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WHEAT AND GLUTEN INTOLERANCE

An overview of the latest scientific insights and possible solutions for the bakery sector

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Contents

The purpose of this report

With this report we aim to present the current state of scientific knowledge of intolerance to gluten and wheat, with reference to other grains such as barley, oats, rye and spelt.
A sound knowledge of this matter is essential for three main groups of people with gluten and/or wheat related intolerances:
Individuals with coeliac disease
Individuals with wheat allergies
A fast-growing group of persons with symptoms experienced after eating wheat products, but not suffering from coeliac disease or wheat allergy.

This report describes in a systematic way the backgrounds of these intolerances and suggests potential solutions that can be used to reduce and perhaps even in time, lower the disease/symptoms prevalence in these three groups of consumers.

In addition to this report we propose research that focuses on the health effects of cereals. Find more details on researches in Chapter 6 of this report.

To align this report to the state of art knowledge, as internationally developed within the European consortium "Health Grain", recently followed by the "Health Grain Forum", a group of internationally recognised specialists in the field of cereals and cereal components in relation to intolerances and health collaborated in this effort.

Acknowledgement

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Summary

Wheat has been used for millennia as a raw material for basic foods. This is partly due to its favourable nutritional composition (good supplier of energy from carbohydrates, protein, dietary fibre, vitamins and minerals), as well as to the presence of gluten. The gluten protein is composed of glutenin and gliadin. Glutenin is especially important for the elasticity of the dough (matrix formation), while gliadin ensures the viscosity. The combination of these properties makes the gluten protein unique and very suitable for the production of tasty (leavened) bakery products for daily consumption.

Since the 50s of the last century, it has been known that a small proportion of the population (0.5-2%) (Mustalahti et al., 2010, Reilly and Green, 2012, Rewers, 2005) does not react well to the consumption of gluten containing food products. At a later stage it turned out that this problem was caused by the gliadine fraction of the gluten protein. This disease is known as coeliac disease (gluten intolerance). If people with coeliac disease eat gluten, this affects their intestinal wall. See also Chapter 2. In addition to coeliac disease, there appears also to be a much rarer disease (estimated at less than 1%) in which the immune system reacts allergically to certain protein fractions from wheat. This results in symptoms such as itching, nausea or sneezing, but it can sometimes take more severe forms. The wheat proteins that may cause allergic reactions are diverse and vary from person to person. Chapter 3 discusses this further.

Finally, fairly recently a third group of people has been added (Non Coeliac Wheat Sensitivity/ Non Coeliac Gluten Sensitivity, abbreviated as NCWS/NCGS) who experience symptoms after eating wheat products, but cannot be diagnosed to suffer from wheat allergy or coeliac disease. It is not known how large this group is, and to what ingredients (of wheat) they react. Because this group is growing rapidly and also because the attention to wheat and gluten sensitivity has recently been increasing considerably, it is important for the bakery sector to study this growing group of people with complaints and to understand what mechanisms lay at the core of the problem. With the existing knowledge about the group of people with coeliac disease and wheat allergy and the new knowledge of the latter group, we can search in a more focused manner for possible solutions.
In the literature, various possibilities to modify raw materials and process technologies have been described for enabling the production of products that potentially may give rise to fewer hypersensitivity reactions to wheat (proteins) or other wheat related components. A number of these solutions can be implemented at short, medium or long term and are of interest to the food sector, in particular the bakery, pasta and breakfast cereal segments.

Food (technology) possibilities	Time frame
Use of alternative grains (such as oats, teff, quinoa, buckwheat and others)	Short
Reduction of the vital gluten	Short
The use of alternatives for gluten, e.g. by the use of whey protein from milk, or the use of oat flour	Medium
The use of sourdough fermentation processes	Medium
Selection and modification of specific wheat lines	Long

In chapter five of this report, these possibilities are described in detail.
It is known that for people with coeliac disease or wheat allergy, eating gluten-free and wheat-free products have thus far been the only solution. This may include the use of alternative grains (technology 1, see above). Perhaps the use of alternatives to gluten could also be a solution. There are promising experiments, but little specific application options (technology 2). In the long term it might be possible to develop less immunogenic wheat varieties for both groups (technology 5). For example, modification of the coeliac disease epitopes making them less harmful. For people suffering from wheat allergy this is a bit more complicated, because it varies from one individual to another to which wheat proteins they react.

The third group of people with NCWS/NCGS is the largest growing group about whom the least is still known. For this group it must first be determined to which ingredients in wheat they react. The literature already contains some information about this, particularly in studies with people with IBS (Irritable Bowel Syndrome). On the basis of this, some hypotheses for the NCWS/NCGS group have been developed. The table below lists the most applicable food technology possibilities for each target group. In the group of NCWS/NCGS, it is also specified which aspects should be investigated first before one can consider possible solutions. Nevertheless, the table, based on current hypotheses, describes what possible solutions may exist for people with NCWS/NCGS.

Condition/main group	Solution
Coeliac disease	Short term: alternative grains Medium term: alternatives to gluten Long-term: selection and modification of specific wheat lines
Wheat allergy	Short term: alternative grains Long-term: selection and modification of specific wheat lines
Non Coeliac Wheat/Gluten Sensitivity	Necessary research: 1) Further research into ingredients of wheat to which they react: - Process: effect of short and long fermentation on modification of protein epitopes and FODMaPs - Raw material: what type of grain is low in FODMaPs and/or in other proteins that can bring about hypersensitivity such as ATIs, lectins, LTPs) - Nocebo effect: to what extent is there a nocebo effect in NCWS/NCGS patients? - Gluten load: lower gluten load by eliminating use of vital gluten 2) Determination of the prevalence of the NCWS/NCGS group in the Netherlands Possible solutions to NCWS/NCGS: Short term: alternative grains Short term: Reduction of the vital gluten Medium The use of fermentation processes with sourdough Long-term: selection and modification of wheat lines

1. Introduction

1.1 History and recent developments

Wheat has been used for millennia as a raw material for basic foods. This is partly due to its favourable nutritional composition (good supplier of energy from carbohydrates, protein, dietary fibre, vitamins and minerals), as well as to the presence of gluten. The wheat grain contains, depending on the variety and growing conditions, 8-15% (average 12%) protein, mainly gluten (80%). Most of this is in the endosperm (the core of the kernel). This core also contains albumin and globulin proteins. The gluten protein is composed of glutenin and gliadin. Here glutenin proteins are particularly important for the elasticity of the dough (matrix formation), while gliadin proteins provide the viscosity. The combination of these properties makes the gluten protein unique and very suitable for the production of tasty (leavened) bakery products for daily consumption.

Wheat has a long history of use (Figure 1). At the very start of the domestication of wheat, farmers made their selections primarily based on grain size. Often different types of wheat were grown together in the fields. This resulted in new hybrid types from which, about 10,000 years ago, the current bread wheat variant developed. One of these is the current bread wheat type, which has a higher starch content and was selected by farmers as more favourable type due to better yield. This wheat type started spreading the world about 10.000 yrs. ago, from the triangle Turkey-Jordan-Iran. Approximately 200-500 years ago, with the development of more voluminous bread products, people also began making selections based on baking quality, and thus indirectly on protein content and composition, as well as the ease with which the grain kernel was released from the ear. It was only 250 years ago that proteins were recognized as separate nutrients, and the reliable measurement of the protein content of wheat has only been possible since the beginning of the 20th century. Hard wheat types, generally grown in area's, which are relatively dry, became used mainly for the production of bread and pasta and contain between 12% and 14% protein. Soft wheat types, grown in more humid regions, were specifically selected on the basis of a lower protein content ranging from 7% - 11%. With an increase in the consumption of wheat products and broader food application, there has also been an increase in the intake of isolated wheat gluten and other wheat components (International Grains Council, 2015).

Since the 50s of the last century, it has been known that some people do not react well to foods containing wheat. Later it turned out that this problem was partially caused by gluten protein fraction called gliadin and the disease became known as coeliac disease (gluten intolerance). It is estimated that approximately 0.5-2.0% (some 150,000) of the population in Europe suffers from this condition (Mustalahti et al., 2010, Reilly and Green, 2012, Rewers, 2005). In addition,

it also proved that people could develop a food allergy to wheat. However, this allergy is relatively rare. Recently, a new syndrome (a cluster of various symptoms) has been identified that is quite different from coeliac disease and wheat allergy. This is currently described as 'non-coeliac wheat sensitivity/non coeliac gluten sensitivity (NCWS/NCGS)'. A conservative estimate is that possibly 5-10% of the Western population suffers from this new form of sensitivity (Brouns 2013, Ludvigsson et al., 2013).

Vital gluten is a by-product of the wheat starch and the beer industry. It is increasingly used as a functional additive in numerous food products. Figures on the actual levels over the years, however, are difficult to obtain. Based on different hypotheses, Kasarda (2013) showed a calculation that the intake of vital gluten has tripled since 1977, from 136 to 408 grams per year or 0.37-1.12g/day, per capita of the population. To what extent this amount is influential, however, is doubtful given the many times higher intake of gluten from wheat flour as used in bread (5-5.5 kg/year or 13.7-15.1 gram/day), says Kasarda (2013). In addition, since the beginning of wheat consumption, the ingestion of gluten, with bread, has been many times higher than the amount of gluten that is required to cause coeliac disease. So the question is whether these relatively small shifts, related to the high average intake are relevant for the development of coeliac disease or NCWS/NCGS.

1.2 Wheat and gluten hypersensitivity

Wheat-related disease syndromes have increased considerably since the 70s/80s of the last century. This was studied thoroughly for coeliac disease (Rubio-Tapia et al., 2012). The increase is often explained as being the result of an increased intake of wheat (proteins), but also through major changes in the overall diet, lifestyle, increased hygiene and medical changes (more antibiotics and vaccines) whereby the immunological sensitivity to certain proteins in food (gluten) has increased.

