no detectable gluten and nongluten wheat allergens (Figure 1; Table 3). It is noteworthy that although these approaches show potential, *in vivo* validation of such fermented wheat products for hypo-/nonallergenicity remains to be done using animal models for preclinical testing and finally clinical testing in wheat allergic humans.

2.2 | Thermal processing of wheat flour can modulate its allergenicity

Primary goals of thermal processing of wheat food products are to make them edible, safe, and shelf stable. However, it can also affect the wheat allergenicity as discussed below (Table 4).

Simonato et al. (2001) were the first to study the effect of thermal processing (i.e., baking at 220°C for 30 min) on wheat allergenicity. They compared the allergenicity of acid-soluble proteins extracted from raw wheat dough versus bread (crumb and crust). They used serum from food allergic subjects, who showed reactions upon consuming wheat products, as a source of IgE antibodies and conducted IgE western blot studies. They found that compared to the raw dough proteins, prolamins in breadcrumb had reduced IgE reactivity. Furthermore, IgE reactivity of α -amylase inhibitor—a major allergen implicated in Baker's asthma, was completely lost upon baking. However, they found that large-sized highly reactive mega allergens were present in the bread crust that were not found in the raw dough or the baked crumb. This suggests that direct exposure of the surface of the bread to high temperature during baking causes wheat allergens to form complex Maillard-like aggregates that form highly IgE-reactive mega allergens made of mostly gluten proteins. Strength of this study is that for the first time it was demonstrated that baking reduces allergenicity of the bread crumb but increases allergenicity of the crust. These data suggest that avoiding eating bread crusts may be a simple way of reducing the risk of exposure to such mega allergens. A major limitation of this study is that they did not conduct *in vivo* allergenicity testing of their breadcrumbs versus crusts in animal models or in humans.

Scibilia et al. (2006) were the pioneers in testing the effect of thermal processing on wheat allergenicity *in vivo* by conducting a double-blind placebo-controlled food challenge study. They compared the allergenicity of cooked (conditions unspecified) versus raw wheat meals with identical contents (wheat flour, water, cocoa, sugar, and lemon aroma syrup). Eleven adult participants were selected based on their clinical reactions upon oral challenge with raw wheat, positive skin prick test (SPT) results

and IgE reactivity to commercial wheat extracts. They found that both cooked wheat meal as well as raw wheat meal elicited food allergic reactions similarly in all subjects. A major strength of this study is that this is the one of the only two reports in the literature in which oral testing was conducted to evaluate the effect of processing on wheat allergenicity. Unfortunately, specific cooking conditions and the variety of wheats used were not reported. Moreover, due to microbial contamination issues, consuming raw wheat or nonheat-treated wheat is no longer recommended. Furthermore, limited sample size ($n = 11$) and unknown cooking conditions, prevent us from making generalizable conclusions on the effect of cooking on *in vivo* wheat allergenicity from this study.

Pastorello et al. (2007) tested the *in vitro* allergenicity of cooked (boiled) versus raw wheat flours. They used serum from wheat-allergic subjects and conducted an IgE western blot study on the albumins, globulins, gliadins and glutenins that had been individually extracted from the boiled versus raw wheat flours. They found similar IgE antibody reactivities of all four families of wheat allergens extracted from raw versus boiled wheat flour. A nonspecific lipid transfer protein (nsLTP, 9 kDa) was the only exception because IgE from some wheat-allergic subjects did not react with cooked nsLTP. Thus, they showed that wheat allergens in general are resistant to change during the boiling conditions used in cooking wheat flour. A major strength of this study is that this is the first comprehensive study to demonstrate the thermostability of all four families of wheat allergens to boiling conditions *in vitro*. Unfortunately, in this study they did not test the *in vivo* allergenicity of their boiled wheat product in wheat allergic subjects. Furthermore, as they had used a mixture of wheat varieties in their product, interpretations of observed effects to specific wheat variety are not possible (Table 4).

De Zorzi et al. (2007) were the pioneers testing the allergenicity of experimental model pasta samples (MPSS). They prepared various MPSS from wheat dough, which were dried at different temperatures (20°C, 60°C, 85°C, 110°C, or 180°C to reach 10% moisture content). The dried MPSS were boiled (10 min) and then used in allergenicity testing (Table 4). Acetic acid-soluble proteins were extracted and used in testing IgE reactivity by immunoblotting and dot blotting methods. They reported that the MPSS prepared at lower drying temperatures (20°C–110°C) showed loss of IgE reactivity of LMW albumins; however, LMW-GS and gliadins retained their IgE reactivities. In contrast, MPSS dried at 180°C exhibited HMW IgE-reacting protein aggregates suggesting the formation of mega allergens. A major strength of their study is that they controlled all the processing conditions so that accurate interpretations about allergenicity could be made. A limitation of

their study is that they did not conduct any in vivo testing of their products in animals or in humans.

De Gregorio et al. (2009) conducted a study to evaluate the impact of commercial processing on allergenicity of a large number of food products (French bread, whole meal bread, white tin loaf bread, whole meal tin loaf bread, toasted bread, whole meal toast bread, white pasta, whole meal pasta, fresh pasta, biscuit, pizza crust, baby cereal food, breakfast cereals, wheat flour, and bran and raw/cooked pasta). Salt-soluble wheat proteins (SSWPs) were extracted from these products and used in testing. Using IgE-positive pooled serum from a group of wheat-allergic subjects, they conducted western blot and ELISA to evaluate the relative IgE reactivity of SSWPs. In addition, they also conducted SPT to evaluate the clinical reactivity of SSWPs. They found that the SSWPs from thermally processed commercial wheat products had a large variation in their allergenicity in vitro as well as in vivo (SPT), thereby providing the direct *proof-of-concept* evidence that the thermal processing does modulate wheat allergenicity (Table 4). Overall, commercial bread and cooked pasta exhibited approximately 50% lower IgE reactivities compared to the respective raw flours. Interestingly, pizza crust, baby cereal, and breakfast cereal were least allergenic. Uncooked pasta, and baked biscuits were as allergenic as the raw flour. The major strength of this study is that they assessed in vitro as well as in vivo (SPT) allergenicity of SSWPs from a large panel of thermally processed wheat food products. It is not possible to deduce effects of specific conditions used in thermal processing on SSPW allergenicity in this study because they used commercial food products to extract SSWPs. The results suggest that some of the least allergenic food products in their study (e.g., breakfast cereals, pizza, etc.) may be tolerated by people who might be allergic only to SSWPs but not to gluteins. However, this remains to be tested in future studies. One limitation of this study is that they tested only the nongluten proteins and did not test gluteins.

Lupi et al. (2019) studied the effect of boiling (100° C) on the allergenicity of purified gliadins. Wheat flour was used to extract the total gliadins, which were then purified to isolate the α -gliadins by reversed-phase high performance liquid chromatography method. The pure proteins were then boiled and used in testing for IgE reactivity. Pooled serum from a group of allergic subjects was used to perform IgE-based dot blotting. In addition, they also tested the ability of boiled proteins to cause degranulation of mast cells in vitro using a cell line assay (RBL-SX38). They found that boiled gliadins completely lost their IgE reactivity as well as their ability to cause mast cell degranulation. Therefore, these data suggest that IgE epitopes on purified gliadins are intrinsically thermolabile and that a simple boiling process may be used to produce a nonal-

lergenic gliadin protein product; however, validating the nonallergenicity in animal models and in humans remains to be done. Furthermore, potential use of such nonallergenic gliadin as a desensitizing allergoid for immunotherapy in wheat allergy also needs to be evaluated. The major strength of this study is that they not only studied the binding of gliadins to the IgE antibodies, but also the functional consequence of this binding as measured by in vitro mast cell degranulation that occurs in vivo during allergic reactions. A limitation of this study is that they did not test the in vivo allergenicity of their presumably nonallergenic boiled gliadin in animal models or in humans.

Overall, the studies discussed above suggest that thermal processing has complex, and different effects on nongluten and gluten wheat allergens as follows (Figure 2): (1) nonglutens lose 50% or more of their allergenicity in commercial foodstuffs (bread, pizza, cereals, boiled pasta, etc.); they also significantly lose their allergenicity in experimental breadcrumbs and in experimental low-temperature dried/boiled pasta; in contrast to nonglutens, gluteins (mostly gliadins were studied) in experimental breadcrumbs or boiled pastas largely retain their allergenicity; (2) interestingly, boiling wheat flour does not significantly change allergenicity of nongluten or gluten proteins; nevertheless, boiling of purified gliadins abolish their allergenicity completely; these results show that IgE epitopes on purified gliadins are intrinsically thermolabile; as boiling of wheat flour does not abolish gliadin allergenicity, it is possible that boiling conditions may promote gliadins to form molecular network with other wheat components (glutenins, nonglutens, carbohydrates) that results in protection of IgE epitopes present on gliadins during the boiling process; (3) pizza crust, baby cereal, and breakfast cereals exhibit least allergenicity of SSWPs—a promising finding toward creating hypoallergenic wheat proteins that might be tolerated by people who are allergic only to SSWPs; (4) in general, very high-temperature treatment (e.g., bread crust, and very high-temperature pasta drying) increases wheat allergenicity due to the formation of highly reactive mega allergens of gluten type; and (5) it may be possible to optimize the thermal processing conditions to create novel hypoallergenic wheat proteins and products; and at the same time, it may also be possible to optimize methods to minimize or prevent inadvertent creation of hyperallergenic mega allergens (Table 4; Figure 2).

2.3 | Enzyme hydrolysis of wheat can reduce or even abolish its allergenicity

Wheat foods, when consumed, undergo gut digestion processes that are expected to influence their allergenicity. Both the de novo allergic immune response to wheat

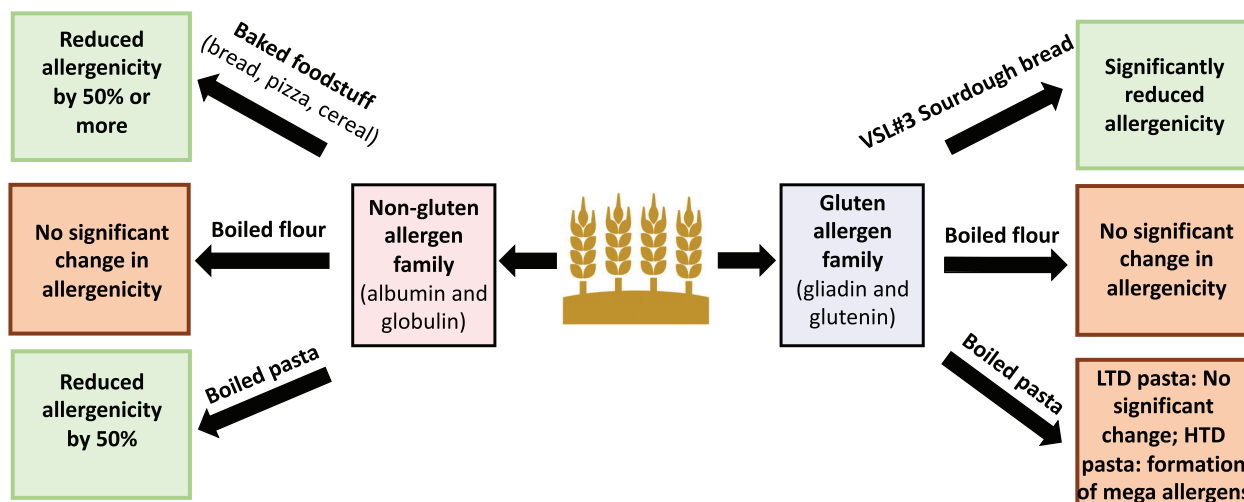


FIGURE 2 Different effects of common thermal processing methods on wheat allergenicity

Note: Left panel: The nongluten allergens (albumins, and globulins) present in baked foodstuffs and boiled pasta lose 50% or more of IgE-binding epitopes compared to those present in the raw wheat flour; in contrast, boiling wheat flour does not markedly reduce the IgE-binding epitopes on nongluten wheat allergens. Right panel: Both gliadins and glutenins retain most of their IgE-binding epitopes in the boiled pasta; gliadins lose significant amount of IgE epitopes in the VSL#3 sourdough bread (glutenins were not studied); boiling wheat flour does not markedly reduce IgE epitopes on gliadins or glutenins. Abbreviations: HTD pasta, high-temperature dried pasta; LTD pasta, low-temperature dried pasta.

allergens as well as elicitation of allergic reaction in pre-sensitized subjects depends on the availability of adequate amounts of allergenic peptides that would have survived the gut digestion. Consequently, allergenicity of wheat food is a function of its ability to resist gut enzyme-mediated hydrolysis. Therefore, it is critical to evaluate the effects of gut enzymes on the stability of wheat allergens. In addition, nondigestive enzymes have also been used not only for industrial production of HWP and HWG for use as an ingredient in food and skin care products (e.g., papain), but also, in preparing experimental hypoallergenic wheat food products (e.g., bromelain, actinase; Tanabe, 2008; Tanabe et al., 1996; Tranquet et al., 2020; Watanabe et al., 1994; Yamamoto et al., 2004). The papain enzyme (derived from papaya fruit) is used as a food additive and bromelain (an enzyme extract derived from the papaya stem, juice, and fruit) is used as a food supplement. Therefore, treatment with these nongut enzymes is a feasible way of developing hypoallergenic wheat flour. Here, we have reviewed the effects of gut digestive enzymes and nongut enzymes on wheat allergenicity (Table 5).