The development of coeliac disease might be related to the food that a child receives early in life. It might be important that intake of small quantities of gluten will start gradually in small quantities between 4 and fore the age of 6 months of age, preferably simultaneously with breastfeeding (Ivarsson et al., 2013). The reason for this would be that the immune modulatory properties of breastfeeding and intestinal flora would contribute to the development and prevention of auto-immune diseases (ESPGHAN, 2009). Patients with coeliac disease do not develop tolerance to gluten, or else they lose this later in life. The effect of breastfeeding and the momentum from which to start additional feeding on this is not clear (Ludvigsson et al., 2012). The most recent advice of the ESPGHAN from 2008 (European Society for Paediatric Gastroenterology, Hepatology and Nutrition) is in line with the information above.

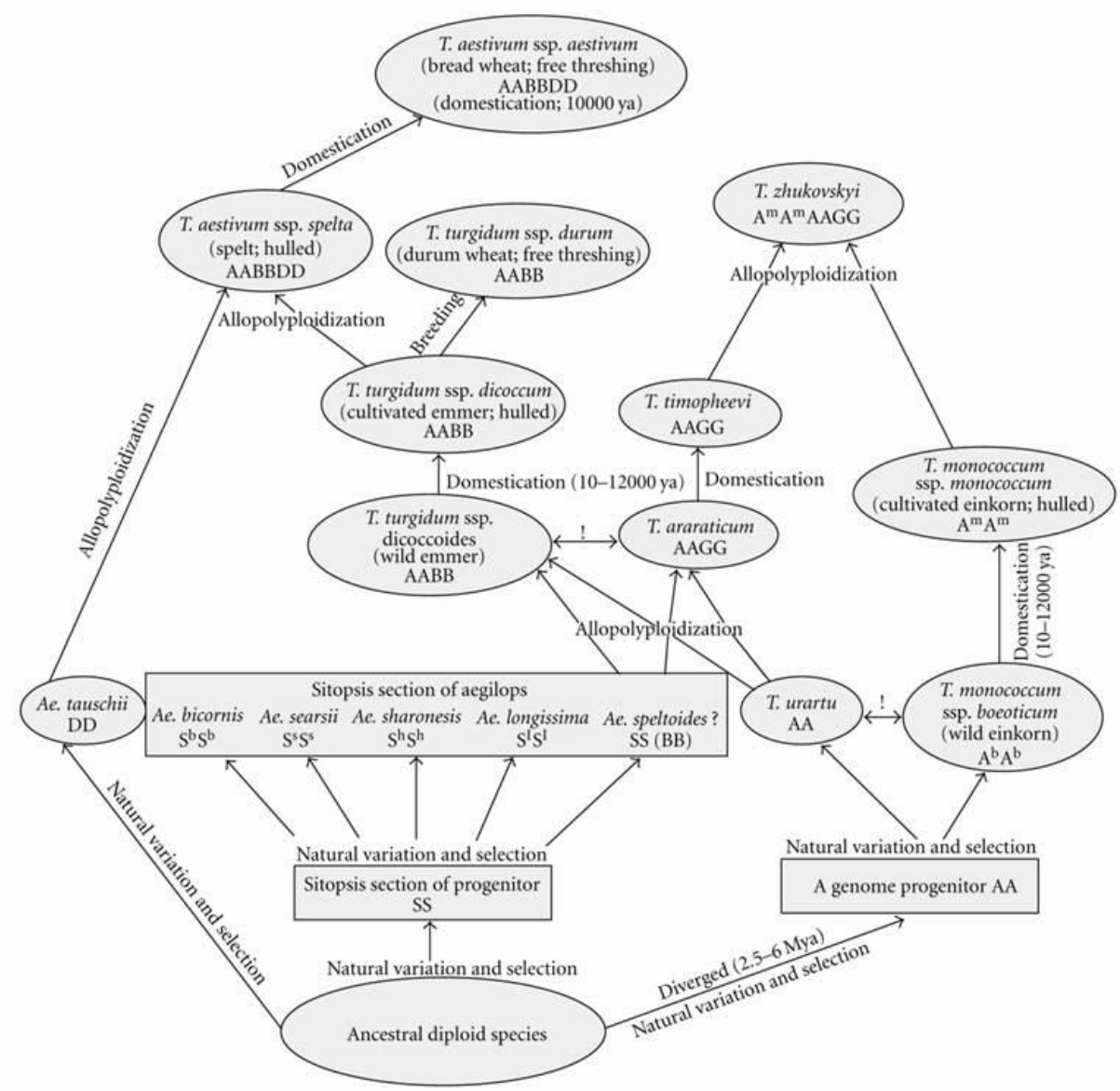

Figure 1. The evolution of wheat varieties (Triticum and Aegilops) (Copied with the authorisation of Gupta et al, 2008).

The growing attention to wheat and gluten hypersensitivity and the trend toward gluten-free products and meals poses a serious challenge for the bakery industry, where wheat and wheat gluten are the basic ingredients. This challenge applies to more than just the development and production of (safe) gluten-free products. There is also a risk of prejudice to the good image of the bakery sector due to increasing negative reports on alleged gluten and wheat effects on the health. Therefore, it is important to obtain a good, clear picture of the scientific basis and the potential scope of wheat-related hypersensitivities. It is also important to obtain a good picture of the more recent processing of wheat and the suggested effects of this on the composition of the ingredients. Is it true that changes have actually occurred in the quantity and the composition of the protein components of wheat? In addition, a good picture is needed of the recent food technology developments with raw materials that are obtained from wheat. It

should be noted that in the last 60 years major innovations have taken place in the field of bakery technology. When we have a more complete overview of the above, optimal, reliable and transparent information can be passed on to bakers and consumers, whose ultimate interests in relation to food and health are paramount.

1.3 Wheat and gluten consumption

The consumption of wheat flour by North Americans shows a decline until 1970 and then an increase between 1970 and 2000 (Figure 2). Since 2000, there has again been a decline in wheat consumption. This might be related to the increase in the number of people with (alleged) NCWS/NCGS. At the same time we see an increase in consumption of gluten-free products appears (Table 1). In many cases, these gluten hypersensitivities also involve the other gluten-containing grains, being barley, rye and spelt. It should be noticed, however, that the consumption of bread wheat or added vital wheat gluten (as added in a wide variety of food products - (also known as 'hidden' ingredients), by far exceeds the relatively small consumption of barley, rye and spelt.

Table 1. Source: http://www.technologijos.lt/

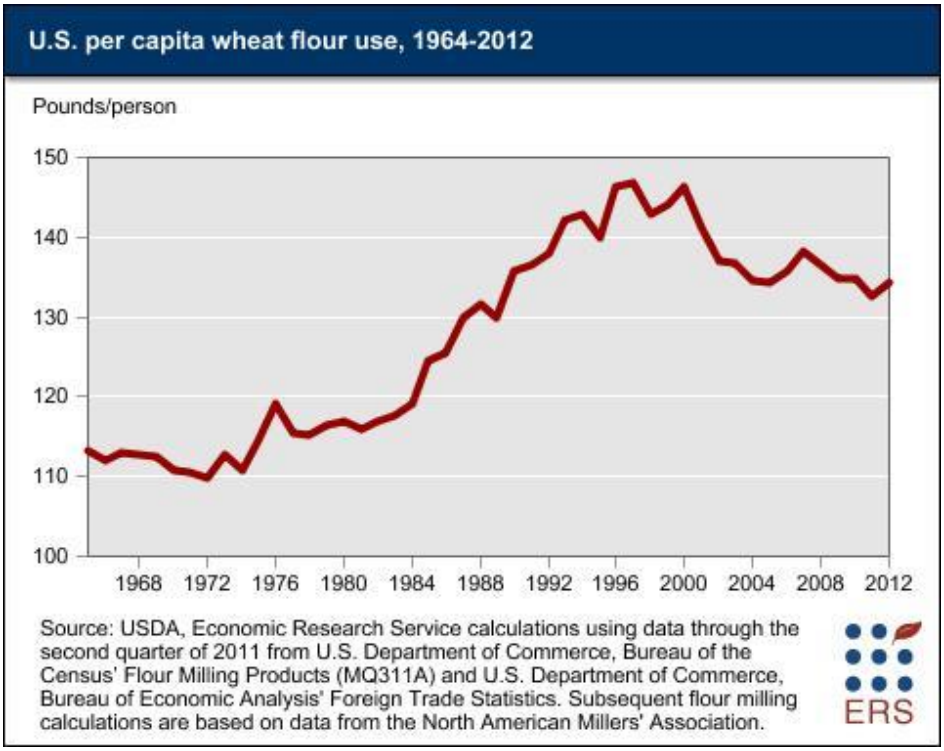

Figure 2. Source: http://www.ers.usda.gov/topics/crops/wheat/wheats-role-in-the-us-diet.aspx

1.4 Gluten-free trend

Both in U.S. and in Europe, for many years we have seen a rising trend toward gluten-free and wheat-free products (Figure 2). An international market research agency (NPD Group) announced in January 2013 that 30% of North Americans indicate that they wish to consume less gluten or even eat an entirely gluten-free diet. Their main motivation has to do with a changing health-awareness. 'It's not that we want health and wellness more but that we are constantly changing how we address health and wellness'. The same research agency reported a strong increase in demand for wheat and gluten free meals at restaurants. In 2013, this would involve some 200 million restaurant visits per year.

In addition, it is speculated that the increased use of wheat (gluten) in food products (including in non-grain products), might lead to exceeding a certain 'sensitivity threshold' in some individuals. For example, in Australia, 30% of labelled food products contain wheat ingredients or gluten (Atchison et al., 2010). In fact, the use of wheat and wheat gluten in a wide variety of food products has increased significantly: the production of gluten has almost doubled in the past decade.

In the following chapters we discuss, for each disease (coeliac disease, wheat allergy and non-coeliac wheat/gluten sensitivity) the possible symptoms, diagnosis, prevalence and the related potentially harmful substances.

2. Coeliac disease

2.1 Introduction

Coeliac disease is an autoimmune disease, i.e. a disease whereby the immune system targets a person's own body. Coeliac disease is caused by a combination of a specific genetic predisposition and environmental factors, of which the consumption of (wheat) gluten is the most important. Not only the genetic predisposition of the immune system but also the immune response over time are of relevance for this disease. Most people with coeliac disease have HLA-DQ2 or HLA-DQ8 receptor genes. Approximately 25-30% of the population have these genes. But it is estimated that only 4% of these DQ2/8 positive people actually develops coeliac disease (Stein and Schuppan, 2014). Other genes and factors also clearly play a role. In addition, age is an important factor. If very young children in whom the intestines are not yet entirely developed are exposed to gluten (piece of bread), they can develop coeliac disease. Coeliac disease can also be manifest in older people, in whom hormonal changes influence the functioning of the immune system. Environmental factors, including our diet, are also of great importance. First, this involves exposure to gluten. Here an increased permeability of the intestinal wall can play a role. Increased permeability may be a consequence of the inflammatory reaction caused by gluten, but also of an intestinal infection, a drug and/or alcohol use. Coeliac disease related Inflammation of the intestinal wall due to gluten cause in a long term a sharp decline in the intestinal wall surface through the disappearance of the surface-increasing villi (Figure 3). As a consequence of this, problems with digestion and the absorption of nutrients occur in individuals that suffer from coeliac disease.

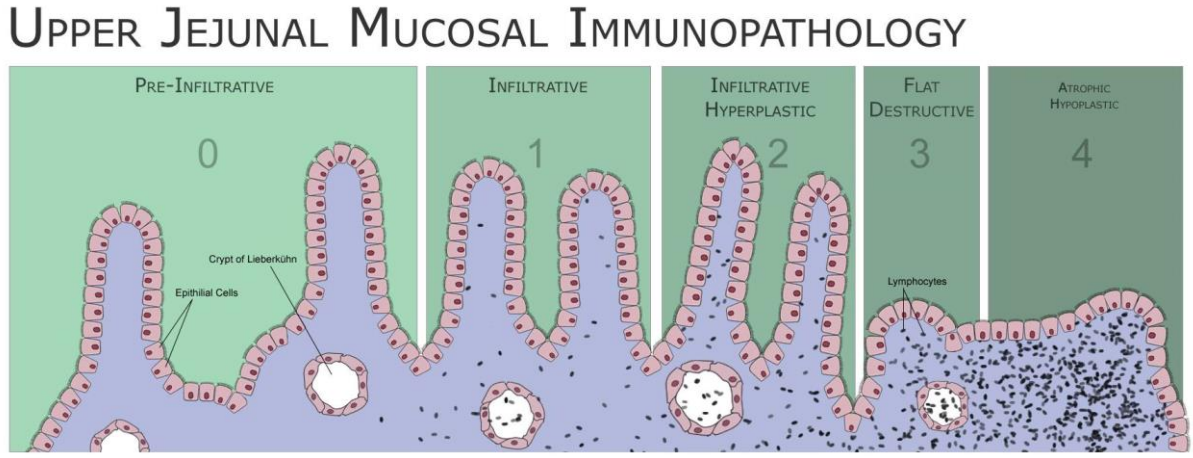

Figure 3. Various stages of damage to the small intestine in coeliac disease are shown schematically. Intact villi are shown on the far left. These are exposed to inflammatory reactions, causing the villi to eventually lose their structure and develop a smooth surface (far right). Source: http://en.wikipedia.org/wiki/Coeliac_disease (classification according to Marsh, 1992)

Resultant symptoms are a.o. (fatty) diarrhoea, weight loss and/or anaemia. In children, also growth retardation, muscle weakness, poor appetite and a tense abdomen have been observed. Children with untreated coeliac disease also often exhibit mood swings and drowsiness. However, this is not the only manifestation of coeliac disease. There are also people with coeliac disease who have the HLA-DQ2 or -DQ8 genes and in whom all the antibodies are present in the blood that are specific to coeliac disease, but have not (yet) have any (verifiable) damage to the small intestine. These people do have one or more general complaints such as chronic fatigue, poor sleep or headaches. Often the complaints of these people are not recognised as possibly representing coeliac disease and as a result such individuals are not tested or diagnosed for this. This is called subclinical coeliac disease. In the literature this is also referred to as potential coeliac disease.

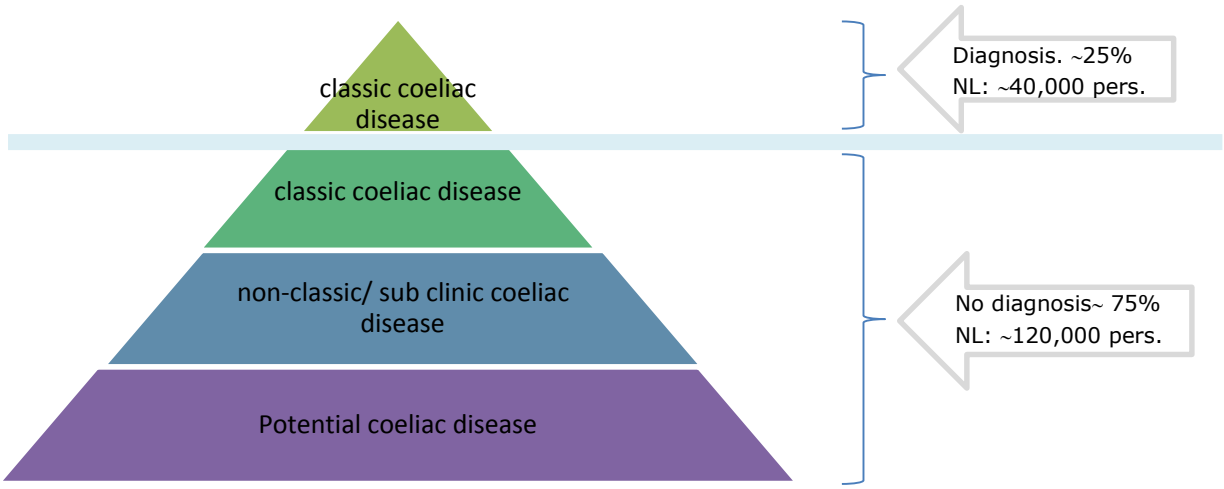

Figure 4. The coeliac iceberg

The development of coeliac disease in short

Due to the high content of proline and glutamine in gluten protein, it is resistant against complete degradation by the enzymes in the stomach and small intestine.

The undigested gluten fragments (amino acid chains) can pass the intestinal wall in people with coeliac disease, probably due to a disturbed permeability of the intestine.

When there is damage to the intestinal wall, an enzyme is released (tissue transglutaminase, tTG or TG2) that converts the glutamine in the undigested gluten fragments into glutamic acid, which can bind more strongly with the HLA-DQ2/8 receptor and then with T-cells of the immune system .

This binding to the T-cells activates an inflammatory response that leads to damage to the intestinal villi.

Because the small intestinal villi are damaged (in severe cases even completely gone, Figure 3), problems arise with the digestion of food and absorption of nutrients, with all the associated consequences.

Inflammatory reactions and substances that thereby can pass through the intestinal wall unwanted can also cause problems elsewhere in the body such as skin diseases (dermatitis herpetiformis) and neurological problems (ataxia).

2.2 Symptoms of coeliac disease

For a list of symptoms associated with coeliac disease, see Table 2 in section 4.2. Here the symptoms of the three diseases (coeliac disease, wheat allergy and non-coeliac gluten sensitivity) are listed.

2.3 Diagnosis

The diagnosis of coeliac disease can be made by analysing the pattern of symptoms; also by blood tests (serology), an assessment of genetic predisposition and finally by histology screening of small intestinal tissue. The guideline for coeliac disease of the Dutch Society for Gastroenterology and Liver physicians (NVMDL, 2008; http://www.mdl.nl/richtlijnen2) says the following regarding the diagnosis of coeliac disease:
The diagnosis of coeliac disease should be considered in patients with 1) symptoms suggestive of coeliac disease, such as chronic diarrhoea, weight loss, mal absorption, abdominal pain, abdominal distension, skid marks in the toilet bowl and growth retardation in children, 2) iron deficiency anaemia and/or osteoporosis, which are reported in coeliac disease and/or 3) in other diseases.
In the absence of the HLA-DQ2 and/or -DQ8, further investigation is not recommended.
In the presence of HLA-DQ2 and/or -DQ8 serological studies need to be carried out (EMA and tTGA).
If scores are positive for EMA and tTGA, further histological examination is required. Here, the state of health of the intestinal biopsy is assessed according to the standard classification of Marsh (see Figure 3).

2.4 Prevalence

It is estimated that approximately 0.5-1.3% of the Western population have coeliac disease (Peumans and Van Damme, 1996). The disease is more common in women than in men, with a ratio of 2-3: 1. In the Netherlands, there are currently about 150,000-200,000 patients with coeliac disease. These are diagnosed and undiagnosed people both with and without complaints. It is estimated that only 1 out of 8 people with coeliac disease (10,000-27,000 people) have been diagnosed (Rewers, 2005, Mustalahti et al., 2010, Reilly and Green, 2012).

2.5 What gluten fragments can be harmful?

In the kernel of a single grain wheat variety, some hundreds of proteins are present.
The gluten proteins from wheat (bread wheat, spelt, khorasan wheat, durum wheat, einkorn wheat, etc.) belong to the so-called prolamins. Gluten is divided into two groups of protein, glutenins and gliadins. Mostly, the gliadin fraction from the gluten can give rise to coeliac disease (gluten intolerance). The gluten components glutenin and gliadin are present in approximately equal quantities. However, depending on the wheat variety this ratio can vary to some extent (the gluten ratio). The gliadin proteins are further classified into α/β, ω and γ-gliadins. In the glutenin proteins, one makes a distinction between proteins with a high molecular weight (HMW-glutenin) and those with a low molecular weight (LMW-glutenin).

Due to the high content of proline and glutamine, gluten proteins at specific sites in the amino acid chain are only partially biodegradable by humans. Healthy people do not suffer from this. In combination with genetic and environmental factors, the remaining undigested protein fragments cause health problems in a small number of people. Research into which gluten proteins cause a hypersensitivity reaction is time consuming and costly, but it has nonetheless led to important insights (DiGiacomo et al., 2013; Ludvigsson et al., 2013; Pastorello et al., 2007; Thatham and Shewry, 2008). Sollid et al., (2012) published the internationally accepted list of gluten fragments, which play a role in coeliac disease.