Simonato et al. (2001) studied the hydrolytic effects of pepsin and pancreatin on the allergenicity of wheat dough, breadcrumb, and bread crust. They found that almost all IgE-reactive protein allergens, with the exception of a 16 kDa allergen, disappeared after the enzyme digestion of dough. Gut enzyme digestion abolished HMW-prolamins (gliadin and glutenin) and nonglutens but

not LMW-glutenins in the baked breadcrumb. Therefore, due to gut digestion, most breadcrumb derived allergens may significantly lose their ability to sensitize and to elicit allergic reactions. Interestingly, large-sized HMW-prolamins/LMW-glutenins that were formed in the breadcrumb upon baking, were resistant to gut enzyme digestion. LMW nonglutens were completely hydrolyzed by digestion. These findings suggest that consuming breadcrumb, and avoiding bread crusts, may be a simple way to reduce the relative risk of exposure to wheat allergens. The major strength of this study is that they tested all four families of wheat allergens. A limitation of the study is that they did not test their products for in vivo allergenicity in animal models or in wheat allergy patients.

De Angelis et al. (2007) compared the effect of pepsin and pancreatin digestion on allergenicity of conventional yeast bread versus yeast plus VSL#3 fermented sourdough bread (prepared with a cocktail of selected LAB as described earlier). Albumins, globulins, and gliadins were extracted from the digested bread for allergenicity testing. They found that the digestion of yeast bread reduced IgE-reactivity of 31 to 45 kDa prolamins. It did not affect 14, 60, and 97 kDa proteins. However, more strikingly, digestion of VSL#3 sourdough bread almost completely abolished IgE reactivity of albumins, globulins, and gliadins. Glutenins were not studied. Therefore, these findings raise the possibility that the VSL#3 sourdough bread may be tolerated by people who are allergic to albumins, globulins,

and gliadins. Thus, a major strength of this study is that they provided a novel method to produce potentially hypo-/nonallergenic sourdough bread. However, *in vivo* testing by preclinical and clinical studies are yet to be performed for validating this potentially hypoallergenic wheat food product (Figure 1).

De Zorzi et al. (2007) tested the effect of pepsin and pancreatin digestion on allergenicity of experimental MPSs dried at different temperatures (20° C–110° C and 180° C). They studied IgE-reactivity of albumins, globulins, gliadins, and glutenins using the western blot and dot blot methods. They found that digestion of pasta made from lower drying temperature abolished IgE-reactivity of all four wheat allergen families. Thus, lower drying-temperature pasta almost completely lose their allergenicity upon digestion. This suggests that all wheat allergens present in such pasta may be unable to elicit sensitization and allergic reactions because all IgE epitopes are expected to be totally digested in the gut. However, pasta made at high drying-temperature exhibited large-sized Maillard-type protein aggregates and one 42 kDa protein that were each highly IgE reactive. Therefore, it may be prudent to avoid high temperature for pasta drying to prevent the creation of mega allergens that resist gut enzyme digestion. The major strength of this study is that they demonstrated loss of allergenicity of all four families of wheat allergens upon simulated gut digestion. A limitation of this study is that the *in vivo* allergenicity was not tested.

De Gregorio et al. (2009) studied the effects of pepsin and trypsin enzyme hydrolysis on IgE reactivity of SSWPs from commercial bread and raw flour. They found that enzymatic hydrolysis reduced the IgE binding of SSWP from raw flour by threefold and reduced the IgE binding of SSWP from bread by 1.6-fold. Gliadins and glutenins were not studied. These results suggest that such commercial products may have lower sensitization and disease elicitation capacities and may be tolerated by patients who might be allergic only to nongluten (albumins and globulins) allergens. Notably, in contrast, to Simonato et al. (2001), these authors did not separate the effects on allergenicity of breadcrumbs versus breadcrusts. Limitations of the study are that the conditions of commercial bread making were not specified, and the *in vivo* allergenicity was also not tested.

Li et al. (2016) used six enzymes (alcalase, α -chymotrypsin, flavorzyme, pepsin, and trypsin) to hydrolyze wheat flour to investigate their proteolytic effects on the allergenic potential of gliadins present in hydrolyzed wheat flour. Gliadins were extracted from hydrolyzed wheat flours, purified by size-exclusion high performance liquid chromatography, and were examined for their allergenicity in an IgE competitive inhibition ELISA. They reported that sequential use of alcalase

and papain significantly reduced the gliadin content of hydrolyzed wheat flour, and the IgE reactivity of its gliadin. Similar results of reduced allergenicity of gliadins from alcalase- and papain-treated flours were reported in a Balb/c mouse model study (Xue et al., 2019). Therefore, two different lines of evidence (human IgE reactivity and mouse model IgE responses) suggest that potentially hypoallergenic gliadins can be produced using alcalase and papain hydrolysis. Such products may have lower sensitization and disease elicitation potencies, which of course, must be confirmed by clinical studies.

Lupi et al. (2019) studied the effect of pepsin digestion on allergenicity of purified gliadins from wheat flour. They found that IgE reactivity of gliadin was reduced after pepsin digestion. However, when gliadins were heated to 100°C for 20 min, and then digested with pepsin, IgE epitopes reappeared; as these epitopes were not involved in mast cell degranulation, they were therefore considered as nonfunctional. These results suggest that simple boiling of purified gliadins may be an inexpensive method to create hypoallergenic gliadins. Whether such gliadins will be unable to cause sensitization and elicitation of allergic reactions is unknown. Once validated, such gliadins might be used in food products as hypoallergenic proteins. The major strength of this study is that they tested IgE binding as well as mast cell degranulation *in vitro*. A limitation of the study is that they did not test the *in vivo* allergenicity.