It turns out that there are big differences between people regarding their response to (combinations of) gluten fragments. So there is not one specific gluten protein or one specific gluten fragment to which all people react in the same way. There are, however, various proteins that can be mentioned to which the largest group of people with coeliac disease react. These are the α-gliadins and to a lesser extent the γ -gliadins. These proteins, in particular, deliver a large number of indigestible immunogenic fragments. People with coeliac disease also react to the gluten proteins from rye (secalins) and barley (hordeins). Much fewer coeliac patients react to glutenins.

Changes in the ratio of undesirable gluten in modern wheat are a frequently heard theory when it comes to explaining the increase in frequency of coeliac disease. There are both pros and cons for this theory. Van den Broeck et al. (2010a) examined the content of the α-gliadin epitopes Glia-α9 and Glia-α20 in several hexaploid wheat varieties used in modern (1986-1998) and older times (1863-1982). Glia-α9 is known as one of the most harmful gluten epitopes for people with coeliac disease, and there is a much smaller group of people that react to Glia-α20. Researchers used Glia-α9 and Glia-α20 epitopes to classify wheat varieties as low, medium or highly reactive. The results showed that only one of the 36 modern wheat varieties had low levels of Glia-α9, compared with 15 of the 50 older varieties. The number of varieties with high Glia-α20 was similar in both groups. In the older varieties, no systematic differences were found

between the hexaploid species (T. aestivum, T. spelta and T. compactum). A few varieties were found which had a relatively low content of these two gliadin epitopes.

Besides the differences between hexaploid wheat varieties, Van den Broeck et al. also looked at the differences in gliadin composition of tetraploid varieties (Van den Broeck et al., 2010b). The tetraploid species include durum wheat, emmer wheat and khorasan wheat (turgidum). This may be interesting because in tetraploid species, the D-genome is missing that introduced a high level of α-gliadin epitopes into modern (hexaploid) bread wheat. The tetraploid varieties, however, did not appear to have substantially lower levels of α-gliadin epitopes than the old hexaploid varieties. But according to the authors, this finding could also be due to the sensitivity of the detection method and the difference would be greater in reality. From this research, supplemented by the work of Salentijn et al. (2013;2009), a few purely tetraploid species were identified with a significantly lower number of epitopes. Colomba and Gregorini (2012) studied the content of immunogenic α-gliadins of two older tetraploid varieties (Graziella Ra and Kamut) in comparison with four more modern durum varieties (Capelli, Flaminio, Grazia and Svevo). They found a significantly higher content of α-gliadins in the older varieties in comparison with the modern varieties, concluding that none of these are suitable for people with coeliac disease. So for the present, the general idea that ancient grains contain fewer immunogenic components than modern bread wheat cannot be confirmed.
Van den Broeck et al. (2010a) emphasise that it is important to carry out research into the gluten composition in a large number of wheat varieties in order to obtain a more complete picture of potentially less coeliac-harmful varieties. So a great deal of further research is needed for the development of wheat varieties with less immunogenic gluten. In any case, these varieties will never be entirely safe for coeliac patients, because the threshold value for a reaction is many times lower than the epitope content in the most favourable variety. Perhaps the use of these varieties may play a role in decreasing the trigger that sets off coeliac disease. Their usage also might decrease the complaints in undiagnosed individuals.

2.6 Threshold value & treatment

For the present, a completely gluten-free diet is the only remedy for people with coeliac disease. Even traces of wheat or other gluten-containing grains (barley, rye, spelt) present in gluten-free food through cross contamination (during cultivation, harvest, transport, production) should be avoided. In the presence of complete avoidance of gluten, the intestinal wall can finally recover and the symptoms disappear. Research has shown that a content of only 10 to 100 milligrams of gluten per day is still safe in most cases (Hischenhuber et al., 2006). In regulatory sense, a maximal gluten content in food products of 20 mg/kg (20 ppm) is maintained as a safe threshold value. Even if one does not immediately experience symptoms from this, a low daily exposure can nonetheless be harmful in the long term. In a very small

number of cases, the disease is already so advanced that avoiding gluten cannot repair the intestinal wall. This is called ‘refractory coeliac disease’.

3. Food allergy to wheat

3.1 Introduction

Wheat allergy is a rare negative response of the immune system to wheat proteins (Figure 5). The body reacts to the protein as if it was a dangerous pathogen that needs to be cleaned up. Via antigen-presenting cells, B-cells are activated to the production of allergen-specific immunoglobulin IgE antibodies. These then bind to mast cells that are present throughout the body. If two or more IgE antibodies on the surface of a mast cell are linked by an allergen, the mast cell is activated and substances are excreted, including histamine (Figure 5). This leads to inflammatory reactions and symptoms such as swollen membranes of the mouth and throat, difficulty in swallowing, shortness of breath, diarrhoea, vomiting, abdominal pain, asthmatic reactions and rashes. A reaction of the entire body may also occur, whereby the blood pressure can drop very rapidly so that patient goes into shock or, in the worst case, may even die (http://www.kennislink.nl/). We call this an anaphylactic shock.

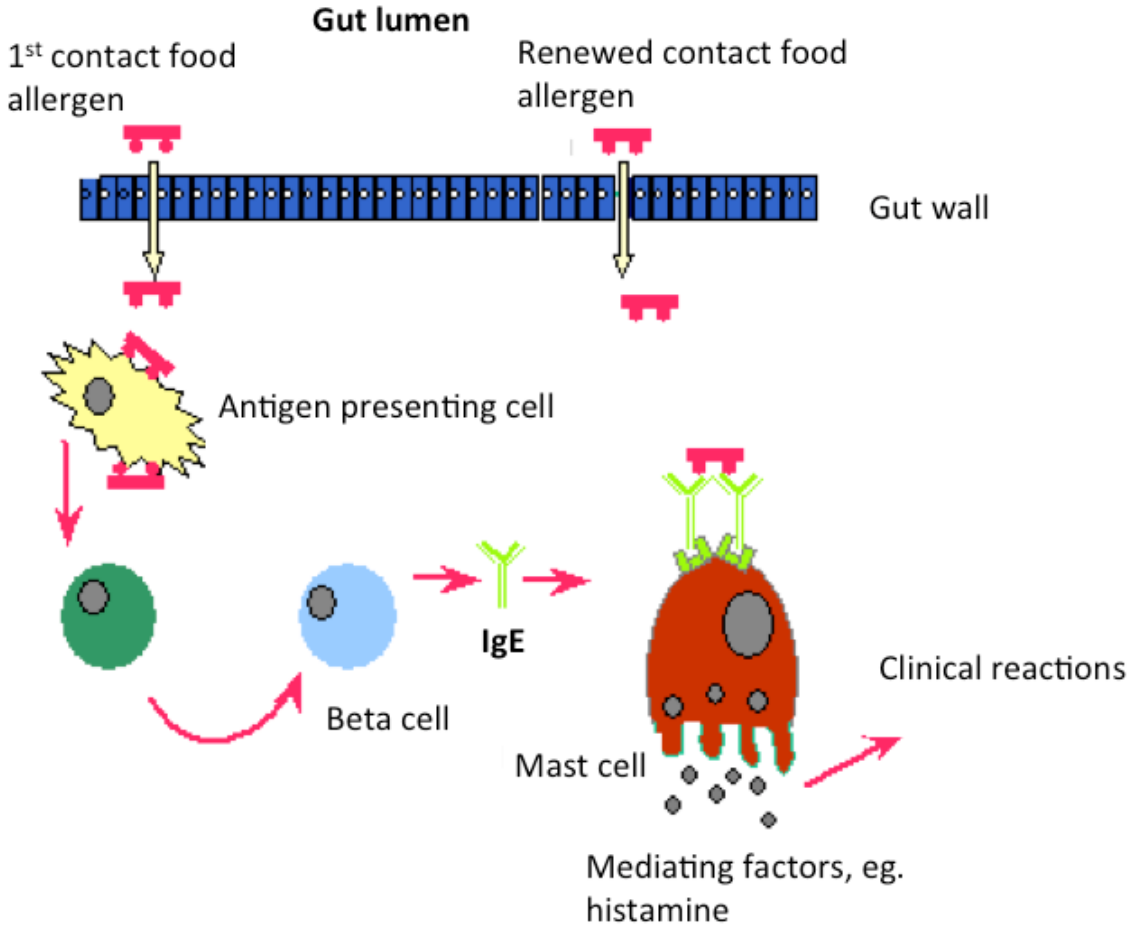

Figure 5. Schematic representation of the response in the body to a food allergy. Adapted from: www.kennislink.nl

In allergy to wheat, a distinction is made between classic food allergies with skin rash, intestinal complaints, respiratory problems and wheat-dependent exercise-related anaphylaxis (Wheat Dependent Exercise Induced Anaphylaxis = WDEIA).
Allergy can occur in bakers through the inhalation of flour (baker's asthma). This is the most common form of IgE-mediated wheat allergy. Bakers with the handicap of baker's asthma will need to be reinstated and/or bought out. In the Netherlands this costs the bakery sector around € 0.5 million per year.

3.2 Symptoms of wheat allergy

For a list of symptoms associated with wheat allergy, see Table 2 in section 4.2. Here the symptoms of the three diseases (coeliac disease, wheat allergy and non-coeliac gluten sensitivity) are listed.

3.3 Diagnosis

Whether a person has sensitivity for wheat, allergy can be determined by the combination of a blood test and a skin test. With the blood test, one can see if there are specific IgE antibodies to allergens (in this case wheat proteins) present in the blood. With the skin test we can see how the skin reacts via the IgE antibodies with a small quantity of the wheat protein. The presence of IgE antibodies against wheat in the blood certainly does not always mean that there is an active (food) allergy. Nor does the skin test yield a conclusive diagnosis (Sapone et al., 2012). So the real evidence is only provided through the so-called food challenge test, executed in a double blind, placebo-controlled set-up to prevent the placebo effect. Such tests have shown that wheat allergy is a relatively uncommon event (Zuidmeer et al., 2008), but that many wheat proteins are immunogenic (producing IgEs), which, however, never have been found to lead to clinical symptoms.

3.4 Prevalence

It is not entirely clear how frequently a food allergy to wheat really occurs. Food allergy in general occurs in more than 6% of children and in almost 3% of adults. Usually this is an allergy to specific proteins from milk, egg and peanut. Figures for wheat allergy amongst children vary from <0.1% to 1%, depending on age and country (Hischenhuber et al., 2006; Kotaniemi-Syrjänen et al., 2010; Sapone et al., 2012). However, a large meta-analysis has shown that the general prevalence is at most approximately 0.2% (Zuidmeer et al., 2008).

About one-half of children 'outgrow their food allergy'. Depending on the type of allergy, this percentage is lower (peanut) or higher (milk). There are reports in the literature that more than 80% of children with a wheat allergy have outgrown this by their 8th year, and 96% before their 16th year (Kotaniemi-Syrjänen et al., 2010). The number of adults with wheat allergy is therefore probably much lower than the number of people with coeliac disease.

3.5 Which wheat proteins?

The wheat proteins that cause allergic reactions are very diverse and different for each person. The two main wheat protein groups with known food allergy are ω5-gliadins and ATIs. To a lesser extent, there are noted to be reactions to LMW-glutenin, lectin (Wheat Germ Agglutinin; WGA) and possibly also Lipid Transfer Proteins (LTPs) (also from corn, rice and barley) (Gilissen et al., 2014). In children with wheat allergies one sees mostly reactions to: α/β- and γ-gliadins (Pastorello et al., 2007; Tatham and Shewry, 2008). In 20% of cases people with a wheat allergy are also allergic to barley and rye (Sicherer, 2001).

3.5.1 The ω5-gliadins

In IgE-mediated wheat allergy, frequently ω-gliadins (particularly ω5-gliadin) are involved. This may involve a cross-reaction with equivalent proteins from other grains.

3.5.2 Amylase trypsin inhibitors (ATIs)

It has long been known that ATIs play a role in baker's asthma (flour dust allergy) and food allergy to wheat (Pastorello et al., 2007; Tatham and Shewry, 2008).

ATIs (α-amylase/trypsin inhibitors) occur mostly in wheat together with gliadins and are often found in gluten extract. These are proteins, which inhibit the action of breakdown/digestive enzymes (α-amylase and trypsin) of insects and mammals. As far as is known, wheat contains at least 11 different ATIs (Junker et al., 2012). Rye and barley also contain similar ATIs. They form a natural defence mechanism of the plant against damage by insects. ATIs form some 80% of the total albumin fraction in wheat and possibly 1% of the total quantity of protein in wheat flour (Cordain, 1999).

ATIs are heat resistant. Research has shown ATIs are also present in cooked wheat (5 minutes at 100°C) and can cause an allergic reaction (Pastorello et al., 2007). Interesting is the observation that there are some studies showing that there are people who have a stronger allergic reaction to wheat that has been heated than to an unheated version (Tatham and Shewry, 2008).

Recent research has indicated that ATIs may play a role in the development of both coeliac disease and non-coeliac-related wheat hypersensitivity (Junker et al., 2012) via the production of inflammatory factors (cytokines) in the intestine.
Growing conditions of the wheat (shade, height, storage conditions) appear to influence the ATI percentage (Prandi et al., 2013). The ATI contents of three different types of durum wheat at three different growing locations in Italy were compared. This showed that there are certainly differences by race (variety?), but that the effect of growing conditions is greater.

3.5.3 LTPs

LTPs (Lipid Transfer Proteins) are fat-carrying proteins present in 'higher' plant species including grains, vegetables and fruits. They are particularly known as heat-resistant allergens from corn, barley and from various fruits such as peach, cherry and apple. They may also play a role in wheat allergy, but this is less well known (Gilissen et al., 2014; Pastorello et al., 2007; Shewry and Tatham, 2008).

3.5.4 Lectins

Lectins in wheat are not known as allergenic proteins. Lectins are specific carbohydrate-binding proteins that are present in almost all plants and their seeds, nuts and fruits. They play a role in important biological processes such as recognition of cells and proteins, and thus protect the plant against external pathogens such as fungi and other organisms. Some cultivated grains and legumes have relatively high concentrations of specific lectins, for example the lectins that are present in the germ of the wheat grain and are called Wheat Germ Agglutinins (WGA).
There is no research known on the content of lectins in different wheat varieties and the possible change in this since the domestication and the recent processing of grains. Potential adverse effects of products made of lectin-rich raw materials are based on animal research in the laboratory (not people) and are usually performed with high doses of extracted (pure and not heat-exposed) lectins. Because WGA is a heat-labile lectin, it is assumed that its biological activity will have disappeared after heating. Studies using pasta have shown that, although some uncooked whole grain pastas in shops do contain active WGA, cooking the food eliminates all the WGA activity (Peumans and Van Damme, 1996). It is not known whether this also applies to other (heat) treatments such as pasteurization, frying and extrusion. In a comprehensive report of the University of Maastricht (Van Buul and Brouns, 2014), a series of recent studies are discussed, which looked mainly at the effects on body weight regulation, autoimmune disease, depression, cancer and chronic bowel disease. This showed that there has been surprisingly little research aimed at obtaining quantitative data on the different types of lectins in food prepared for consumption, as well as on the effects of ingestion by humans in the short and long term.

3.6 Threshold value & treatment

It is necessary for people with wheat allergy to completely avoid products with wheat (and possibly other grains to which they react). Depending on the severity, they must also to a greater or lesser extent be aware of cross-contamination with traces of wheat. In some cases of allergic cross-reactivity with other grains (wheat, barley, rye), and even with fruit can occur. This is rare, however. (Gilissen et al., 2014). Research has shown that the tolerance of adults is generally higher than that of children. Several to ten's of grams of wheat protein are tolerated by most adults, without any complaint. This is (much) higher than in people with coeliac disease. In one study a group of adults was given wheat gluten as the only protein source for a period of 50 days and no adverse effect were observed (Van Buul and Brouns, 2014). For most of these people the risk of cross-contamination therefore seems to be less important than for those with coeliac disease. However, there are large individual differences and there will also be patients who do react to smaller quantities. Children with wheat allergies usually react to smaller amounts of wheat than adults. In literature it is reported that 80% of children react to less than

two grams of wheat protein. For a small subgroup, less than ten milligrams is even a problem. On the other hand, the nature of the complaints in children is often less severe (mainly rashes, respiratory and intestinal cramps) than in adults (more frequent anaphylactic shock, facial oedema and severe intestinal symptoms and oesophageal irritation) (Hischenhuber et al., 2006). Clinical allergy symptoms, however, are rare phenomena (Zuidmeer et al., 2008).

4. Non-coeliac wheat/gluten sensitivity (NCWS/NCGS)

4.1 Introduction

For several decades, a third group of people has been classified who experience symptoms after eating wheat products, but who cannot be diagnosed with wheat allergy or coeliac disease and wherein the gastrointestinal symptoms of irritable bowel syndrome (IBS)-like complaints improve on a gluten-free diet. This group is referred to as 'non-coeliac gluten/wheat sensitivity' (NCWS/NCGS). Consensus has been reached about the name and the diagnostic criteria rather recently. Despite the word 'gluten' in the current definition of NCWS/NCGS, it is far from certain that the gluten is the (main) cause of symptoms in this group of people. However, wheat as direct cause is also unclear.

4.2 Diagnosis

What makes the definition of NCWS/NCGS difficult is that people report both symptoms that may indicate coeliac disease as well as symptoms that occur with wheat allergy (see Table 2). Often these are self-diagnosed. Well-defined biomarkers are still lacking for NCWS/NCGS. For example, in people with NCWS/NCGS there is no damaged intestinal wall (biomarker for coeliac disease) and in these people no wheat-allergen specific IgE antibodies are encountered in the blood (biomarker for wheat allergy). A more stringent hallmark could be the absence of an allergic or immune reaction, or else a positive reaction on a double-blind, placebo-controlled gluten challenge (Sapone et al., 2012, Ludvigsson et al., 2013).

4.3 Symptoms of non-coeliac wheat/gluten sensitivity

As mentioned above, people with NCWS/NCGS can report both symptoms that may indicate coeliac disease as well as symptoms that occur with wheat allergy. For a list of symptoms associated with all three diseases (coeliac disease, wheat allergy and non-coeliac gluten sensitivity), see Table 2 below.

	Coeliac disease (Symptoms due to a chronic immune reaction)	Wheat allergy (Acute immune-IgE mediated complaints)	Non-coeliac gluten/wheat hypersensitivity (Chronic symptoms)
Gastrointestinal complaints	Abdominal pain Diarrhoea Constipation Nausea Emesis Abnormal stool Weight loss	Abdominal pain Intestinal cramps Diarrhoea Nausea Emesis	Abdominal pain Diarrhoea Constipation Nausea Distended abdomen
Neurological/ pain complaints	Headache Bone or joint pain 'woolly' in the head Tingling or numbness of hands and feet Fatigue Ataxia (= coordination disorder) Depression Seizures Anxiety/tension	Dizziness Headache	Headache Joint or muscle pain 'woolly' in the head Tingling or numbness of hands and feet Fatigue Depression
Other	dermatitis herpetiformis (skin condition) Unexplained iron deficiency (anaemia) Osteoporosis Reduced fertility Irregular menstrual cycle Increased risk of abortion Dental problems Canker sores	Eczema Asthma Rhinitis (inflamed nasal cavities) Itching Difficulty breathing Swollen mouth and throat Anaphylactic shock Angioedema	Skin rash

Table 2. Possible symptoms resulting from wheat consumption; taken over by and translated from (Capili et al., 2014)

4.4 Prevalence

An international market research agency reported in January 2013 that 30% of adult North Americans indicated that they are reducing their gluten intake or following a completely gluten-free diet. According to the agency, this was around 25% in 2010. Such high numbers are in stark contrast with results of data from large population studies. In the US National Health and Nutrition Examination Survey 2009-2010 (n = 7762, coeliacs excluded) only 0.55% answered 'yes' to the question 'are you on a gluten-free diet?' (DiGiacomo et al, 2013). Rubio-Tapia et al., (2012) reported a similar number: according to their research some 0.63% of North Americans follow a gluten-free diet.