A group of Japanese scientists reported several studies on producing potentially hypoallergenic wheat flour using nongut enzymes as follows. Watanabe et al. (1994) reported that treatment of hard wheat flour (Eagle brand, Nihon Flour Milling Co.) and soft wheat flour (Heart brand) with actinase, and collagenase results in hypoallergenic flours with reduced *in vitro* IgE antibody reactivities of both salt-soluble (nongluten) and salt-insoluble (gluten) proteins; soft wheat product showed better results than the hard wheat flour. Tanabe et al. (1996) found that bromelain-treated soft wheat flour lost IgE reactivity of a major epitope on glutenin (Gln-Gln-Gln-Pro-Pro) implicated in atopic dermatitis among wheat allergic children. Using this flour, they prepared a hypoallergenic bread that resembled English muffins. Subsequently, Yamamoto et al. (2004) prepared a hypoallergenic cupcake using actinase-treated wheat flour that had lost all IgE reactivity *in vitro* and tested its safety and efficacy in a group of atopic dermatitis children ($n = 9$) who had wheat allergy. They reported that upon oral provocation with the hypoallergenic cupcake, only two of nine patients showed clinical reactions (eruption, urticaria, and/or wheezing). Furthermore, more than half of the patients who tolerated this cupcake were later able to ingest normal wheat products without reactions suggesting desensitization and induction of immune tolerance to wheat allergens (Tanabe,

2008). Although these studies are exciting, sample size was limited ($n = 9$) in this study. Whether this product may be tolerated by adult wheat allergic patients is also unknown. Furthermore, this study was based on inactivating a major IgE epitope on glutenin involved in atopic dermatitis in this group of children. Therefore, it is unclear whether or not this product may be tolerated by patients allergic to nonglutenin family of wheat allergens. Overall, this study supports the idea of creating hypoallergenic wheat products for wheat allergic patients using nongut enzymes.

In summary, available published studies on *in vitro* enzyme digestion show that: (1) enzymes present in the digestive tract have the capacity to abolish the allergenicity of nongluten wheat allergens present in conventional yeast breadcrumb and the VSL#3 sourdough bread; further, whereas gliadin allergenicity in yeast bread is slightly reduced and that of the VSL#3 sourdough bread is completely abolished; however, glutenins in both types of breads appear to resist gut enzyme digestion; (2) gut enzymes can completely abolish the nongluten allergens and gluten allergens present in the boiled pastas that had been prepared at low drying temperatures; however, mega allergens (mostly gliadins and glutenins) created at high-temperature processing in yeast breadcrumb or in high-temperature dried pastas resist gut enzyme digestion; (3) nondigestive enzymes (alcalase, papain, bromelain, actinase) have the capacity to significantly reduce or eliminate the allergenicity of purified gliadins, as well as, that of gluteins (gliadins and glutenins) present in wheat flour; (4) most importantly, hypoallergenic cupcakes made from actinase-treated wheat flour was tolerated by the majority of children (seven of nine) with atopic dermatitis associated with wheat allergy; subsequently, these children were able to eat regular wheat products without clinical reactions suggesting that hypoallergenic cupcake was able to cause desensitization; and (5) further, preclinical and larger clinical studies are needed to validate such hypoallergenic wheat products produced by enzyme treatment of wheat flour (Table 5).

2.4 | Industrial processing can be used to produce hypo-/nonallergenic hydrolyzed wheat products

Hydrolyzed wheat products such as HWP and HWG are produced by industrial processing of wheat protein with acid, alkali, enzymes, or steam (Tranquet et al., 2020). These products are used as functional ingredients in food and cosmetics because they can provide emulsifying and foaming attributes (Gabler & Scherf, 2020). Their use in cosmetics include adding to skin and hair-conditioning products in a large number of personal care products and

in some veterinary skin and hair products (Table 2). These HWP and HWG are mixtures of amino acids and peptides of varying lengths. There is a wide variation in the distributions of the sizes of peptides and polypeptides in such products depending upon the company that produce them (Gabler & Scherf, 2020). However, in general, molecular weights of the hydrolyzed proteins range from 0.5 to 30 kDa (Burnett et al., 2018; Gabler & Scherf, 2020).

If the HWP and HWG products contain peptide sizes larger than 3.5 kDa, then they pose an allergenicity risk in humans via both skin and mucosal (eye, oral, respiratory) routes of exposures. In one specific case of a Japanese facial soap, HWP made from partial hydrolysis of gluten with hydrogen chloride (95°C for 40 min) had been used. This product contained large sized proteins (40–50 kDa). Unfortunately, this product caused anaphylactic sensitization to wheat in a large number (>1000) of Japanese people (Chinuki & Morita, 2012; Chinuki et al., 2013; Fukutomi et al., 2011; Nakamura et al., 2013; Noguchi et al., 2019; Tranquet et al., 2017; Yokooji et al., 2013). An expert committee that reviewed the allergenicity of HWP and HWG concluded that these products are safe only when they are formulated to minimize peptide lengths greater than 30 amino acids (~3.3 kDa; Burnett et al., 2018). Furthermore, current advisory is that the cosmetic products containing HWP and HWG should not be used on damaged skin or in products that may come in contact with the mucosal surfaces such as eyes and airways. Thus, it is possible to produce industrially processed hypo-/nonallergenic wheat proteins for use in cosmetics.

3 | THE EFFECT OF PROCESSING ON WHEAT ALLERGENICITY: MOLECULAR MECHANISMS

Wheat allergenicity depends on two phases of immune responses to wheat protein allergens that occur in sequence: Phase 1: exposure of nonallergic, but genetically prone, healthy subjects to wheat allergens results in the production of wheat allergen-specific IgE antibodies most of which remain in the body bound to the receptor present on mast cells and basophils; this process is termed sensitization; and Phase 2: re-exposure of presensitized subjects to wheat allergens results in physicochemical binding of the cell-bound IgE antibodies to the allergens that initiates the disease; this process is known as allergic reaction elicitation. Without Phase 1, disease elicitation is not possible (Jin et al., 2019; Renz et al., 2018).

Food processing can modify the structure of wheat allergens. Therefore, it can potentially affect the capacity of altered allergens to initiate sensitization (i.e., Phase 1) as well as the disease elicitation (i.e., Phase 2). It is

noteworthy that all the reports reviewed in this paper focused entirely on the Phase 2 of the process (i.e., interaction of IgE antibody with processed wheat proteins *in vitro* in all studies, and mast cell degranulation and clinical reactions in three studies). Therefore, the impact of processing on the Phase 1 of wheat allergenicity is largely unknown at present. Accordingly, the mechanisms discussed here refer only to the Phase 2 of the wheat allergenicity process.

Wheat proteins can function as allergens because of their epitope structures that bind to IgE antibodies through a complementary three-dimensional matching of molecular shapes (Battais et al., 2005; Battais et al., 2008). These IgE epitopes are of two general types: conformational and linear. The quaternary structures including the intra/interchain disulfide bonds and carbohydrates present on the proteins contribute to the integrity of conformational epitope structure. The linear epitopes are determined primarily by the sequence of the amino acids in the polypeptide chain. Consequently, if these epitope structures can be abolished or altered by food processing, then one would expect corresponding changes in IgE antibody-binding capacity of wheat allergens, thereby influencing Phase 2 of the wheat allergenicity process.

It is estimated that a functional protein allergen possesses at least two epitopes of 15 amino acids each in length that can bind to one IgE antibody molecule. Such epitopes can be either linear or conformational in nature (Huby et al., 2000). When a person has at least two or more wheat specific IgE molecules bound on the mast cells and basophils in the body, that person is deemed sensitized to the wheat allergen. Subsequently, allergen binding and cross-linking of at least two IgE molecules present on mast cells and basophils is required to activate them to cause release of vasoactive chemicals like histamine to trigger an allergic reaction (also known as type I hypersensitivity reaction). Therefore, the primary requirement for a wheat product to function as hypoallergenic, it must contain markedly fewer IgE-binding epitopes compared to the conventional wheat product. If all the IgE-binding epitopes are abolished, then the resulting product may be considered nonallergenic. However, both types of products must be confirmed for hypo-/nonallergenicity by preclinical and clinical testing. These definitions are operationally used in all the studies we have reviewed in this article.

Existing evidence shows that processing conditions can affect the wheat allergen and IgE-binding interactions as measured by not only *in vitro* methods, such as western/dot blot, ELISA, and mast cell degranulation assays, but also *in vivo* methods, such as skin prick testing (in one study) and oral allergen challenge (in two studies; Verhoeckx et al., 2015). Combined effects of thermal processing plus gut enzyme digestion must be considered to deduce the molecular effects on wheat allergenicity. Ther-

mal processing methods (baking and pasta drying and boiling) alone appear to reduce the IgE-binding ability of most nongluten wheat allergens, suggesting significant destruction of relevant IgE epitope structures (Table 4; Figure 2; Cabanillas & Novak, 2019). Gut enzyme digestion completely abolishes the IgE epitopes on nongluten allergens in bread and boiled pasta. Therefore, almost all IgE epitopes present on nonglutens in baked foodstuffs and in boiled pasta will be expected to be nonfunctional in the gut when such wheat products are consumed. In contrast, with the exception of breadcrumb, IgE epitopes on gluten allergens generally resist thermal processing such as baking and pasta boiling (Figure 2). However, most IgE epitopes on gluten allergens present in VSL#3 sourdough bread and in boiled pasta are easily destroyed by gut enzyme digestion. Therefore, most IgE epitope structures on gluten in such products are expected to be nonfunctional in the gut when they are consumed. These molecular mechanisms suggest that *in vivo* testing of these products is warranted to confirm the potential clinical benefits of such dramatic molecular changes in wheat allergens induced by the combined effects of thermal processing and gut enzyme digestion.

Boiling of wheat flour, in general, does not induce major changes to IgE epitopes present on gluten or nongluten wheat allergens (Figure 2). In contrast, in the wheat pasta matrix, almost 50% of IgE epitopes on nongluten allergens are destroyed by boiling but, almost all IgE epitopes on gluten allergens resist boiling. Nevertheless, boiling purified gluten (gliadins) results in complete loss of their IgE epitope structures suggesting intrinsic thermolability of IgE epitopes present on purified gliadins. These observations, together suggest that IgE epitopes of both gluten and nongluten allergens, when present in the wheat flour matrix, resist boiling possibly due to their protection conferred by multi-molecular networks formed among gliadins, glutenins, albumins, globulins, and polysaccharides during the boiling process. Similarly, in the pasta matrix also, same mechanism may protect most IgE epitopes on gluten allergens and 50% of IgE epitopes on nongluten allergens.

Enzymes produced during fermentation reactions can be used to destroy IgE epitopes by hydrolyzing larger proteins into small peptides. Such enzymatic hydrolysis of gluten commonly occurs in the production of fermented foods, in which the acid produced during microbial metabolism activates enzymes that degrade the protein; the net effect on allergenicity would depend on the enzyme specificity and the degree of hydrolysis (Thiele et al., 2004; Wang et al., 2007).

Sourdough fermentation of wheat flour, as opposed to the traditional yeast fermentation, appears to markedly reduce wheat allergenicity (Figure 1; Table 3). It is

noteworthy that, the yeast fermentation of wheat flour does not significantly change most of the IgE epitopes structures on glutens or nonglutens (De Angelis et al., 2007); however, use of LABs alone or along with the yeast during the fermentation process appears to consistently reduce the density of IgE epitopes. Furthermore, Japanese soy sauce fermentation method that uses molds, yeasts, and LAB, progressively destroys all IgE epitopes present on both gluten and nongluten wheat allergens (Kobayashi et al., 2004). These results together demonstrate the extreme vulnerability of the molecular IgE epitope structures of wheat allergens to the effects of microbial fermentation. Therefore, these effects can provide strong molecular basis and rationale on using combination of microbes to reduce or even to eliminate wheat allergenicity.

Several mechanisms are proposed to explain this significant destructive effects of microbial fermentation on wheat allergenicity (Figure 1): (1) enzymatic hydrolysis: acidic pH from the fermentation reaction can activate the proteolytic activity of proteases released by LABs; these enzymes can then break the large allergen molecules into smaller sized proteins, polypeptides, oligopeptides and amino acids, causing progressive changes to and loss of the linear as well as conformational epitopes that are required for IgE binding; (2) nonenzymatic hydrolysis under acidic pH conditions can denature the epitopes by breakage of disulfide bonds and disruption of noncovalent interactions among amino acids. In those cases, where there is complete loss of allergenicity, the mechanism may be due to complete loss of epitope structure present on large-sized protein products of >3.5 kDa, or complete degradation of the protein to peptides less than 3.5 kDa that are incapable of binding to the IgE antibodies (Akiyama et al., 2006; Chahal, 2014).