Between 2004 and 2010, 5,896 patients visited the Centre for Coeliac Disease of the University of Maryland (USA) with 'coeliac/wheat allergy-like symptoms'. Of these patients, 6% met the criteria for NCWS/NCGS (Sapone et al., 2012). Judging from these data, the prevalence in the general population may therefore be much lower than 6%, because 5,896 patients are not a representative sample of the population, but came in to the Centre complaining of abdominal pain. The most useful data can be obtained from research on food hypersensitivity in people with IBS. Several indications have been observed that some of the people with IBS may react in a hypersensitive manner to gluten/wheat and that this number is probably higher than can be explained with the diagnosis coeliac disease. In 1982, Alun Jones and colleagues (Alun Jones et al., 1982) found that of the 21 patients, nine reported complaints when eating wheat without the presence of coeliac disease (according to the then applicable diagnostic criteria). Cash et al. (2011) found antibodies that could be related to coeliac disease (specifically against gliadin) in 7.3% of the IBS patients (total: n=492) that they examined. However, in only 0.4% was the diagnosis of coeliac disease confirmed. Carroccio et al. (2012) found that 30% of the people with IBS (n=920), without coeliac disease or wheat allergy reacted to exposure to wheat in a double blind study. Most of these (more than 22.4 %) also showed, in addition to wheat, hypersensitivity to cow's milk (all), egg (n = 120) and/or tomato (n = 112). Only 7.6% (n=70) reacted only to wheat. The first group showed symptoms that were more similar to those in wheat allergy; in this group there was also more frequently a history of food allergy in childhood. The group that reacted only to wheat demonstrated more correspondence with coeliac disease. For example, 75% of them had the HLA-DQ2/8 gene and 94% of these demonstrated lymphocytes in the duodenum. In addition, the intestinal biopsy in one-third of this group of patients was positive for EMA. So this might also point to a pre-stage of coeliac disease.

Due to the current broad consensus definition (see 4.1), it is not yet possible to make a reliable estimate of the number of people suffering from NCWS/NCGS. It is expected that this will be higher than the number of people with coeliac disease, but concrete figures that give an (more or less substantiated) estimate are scarce and range from 0.5 to 10% of the population (Ludvigsson et al., 2013).

It is estimated that approximately 15% of the Dutch population have IBS (Dutch College of General Practitioners, NHG, 2011; https://www.nhg.org/standaarden/volledig/nhg-standaard-prikkelbaredarmsyndroom-pds). On this basis and the investigation of Carroccio and others (2012) some 4.5% of the Dutch population might have a hypersensitivity to wheat without the presence of coeliac disease or wheat allergy. More research among the Dutch population is needed to substantiate this estimate further.

4.5 Which components of wheat?

For the group of people with NCWS/NCGS it must still be determined to which components of wheat they react. The literature already contains a number of documents on the subject, particularly in studies concerning people with IBS.

Gluten or wheat?

Over the last two years, a relatively large number of publications on NCWS/NCGS have appeared. Several intervention studies were carried out that looked at the reaction of people with (alleged) NCWS/NCGS on a diet with and without gluten and/or wheat in comparison with a control group. Most of the studies were done in groups of patients with IBS, who reported to benefit from avoiding wheat. In the studies that showed significant differences, these mainly involved complaints reported after exposure to wheat/gluten in blind challenges studies (the participant in the test did not know whether wheat/gluten or placebo was present in the food). In most studies, no changes were observed in intestinal permeability or in specific (immunological) biomarkers that could explain something about the underlying cause of NCWS/NCGS. In a few of these studies, coeliac disease could not be ruled out (Alun Jones et al., 1982; Bucci et al., 2013; Sapone et al., 2011) because there was no information about the tissue status of the small intestine. In some other studies, people were involved with mild intestinal damage, so they would be consistent with the Marsh 1 stage of coeliac disease (Figure 3). These people were then erroneously labelled as NCWS/NCGS patients and would probably be diagnosed with coeliac disease after further investigation. However, such slight damage of the intestinal wall and its associated permeability can also occur after extreme physical exercise, drug and/or excessive alcohol use. In short, there are doubts about the reliability of the data currently available. According to various researchers, there is mainly a so-called 'nocebo' effect1. The relationship with gluten was not confirmed by research.

There have also been several studies with wheat (products) and therefore not only with gluten/gliadin (Biesiekierski et al., 2013, Vazquez-Roque et al., 2013). So, in these studies,

[1]The nocebo effect is a negative expectation effect and the counterpart of the positive expectation effect that is known as the placebo effect. Nocebo is often seen as part of the placebo effect. Stated simply, `Fear makes us sick`.

people were also exposed to other wheat components such as LTPs, ATIs (for further explanation see also Chapter 3). To date there have been no studies carried out in which wheat-hypersensitive people were tested on their reaction to these individual components in relation to each other.

FODMaPs

In some patients with IBS there appears to be a link between the consumption of food with a high content of fermentable saccharides (fermentable oligo, di, monosaccharides and polyols; FODMaPs). Studies that looked specifically at the effect of a FODMaP-free diet (that is to say, not only wheat-free) reported quantities of around 75% of the IBS patients that reacted well to this (Barrett and Gibson, 2012; Staudacher et al., 2011; Van der Waaij and Stevens, 2014). Most patients, however, did not react to all FODMaPs, but only to one or two specific ones. Barrett and Gibson (2012) listed the following percentages for the number of IBS patients that would have problems in digesting specific FODMaPs: This is 20% for mannitol, 57% for sorbitol, 45% for fructose and 25% for lactose. Fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are dietary fibres and therefore, by definition, make their way undigested into the large intestine where they are fermented. These types of indigestible carbohydrates can all have a laxative effect when ingested in large amounts. They are also rapidly fermented, so that a relatively large amount of gas may be released. People with IBS may react strongly to these with clinical symptoms. Most wheat varieties and species have a relatively high content of these FODMaPs (Biesiekierski et al., 2011).

In Australia, there have been studies of the content of FODMaPs in various vegetables, fruits, legumes, seeds and grain products, including various types of bread (Biesiekierski et al., 2011). Here it proved that the FODMaP content of (dark) rye bread was the highest, followed by whole wheat bread and white wheat bread. Bread made with spelt had a significantly lower content of FODMaPs, namely 0.24 grams per 100 grams compared to 1.96 grams per 100 grams of dark rye bread and 1.36 grams per 100 grams of whole wheat bread. In the breakfast cereals and pasta, as well, the variants scored on the basis of wheat, corn and/or rye higher than variants with oats, quinoa or rice as the main grain ingredient. Further research with a broader product range should confirm these results. See Figure 6 for examples of foods that contain little or a large amount of FODMaPs.

FODMaP	Food products high in FODMaPs	Product group	Food products low in FODMaPs
Fructose	Honey, apple, cherry, pear, mango, water- melon, asparagus	Sources of starch	Spelt bread, cornflakes, oats, buckwheat, potatoes, rice
Lactose	Milk, buttermilk, yoghurt, custard	Dairy sources	Gouda cheese, soy milk, lactose free milk, meat, fish, egg, poultry
Oligosaccharides	Wheat (pasta, bread), rye, water-melon, plum, cabbage, leek, onion, legumes	Vegetables	Carrots, lettuce, tomato, chicory, pepper, endive, cucumber, green beans
Polyols	Apple, pear, plum, cauliflower, mushroom, sugar free chewing gum	Fruits	Strawberry, pineapple, orange, grapes, kiwi fruit, banana, Galia melon

Table 3. Examples of foods that contain little or a large amount of FODMaPs. Adapted from Van der Waaij and Stevens (2014).

There is no comparative study known on the total content of FODMaPs in wheat or other grains. Research has, however, been done within the European HEALTHGRAIN project concerning the difference in fibre content between different grain types and old and newer varieties of these. This study specifically looked at the content of arabinoxylans, the water-extractable fraction (WE-AX), which is fermentable in the large intestine. These arabinoxylans can also act as a carbon source to the gut microflora. The highest concentrations of WE-AX were found in rye, and the lowest levels in barley, oats and T. dicoccum. This was true for both the flour and the bran parts of these types of grains (Figure 6). WE-AX does not, however, belongs to the FODMaPs, as opposed to arabinoxylooligosaccharides (AXOS) that industrially, using enzymes, can be produced from AX.

More research is needed in which different varieties, also harvested after similar growing conditions (which may affect the fibre content in the grain) are compared with each other on the content of total fibre and (specific) fermentable fibres (and therefore FODMaPs), in order to make reliable statements about actual consistent differences between the different grain types. It needs to be noticed here that a significant portion of fructo-oligosaccharides present in wheat flour are being fermented by the yeast and/or active starter culture during dough preparation, In the case of the latter disappearance may be complete.

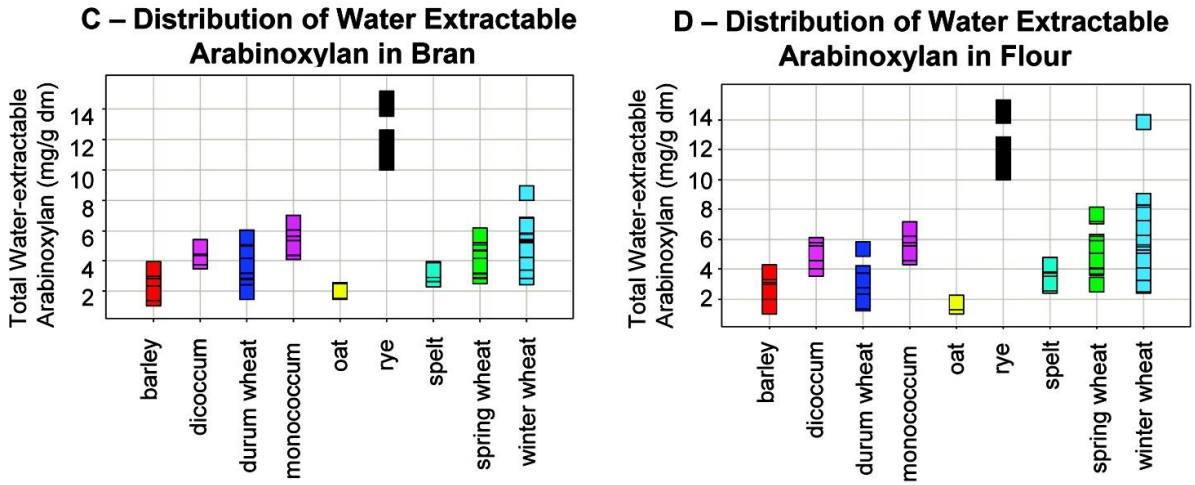

Figure 6. Fermentable arabinoxylans in various grains as measured in the HEALTHGRAIN Cereal Diversity Screen (reprinted with permission from Ward et al., 2008).