Evidence in the literature shows that nongut enzymes, such as bromelain, papain, alcalase, collagenase, and actinase, also can reduce or eliminate wheat allergenicity (Table 1). The mechanism includes proteolysis and breakdown of larger protein molecules into smaller sized peptides and amino acids with consequent loss in total quantity of allergenic proteins associated with loss of both linear and conformational IgE epitopes. Dramatic effects of bromelain and actinase in destroying most IgE epitope structure is illustrated by the resulting wheat flour testing negative for any detectable allergenicity in *in vitro* assays (Tanabe, 2008; Yamamoto et al., 2004). Furthermore, actinase-treated hypoallergenic wheat flour was used to produce cupcake that was tolerated by most wheat allergic children. It also induced clinical desensitization enabling patients to eat common wheat products without problems a year later. This suggests that immune tolerance-inducing nonallergenic wheat proteins can be produced by treating wheat flour with the actinase enzyme.

The mechanism of reduction in allergenicity of industrially produced HWP and HWG involves the hydrolytic breakdown of large-sized wheat allergens into products containing peptides less than 3.5 kDa that simply lack the ability to bind to IgE antibodies. Therefore, industrial production of nonallergenic HWP and HWG is possible when the products contain protein sizes of less than 3.5 kDa (Burrnett et al., 2018). It is noteworthy that partial hydrolysis of wheat proteins using acids does not reduce allergenicity. It is thought to be due to the re-organization of IgE epitopes by the interchange of disulfide bonds, entanglements of chains or due to formation of noncovalent bonds. Such protein products are soluble, contain lots of IgE epitopes and therefore, can trigger serious allergic reactions (Bouchez-Mahiout et al., 2010). Therefore, inadvertent production of such epitopes must be avoided.

4 | CREATING HYPO-/NONALLERGENIC WHEAT PRODUCTS USING FOOD PROCESSING METHODS: POTENTIAL OPPORTUNITIES, AND CHALLENGES

The primary objective of this study was to evaluate the published literature on the effect of food and industrial processing of wheat so as to establish the evidence-based potential for creating novel hypo-/nonallergenic wheat products. Other objectives included identification of the knowledge-gaps and challenges preventing advancements, to inform the future research agenda.

A professionally developed and widely accepted definition of hypo-/nonallergenicity for wheat products does not exist at present. The *in vitro* studies reviewed here used reduced binding or loss of binding to IgE antibodies obtained from wheat allergic subjects as operational definitions in their experiments. The three *in vivo* studies in humans used the frequency and severity of clinical reactions (one study, skin reaction to intradermal injection; two studies, oral reactions) as evidence for hypoallergenicity. The US FDA does not have an approved definition for “hypoallergenicity” (US FDA, 2020). Nevertheless, for successful development of hypoallergenic infant milk products, pediatric clinical scientists, along with the infant food industry professionals have developed clear guidelines for preclinical and clinical testing of candidate hypo-/nonallergenic products, that includes the following definition: “These tests should, at a minimum, ensure with 95% confidence that 90% of infants with documented cow’s milk allergy will not react with defined symptoms to the product under double-blind, placebo-controlled condition” (Baker et al., 2000; Høst & Halken, 2004). We suggest that a similar professional guideline and definition

TABLE 6 Three genetically distinct genotypes of wheat are commonly used in food products and/or animal feed

Genome	Common name/s	Species ^a	Common uses
AA	Wild einkorn	<i>Triticum boeoticum</i>	Cookies, brownies, cakes, bread, pizza, rolls, soup, cereals
AABB	Emmer	<i>Triticum dicoccon</i>	Soup, beer, animal feed
	Durum	<i>Triticum durum</i>	Pizza, pasta, bread, semolina, upma, couscous, pudding
	Oriental wheat	<i>Triticum turanicum</i>	Bulgar, pasta, cereals, bread, waffles, beer,
	Persian wheat	<i>Triticum carthlicum</i>	Bread
	Rivet wheat	<i>Triticum turgidum</i>	Feed
AABBDD	Common wheat	<i>Triticum aestivum</i>	Bread, cookies, crackers, cereals, and so on.
	Spelt	<i>Triticum spelta</i>	Pasta, crackers, bread, cereals, risotto, cookies, stews
	Clubed wheat	<i>Triticum compactum</i>	Cake, crackers, cookies, pastries

^aTaxonomy based on Integrated Taxonomic Information System (ITIS, 2021).

of hypoallergenicity be established for developing hypo-/nonallergenic wheat products also.

Genetically, the modern wheats are derived from three genomes—A, B, and D (Juhász et al., 2018). Commonly consumed wheats are either diploid (AA; e.g., Einkorn), tetraploid (AABB; e.g., durum), or hexaploid (AABBDD, e.g., bread wheat; Table 6; Appels et al., 2018; Juhász et al., 2018; Shewry, 2018). There are many species within hexaploid and tetraploid wheat, and there are hundreds and thousands of varieties and accessories within each species. As allergenic protein content and their structure are genetically encoded, one can expect variation in the allergenicity of wheat at the ploidy level, at the species level as well at the variety/accession levels (Gao et al., 2019; Nakamura et al., 2005). However, not every study we reviewed specifies the wheat variety used in testing. Furthermore, commercial flours most likely contain mixture of several species/varieties/accessions. Therefore, it is critical that future studies on wheat allergenicity consider using standardized material and document genetic details on the wheat used so as to advance the ultimate goal of consistently creating hypo-/nonallergenic wheat products.

Studies show that the wheat proteins (both gluten and nongluten) present in wheat flour exhibit high levels of intrinsic allergenicity in vitro as well as in vivo in humans. Wheat flour is not consumed as such but is subjected to hydration and thermal processing (with or without pressure) or to fermentation followed by thermal processing. Wheat-based food further undergoes gut digestion upon consumption. Not all studies have done this. Therefore, ideally future studies on in vitro assessment of the effect of food processing on wheat allergenicity should involve not only testing the IgE-binding capacity of processed wheat, but also the effect of gut digestion (acidic pH plus gut enzymes) on processed wheat products.

Upon fermentation with Baker's yeast, allergenicity of wheat proteins in dough is not reduced in a significant way. When fermented dough is baked to make conven-

tional bread, major changes in the allergenicity of wheat proteins occur. Allergenicity of albumins and globulins is significantly reduced. Gluten proteins in the breadcrumb also lose a significant degree of allergenicity. However, newer and large-sized gluten allergens are formed within the bread crust (Simonato et al., 2001). Digestion studies have shown that the albumins, globulins, and gliadins, but not glutenins, present in the breadcrumb are susceptible to human digestive pH and enzymes and therefore lose much of their allergenicity during digestion. In contrast, gluten proteins present in the bread crust resist digestion. Furthermore, nongluten proteins extracted from commercial bread (crust and crumb combined) also appear to have reduced allergenicity in skin prick testing. These pieces of evidence together suggest that the conventional breadcrumb may have lower potential to cause sensitization and disease elicitation. Therefore, this needs to be tested in pre-clinical and clinical studies.

Sourdough breads prepared using cocktails of selected strains of LAB (alone or along with yeast) show markedly reduced allergenicity in vitro; the extent of reductions is much more dramatic than the effects seen in conventional bread studies (Figure 1; Tables 3 and 5). Interestingly, all the residual allergens (albumins, globulins, and gliadins) present in such sourdough breads are much more sensitive to gut digestion compared to that of the conventional yeast-based breads (De Angelis et al., 2007). Therefore, such sourdough bread is expected to have dramatically reduced ability to cause sensitization and disease elicitation compared to the conventional bread. Therefore, future preclinical and clinical testing of such product is warranted.

Based on in vitro ELISA testing, researchers have reported that Japanese soy sauce products contain no detectable gluten nor nongluten wheat allergens (Table 1). Therefore, these products may be tolerated by all wheat allergic subjects. Furthermore, these products may also be incapable of causing allergic sensitization. However, preclinical and clinical testing of these products in wheat

allergic patients have not been reported so far. It is important to conduct such studies urgently as the *in vitro* data are very compelling. As these products may vary widely based on the producer, it is also important to evaluate whether other types of soy sauce products (prepared by different methods, other companies, other countries) are also hypo-/nonallergenic.

It is noteworthy that the fermentation studies (except soy sauce studies, that tested both soluble and insoluble wheat allergens) extracted and tested the soluble proteins in IgE binding. Therefore, the question remaining to be clarified is whether or not the insoluble proteins present, the ones not extractable by the methods used, also show reduced allergenicity. This can be tested by conducting oral feeding of the food slurries (without extracting any specific proteins) in validated animal models and eventually in humans. Thus, fermentation of wheat using LAB plus yeast (as in sourdough bread), and mold plus LAB plus yeast (as in soy sauce), provide highly promising methods to create potentially hypo-/nonallergenic wheat products.

Several commercial baked wheat products in addition to the bread have been tested for allergenicity of nongluten proteins. Interestingly, reports show that nongluten protein obtained from toasted bread exhibited no allergenicity and the allergenicity of nongluten protein from pizza crust, baby cereal, and breakfast cereal were minimal (Tables 4 and 5; De Gregorio et al., 2009). Although these results are encouraging, a key question would be how much of the nongluten protein was actually extractable from such products because the processing conditions may make the proteins insoluble and nonextractable with saline. Moreover, such nonextractable proteins may still be allergenic or even worse, could be hyperallergenic due to the creation of high temperature-induced mega allergens. Nevertheless, if all the nongluten family of allergens had been indeed destroyed, such products might be tolerated by those who are allergic only to nongluten wheat allergens. Therefore, further *in vivo* testing of these products would be critical to validate the results.

Available evidence shows that commercial raw pasta is intrinsically as allergenic as the raw flour. However, boiled pasta appears to lose up to half of the allergenicity of its extractable nongluten proteins. Furthermore, digestion using gut enzymes almost completely eliminated allergenicity of the albumins, globulins, gliadins and glutenins present in the cooked pasta made using relatively lower drying temperature. Therefore, available evidence suggests that lower temperature drying conditions of pasta must be precisely defined to favor the creation of potentially hypoallergenic products.

There is compelling evidence that treatment of soft wheat flour with nondigestive enzymes (bromelain and actinase) can produce hypoallergenic wheat flour that can

be used to make bread and cupcake products. Furthermore, such a cupcake is not only tolerated well by most children with atopic dermatitis (seven of nine tested), but also subsequently induce immune tolerance in more than half of the patients to enable consumption of regular wheat products. Although this was a small study, it supports a strong rationale to conduct further preclinical and clinical testing of such products in the immediate future.

Boiling of wheat is a very commonly used processing method. Evidence shows that both gluten and nongluten wheat allergens resist degradation upon boiling and retain their allergenicity *in vitro*; however, boiling abolishes allergenicity of purified gliadins *in vitro* (Table 4). In the real world, consumers are typically exposed to gliadin in a food matrix. These studies together suggest that extrapolating data from pure protein studies to the whole foods can be misleading. Therefore, to determine the real effects of food processing on wheat food products, testing of wheat protein allergenicity must be done using the wheat food rather than purified single wheat allergens. Nevertheless, when purified wheat proteins are intended for use as a food ingredient or as an ingredient in cosmetic products, testing the allergenicity of those purified proteins might be appropriate.

5 | FUTURE DIRECTIONS AND SUGGESTED RESEARCH AGENDA

Overall, we found evidence that: (1) in contrast to the effect of food processing on other allergenic foods such as peanuts and tree nuts, effect on wheat allergenicity has been modestly studied (Ortiz et al., 2016; Palladino & Breiteneder, 2018; Zhang et al., 2018); (2) effects of processing have been studied mostly using *in vitro* methods; (3) food processing has significant but variable effects on wheat allergenicity, with reports showing anywhere from a reduction in allergenicity or even abolishment of allergenicity of specific wheat allergen protein families, all the way to increasing allergenicity by creating newer and larger mega allergens; (4) among the processing methods, fermentation and enzyme hydrolysis offer the strongest promise to produce novel hypo-/nonallergenic wheat proteins and products; however preclinical and clinical validations are urgently needed; and (5) several scientific concepts can provide fertile ground for future research and development to create hypo-/nonallergenic wheat proteins for applications in food, medical, and cosmetic industries (Kohno et al., 2016).