4.6 Threshold value & treatment

There is no reliable information on the threshold of wheat consumption at which people develop complaints related to NCWS/NCGS. It could be that this threshold is (many times) higher than in coeliac disease and wheat allergy, but this has never been properly investigated. The threshold value will in any case, depend strongly on the causative component, and the (specific) sensitivity of the patient.

Di Sabatino and Corazza (2012) emphasise in an opinion article that NCWS/NCGS is not a homogeneous disease syndrome (such as coeliac disease and wheat allergy), but rather a heterogeneous syndrome. Probably the underlying cause and the mechanism is not the same for all people with NCWS/NCGS. So it is quite possible that the term NCWS/NCGS will soon no longer suffice, but that different (new) disease syndromes will be defined on the basis of a hypersensitivity reaction to different components of wheat or grain (products). The approach to the treatment or prevention of these conditions will therefore become more specific.

With regard to dietary fibre, there are two recommendations: 1) For healthy intestinal flora and intestinal function, the consumption of fermentable carbohydrates is generally recommended. With consumption of more than five grams of rapidly fermentable carbohydrates per meal, (harmless) complaints may occur due to gas formation leading to a ‘somewhat bloated feeling’ in the abdomen. 2) Because people with IBS can be very sensitive to quantitative gas formation in the intestine, they are, however, advised to eat few rapidly fermentable fibres. Whether the consumption of just 0.5 to a little more than 1 gram of FODMaPs, as present in most bread, can indeed lead to disruptive gas formation is still unclear. For the present, however, in many cases of NCWS/NCGS in IBS patients, a gluten and/or wheat-free diet is advised (Nijeboer et al., 2013).

As mentioned above spelt wheat has a relatively low FODMaP content (Biesiekierski et al., 2011). To what extent this could play a role in the studied spelt products for some of the people with IBS who say they benefit from eating spelt products instead of standard wheat products deserves further investigation.

5. Nutrition-technological solutions to help reduce wheat-related intolerances

5.1 Introduction

The entire grain supply chain is particularly interested in solutions that are applicable in standard bakeries. Solutions for patients with coeliac disease and wheat allergy about whom it is certain that they react to even the smallest traces of gluten-containing grains can already be considered not to apply in this setting. This is why, in this chapter, we mainly discuss strategies directed at making products with a decreased content in substances that may potentially cause complaints.
Based on the literature, various solutions become apparent that can already be implemented in the relatively short term (Gilissen et al., 2014). These are subdivided into short, medium and long-term solutions.

Nutrition (technology) possibilities	Time frame
Use of gluten free grains (such as e.g. oats, teff, quinoa, buckwheat and millet)	Short
Reduction of the vital gluten	Short
The use of alternatives for gluten, e.g. by the use of whey protein from milk, or the use of oat flour	Medium
The use of fermentation processes with sourdough	Medium
Selection and modification of wheat lines	Long

Table 4. Short, medium and long-term solutions

5.2 Short term

5.2.1 Use of other grains

Gluten containing grains are wheat, barley, rye and spelt. That is to say, these grains contain gluten proteins with immunogenic peptides, which may cause coeliac disease. Other closely related grains include oats, teff, rice and ragi. Slightly less closely related, but still belonging to the grasses are maize, sorghum and millet. The seeds of all these cereals store gluten-like proteins in their seeds as a reserve food for the young germ. However, these proteins do not contain the toxic epitopes and are thus safe in a gluten-free diet. Oatmeal has long been on the

list of banned products until it became clear that the problems with oats were actually caused by contamination with wheat, barley or rye form the field or during transport and processing. The gluten-like oat protein avenin does not contain any of the known toxic epitopes from wheat, barley or rye (Londono et al., 2013). According to European laws and regulations, oat products with less than 20 ppm. gluten contamination may now be labelled and sold as gluten-free (EC-Regulation 41/2009). This makes oats a good alternative to wheat and is a major source of healthy dietary fibre (β-glucan) in the daily diet. A daily intake of at least three grams of oat β-glucan helps lowering cholesterol levels and ensures good bowel activity.
There has been not yet confirmed evidence from Finland that the immunogenicity of rye seems to be much lower than that of wheat. Large-scale replacement of wheat with rye products could possibly reduce the prevalence at a population level.

Combining regular bread wheat with grains that are known for their low levels of specific gliadins epitopes and/or gluten, ATIs and/or FODMaPs ensures a lower exposure to these substances and thus possibly to a decrease or even disappearance of mild complaints. Spelt might be interesting to use in bakery products for people with bread wheat hypersensitivity (NCWS/NCGS; IBS). The availability of these grains for commercial cultivation and application is a consideration here. It is also interesting to get solid data on specific gliadin epitopes, ATIs and/or FODMaPs in durum and various ancient wheats such as einkorn wheat and emmer.

5.2.2 Reduction in the addition of vital gluten

Not adding small quantities of vital gluten to improve texture, to many products from which the consumer does not expect it, such as e.g. to cold cut meat products, would be a relatively simple measure to make these products suitable again for consumption by persons who need to avoid gluten. Of course this will affect the quality of the end product, which means that adequate replacement proteins and /or food technology solutions need to be found.

5.3 Medium term

5.3.1 Fermentation Process

Sourdough technology is very old in the history of bread making. If the sourdough culture has protease activity (contains protein digesting enzymes), it results in a ‘pre-digesting’ effect on gluten protein. That on its turn, affects inactivation of epitopes and perhaps, as well, the decrease of possible harmful effects of other components such as FODMaPs, as indicated in preliminary results from the HealthBread project. In Australian studies of the fermentation of rye dough, as well, a minimal decrease (10-15%) in FODMaP content is described. Which

FODMaPs specifically and which FODMaP-containing products exactly cause the complaints in IBS patients has still been insufficiently demonstrated (Costable et al., 2014).

Costable et al. (2014), also investigated the effect of different types of bread and preparation methods (yeast and sourdough, including different fermentation times) on the composition of the faeces of healthy test subjects and people with IBS. In the study, various parameters were studied (in vitro), two of which showed a striking result. Two groups of 'bad' intestinal bacteria decreased with longer fermentation of sourdough bread (15 hours or more), whilst the ‘good’ bifidobacteria showed a significant increase in healthy subjects. It was also remarkable that sourdough bread gave a lowered and more gradual gas formation by the faecal bacteria. For these reasons, the researchers conclude that sourdough bread with a long fermentation time (from 15 hours) probably leads to fewer negative symptoms in patients with IBS.

Since the fifties of the last century the Netherlands has eaten less and less sourdough bread, in favour of bread made with a fast rising process using yeast. Since that time, as well, increasing amounts of vital gluten have been used as bread upgrader in order to realise a lighter bread with a greater volume. The existence of a direct link between an ‘accelerated' bread making process and an increase in the number of cases of coeliac disease and NCWS/NCGS has not been established unambiguously. In the Netherlands, bread has been prepared using yeast since at least the 1950s. As mentioned above, there are indications that long-term fermentation of wheat dough, can change the composition such that certain nutrients become more readily available for human digestion and absorption. In this context it is interesting to note that the prevalence of coeliac disease in Germany, which has a long tradition of eating sourdough bread (and also rye bread), is significantly lower than in other European countries, such as the Netherlands (0.3% versus 1-2% of the population). However, from this observation, no causal relation can be concluded.

5.3.2 Alternatives to gluten

Another approach involves the development of alternatives to gluten in order to prepare a good visco-elastic dough. One such alternative is based on the use of whey protein from milk. The first experiments using this protein in a starch blend yielded a dough-like structure (Van Riemsdijk et al., 2011). Subsequent experiments, as well, with the preparation of dough from oat flour are promising (Londono et al., 2014).

5.4 Long-term

5.4.1 Selection low-immunogenic wheat lines

Many thousands of wheat varieties and species have been collected in gene banks around the world. These include modern and ancient hexaploid and tetraploid varieties, local varieties, wild species, cultivated and diploid species, including the ancestors of the current hexaploid bread wheat with the A, B and D genome (Molberg et al., 2005; Salentijn et al. 2013; Salentijn et al., 2009; Spaenij-Dekking et al., 2005; Van den Broeck et al., 2010a; Van den Broeck et al., 2010b; Van Herpen et al., 2006). These collections provide a useful source for further research into gluten proteins (and their genes) that cause coeliac disease and the identification of wheat lines with a decreased coeliac response. Such research has already shown that the α- and γ-gliadins from the D-genome contain most of the immunogenic epitopes, followed by those from the A-genome, whereas the gliadins from the B-genome are free of these epitopes. Based on protein analysis and gene expression, several tetraploid wheat lines have now been found with an epitope profile that indicates significantly reduced coeliac immunogenicity. These results should be further confirmed with patients' panels. Additionally, a diploid species (the einkorn wheat 'Monlis') was selected that was already expected to be tolerated well by patients (Zanini et al., 2013). Such lines with favourable epitope profiles could be very useful in breeding programmes.

5.4.2 Deletion lines

Deletion lines are developed by natural mutagenesis. It involves removal of certain pieces of chromosomes, allowing us to gain a better understanding of missing genes (Endo and Gill, 1996). The genes for the α-gliadins in wheat are located on the short arm of chromosome 6. Elimination of the relevant piece of chromosome 6 of the D-genome of hexaploid wheat (in this case, the wheat line Chinese Spring) resulted in a strong reduction of immunogenic epitopes, and even an improvement of the dough quality. The dough of Chinese Spring wheat is usually very sticky and sensitive to being mixed too long. Elimination of γ and ω gliadins (genes are located on chromosome 1) showed a reduction of immunogenic epitopes, but the baking quality remained. Further studies also showed that the quality could be improved even more by the addition of avenin protein from oat, which is free of CD-epitopes (Van den Broeck et al., 2011). Chinese Spring wheat is a model wheat line and the deletion lines from this were developed decades ago for research purposes. It is not expected that these lines will be developed into commercial varieties. This would require working with different hexaploid wheat starting material. There are also deletion lines of more modern bread and pasta wheats (MacRitchie and Lafiandra, 2001).