This review shows that there is significant potential for creating novel hypo-/nonallergenic wheat products by optimizing specific food processing methods. These findings also inform the following specific scientific concepts

for research and development in this exciting area of food science:

- (1) Establishing professional guidelines for the preclinical and clinical testing of potentially hypoallergenic wheat products. To advance the goal of creating such products for wheat allergic patients, as well as for the general public, precise professional guidelines for preclinical and clinical testing including definitions for hypo-/nonallergenicity, similar to the model developed for hypoallergenic infant milk products, are immediately needed.
- (2) The *in vivo* validation of the effects of processing on wheat allergenicity. Currently, the majority of studies have reported *in vitro* data on allergenicity. Although valuable as a first level screening method, such methods must be followed by preclinical testing (in appropriate animal models) and human clinical trials (Gonipeta et al., 2015; Jin et al., 2019). Previous studies have already identified few potential hypo-/nonallergenic wheat products (e.g., Japanese soy sauce, VSL#3 sourdough bread, cupcakes from actinase-treated wheat flour, etc.). Oral challenge studies can be conducted in both animals and humans to determine the allergenicity of processed wheat foods versus extracted allergens so that a comprehensive picture on the effect of processing can be drawn. For testing allergenicity of processed wheat proteins used in skin care products, appropriate dermal exposure allergenicity studies may be conducted using an adjuvant-free model described recently (Jin et al., 2020).
- (3) Does food processing differently affect the allergenicity of wheat products prepared from different wheat species, varieties, and accessions? Commonly consumed wheats belong to different species, varieties, and accessions that are expected to be different in the allergenic protein contents and their susceptibility to the effects of food processing (Gao et al., 2019; Nakamura, Tanabe, Watanabe, & Makino, 2005). Published studies do not always mention the genetic details of the wheat used. To advance the goal of consistently producing hypoallergenic wheat products, it will be important to relate the effects of food processing at each of these genetic (i.e., species, varieties, and accessions) level (Gao et al., 2019; Nakamura, Tanabe, Watanabe, & Makino, 2005).
- (4) Does processing alter the sensitization capacity of wheat allergens and convert them to immune tolerance-inducing wheat proteins? The immune process of wheat allergy involves the first phase of sensitization (i.e., production of IgE antibodies) to wheat allergens followed by the second phase of IgE binding with consequent clinical reactions upon

re-exposures (Renz et al., 2018). Currently, wheat allergenicity studies have mostly used the IgE-binding methods to assess allergenicity, which relates to second phase. It will be important to determine whether hypo-/nonallergenic wheat products will be inferior in eliciting sensitization in healthy subjects, and whether they will be able to desensitize wheat allergic subjects by inducing immune tolerance as suggested by the hypoallergenic cupcake study (Tanabe, 2008; Yamamoto et al., 2004). Future studies should evaluate the immunotherapy-relevant effects of processing on wheat allergens.

- (5) Determining the effects of additional methods of processing on wheat allergenicity. All reported studies reviewed in this paper examined the effects of the commonly used methods of thermal processing, fermentation, and enzyme/acid hydrolysis on wheat allergenicity. There is ample opportunity to test the effects of several other methods of processing, including extrusion processing, high-pressure processing, plasma treatment, ultraviolet treatment, and so on, in future studies (Boreddy et al., 2019; Kim, 2017).

6 | CONCLUSIONS

There is significant evidence in the literature to suggest that wheat allergenicity can be altered (increased, decreased, or abolished) using common food and industrial processing methods. There is significant potential for creating novel hypo-/nonallergenic wheat products using optimal fermentation and enzyme hydrolysis methods. Almost all of the studies (with the exception of three) have evaluated the allergenicity of processed wheat products using *in vitro* IgE-binding methods. Furthermore, the effect of processing methods on the sensitization capacities of wheat allergens has not been widely studied. Current focus has been largely to study the effects on binding of IgE antibodies from wheat allergic subjects, and in just three cases allergic reaction elicitation. Therefore, future work is required to validate the effects of processing on wheat allergenicity, both sensitization (i.e., *de novo* IgE production) as well as disease elicitation, by conducting preclinical testing using appropriate animal models and finally human clinical trials. These concepts provide fertile grounds for future research and development to create hypo-/nonallergenic wheat proteins for applications in the food, medical, and cosmetic industries. Availability of hypo-/nonallergenic wheat products is expected to increase the consumer-base for the wheat industry that is seriously challenged currently by the ongoing increases in adverse health effects of wheat products on a global scale (Yang & Kulis, 2019). Furthermore, such efforts will be

consistent with the emerging concepts of precision food, nutrition, and health as envisioned by the US National Institutes of Health (NIH, 2021).

ACKNOWLEDGMENTS

This research was funded by the United States Department of Agriculture (USDA)/National Institute of Food and Agriculture (NIFA); Hatch project M1CL02486 (Accession Number: 1012322), Hatch project M1CL01699; Agricultural and Food Research Initiative Competitive Program, Grant Number: 2018-67017-27876; and Project GREEN (Michigan State University). Rick Jorgensen was supported by the John Harvey Kellogg Graduate Assistantship and the Academic Achievement Graduate Assistantship from the Michigan State University. We would like to thank Les Bourquin, Eric Olson, Sarah Comstock, and Dechun Wang for mentoring Haoran Gao and Rick Jorgensen.

AUTHOR CONTRIBUTIONS

Venu Gangur, Haoran Gao, Rick Jorgensen, and Rajsri Raghunath: Conceptualization. Venu Gangur, Haoran Gao, Rajsri Raghunath, Rick Jorgensen, and Siddharth Nagisetty: Methodology; validation; formal analysis; and investigation. Venu Gangur and Perry K. W. Ng: Resource; funding acquisition. Haoran Gao, Venu Gangur, Rajsri Raghunath, Rick Jorgensen, and Siddharth Nagisetty: Writing—original draft preparation. Venu Gangur, Haoran Gao, Rick Jorgensen, Siddharth Nagisetty, and Perry K. W. Ng: Writing—review and editing. Venu Gangur: Supervision and project administration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Venu Gangur  <https://orcid.org/0000-0001-7985-7514>

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How to cite this article: Gao, H., Jorgensen, R., Raghunath, R., Nagisetty, S., Ng, P. K. W., & Gangur, V. (2021). Creating hypo-/nonallergenic wheat products using processing methods: Fact or fiction? *Compr Rev Food Sci Food Saf*, 1–27. <https://doi.org/10.1111/1541-4337.12830>