5.4.3 Synthetic hexaploids

By crossing tetraploid wheat lines (emmer and durum type, with AABB genome composition) with diploid lines with the D-genome, so-called synthetic hybrids can be obtained with the AABBDD genome composition. This type of work was begun at the CIMMYT and the former Centre for Plant Breeding and Reproduction Research in Wageningen (CPRO-DLO) (Lange and Jochemsen, 1992) and is now being continued on a large scale by the NIAB in the UK. All current commercial bread wheat varieties contain the D-genome of probably one ancestor. This D-genome contains many epitopes. Research is now underway to find other D-genome parental lines (read: other diploid wheat species with the D-genome) that contain much fewer immunogenic epitopes and thus can make a synthetic hexaploid bread wheat with a substantially reduced coeliac immunogenicity (Collaborative Wageningen UR NIAB in the UK). Crossings are often required to obtain lines with good agronomic and technological qualities. An interesting question that remains is the baking quality of these new hybrids with a decreased immunogenic epitope content. By now the NIAB has thousands of pre-breeding lines in the test phase. The first commercial synthetic varieties are expected to be on the market around 2020 (www.niab.com).

5.4.4 Genetic modification targeted at less 'harmful' varieties of wheat

With the aid of so-called ‘gene-silencing’ technology, a gene can specifically be shut down so that the protein in question (for example, a gluten protein in the seed of grain) is not formed. This method is based on the breakdown of specific RNA by RNA interference (RNAi; RNA silencing). In this way several wheat lines have been produced that no longer contain gliadins and thus also less immunogenic epitopes. The baking quality of these lines proved to be fairly well preserved (Gil-Humanes et al., 2012; Piston et al., 2011). It is also possible through another molecular biological approach to block the DNA of specific genes in such a way that it cannot be transcribed (Wen et al., 2012). If the agronomic properties, the yield and the technological quality of such lines are acceptable, they could be applied in the production of ‘gluten-free’ or ‘low-in-gluten’ foods. Here it should be noted, however, that this involves specific forms of genetic modification. Not all European countries feel positively concerning this topic. The laws and regulations in Spain are more relaxed. This is why a great deal of research on GM wheat takes place there. Thus far there have been no GM wheat products on the European market (Gil-Humanes et al., 2014a; Gil-Humanes et al., 2014b; Gil-Humanes et al., 2008; Gil-Humanes et al., 2012; Gil-Humanes et al., 2010; Pistón et al., 2011; Van den Broeck et al., 2011; Van den Broeck et al., 2009; Zörb et al., 2013). These technologies could also be applied for the reduction of the levels of ATIs and (indirectly) for FODMaPs. However, many years of research will be

required in order to develop varieties in which the proper genes are eliminated but still remain suitable for commercial cultivation and food production.

6. Possible nutritional technology solutions; current and planned studies

6.1 Introduction

All the solutions mentioned in chapter 5 are aimed at reducing exposure to wheat ingredients that may cause coeliac disease or other hypersensitivity, but not necessarily at eliminating them completely. We assume that a lower exposure to these components is favourable for health.

It is known that for people with coeliac disease or wheat allergy, eating gluten-free and wheat-free products have thus far been the only solution. This may include the use of alternative grains (5.2.1, see above). Perhaps the use of alternatives to gluten could also be a solution (5.2.2 and 5.3.2, see above). There are promising experiments, but thus far few specific application options. In the long term it might be possible to develop less immunogenic wheat varieties for both groups (5.4, see above). In coeliac disease this would involve modification of the coeliac epitopes, making them less harmful. In people with wheat allergy this is a bit more complicated, because it varies from one individual to another to which wheat proteins they react.
The third group of people with NCWS/NCGS is the largest growing group about whom the least is still known. For this group it must first be determined to which ingredients in wheat they react.

The table below lists possible solutions per target group. In the group of NCWS/NCGS, it is also specified which aspects should be investigated first. Nevertheless, for this group, Table 4 lists on the basis of existing hypotheses nutritional technological solutions.

For the NCWS/NCGS group it is also stated in Table 5 what investigations are needed in order to delineate the components to which this group reacts. Some of these studies are already underway or are in preparation in a large international research consortium and are listed briefly below:
Effect of process and raw material: In 2015/2016*, a study will be carried out on the effect of short and long fermentation with different starter cultures and types of grain on CD epitope modification and the quantity of FODMaPs present (2014/2015).

Effect of process, raw material and nocebo effect*:
This concerns a double blind placebo controlled human intervention study on effects of different grains (wheat, spelt, emmer) and the effects of processing (short and long fermentation, yeast versus sourdough). Testing will involve a number of markers that are

related to wheat food hypersensitivity. Within the same proposal, an in vivo study will also be carried out that measures the effects (functional and metabolic) of the consumption of products prepared by a different process on post-consumption complaints/symptoms in IBS patients (2015/2016 and beyond). It will also be studied to what extent a nocebo effect might be at play here.

* for information about these studies, conducted in a consortium under coordination of Maastricht University, NL, Wageningen University, NL, Rothamsted Research Institute, UK, and the Dutch Bakery Center (NBC), NL, contact Fred.Brouns@maastrichtuniversity.nl
Regarding the effect of reducing a possible gluten load, to our knowledge no studies have been started at the moment, nor are any in preparation.

In conclusion

Especially for the short and medium term, for people with coeliac disease and wheat allergy concrete solutions are available with which the bakery sector can immediately get to work. This involves the use of alternative grains and alternatives to gluten. For the group of NCWS/NCGS patients, more studies must be carried out first in which existing hypotheses are tested for their validity.

Condition/main group	Solution
Coeliac disease	Short term: alternative grains Medium term: alternatives to gluten Long-term: selection and modification of wheat lines
Wheat allergy	Short term: alternative grains Long-term: selection and modification of wheat lines

Non Coeliac Wheat/Gluten Sensitivity	Necessary research: 1) Further research to which ingredients of wheat they react: - Process: effect of short and long fermentation, on modification of protein epitopes and FODMaPs - Raw material: what type of grain is low in FODMaPs and/or in other proteins that can bring about hypersensitivity such as ATIs, lectins, LTPs) - Nocebo effect: to what extent is there a nocebo effect in NCWS/NCGS patients? - Gluten load: effects of lower gluten load by use of alternative grains and/or use of less vital gluten 2) Determination of the prevalence of the NCWS/NCGS group in the Netherlands Possible solutions for NCWS/NCGS: Short term: alternative grains Short term: Reduction of the vital gluten Medium term: The use of fermentation processes with sourdough and protease activity Long-term: selection and modification of wheat lines

Table 5. Overview of solutions by main group

7. References

- Alun Jones, V., Shorthouse, M., McLaughlan, P., Workman, E., Hunter, J.O., 1982. Food intolerance: A major factor in the pathogenesis of irritable bowel syndrome. The Lancet 320, 1115-1117.
- Atchison, J., Head, L., Gates, A., 2010. Wheat as food, wheat as industrial substance;
- comparative geographies of transformation and mobility. Geoforum 41, 236-246.
- Barrett, J.S., Gibson, P.R., 2012. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMaPs) and nonallergic food intolerance: FODMaPs or food chemicals? Therap. Adv. Gastroenterol. 5, 261-268.
- Biesiekierski, J.R., Rosella, O., Rose, R., Liels, K., Barrett, J.S., Shepherd, S.J., Gibson, P.R., Muir, J.G., 2011. Quantification of fructans, galacto-oligosacharides and other short-chain carbohydrates in processed grains and cereals. J. Hum. Nutr. Diet. 24, 154-176.
- Biesiekierski JR, Muir JG, Gibson PR (2013) Is gluten a cause of gastrointestinal symptoms in people without coeliac disease. Curr. Allergy Asthma Rep. DOI 10.1007/s11882-013-0386-4 Boettcher, E., Crowe, S.E., 2013. Dietary Proteins and Functional Gastrointestinal Disorders. Am. J. Gastroenterol. 108, 728-736.
- Brouns FJPH, van Buul VJ, Shewry PR. Does wheat make us fat and sick? Journal of Cereal Science (0). doi: http://dx.doi.org/10.1016/j.jcs.2013.06.002
- Bucci, C., Zingone, F., Russo, I., Morra, I., Tortora, R., Pogna, N., Scalia, G., Iovino, P., Ciacci, C., 2013. Gliadin Does Not Induce Mucosal Inflammation or Basophil Activation in Patients with Non-Coeliac Gluten Sensitivity. Clin. Gastroenterol. Hepatol. 11, 1294-1299.e1.
- Cabrera-Chávez, F., Calderón de la Barca, A.M., 2010. Trends in wheat technology and modification of gluten proteins for dietary treatment of coeliac disease patients. J. Cereal Sci. 52, 337-341.
- Capili, B., Chang, M., Anastasi, J.K., 2014. A Clinical Update: Noncoeliac Gluten Sensitivity—Is It Really the Gluten? J. Nurse Pract. 2014, 10, 666-673.
- Carroccio, A., Mansueto, P., Iacono, G., Soresi, M., D'Alcamo, A., Cavataio, F., Brusca, I., Florena, A.M., Ambrosiano, G., Seidita, A., Pirrone, G., Rini, G.B., 2012. Non-Coeliac Wheat Sensitivity Diagnosed by Double-Blind Placebo-Controlled Challenge: Exploring a New Clinical Entity. Am. J. Gastroenterol. 107, 1898-1906.
- Cash, B.D., Rubenstein, J.H., Young, P.E., Gentry, A., Nojkov, B., Lee, D., Andrews, A.H., Dobhan, R., Chey, W.D., 2011. The Prevalence of Coeliac Disease Among Patients With Nonconstipated Irritable Bowel Syndrome Is Similar to Controls. Gastroenterology 141, 1187-1193.
- Colomba, M.S., Gregorini, A., 2012. Are Ancient Durum Wheats Less Toxic to Coeliac Patients? A Study of α-Gliadin from Graziella Ra and Kamut. ScientificWorldJournal 2012, 8.

- Cordain, L., 1999. Cereal grains: humanity's double-edged sword. World Rev. Nutr. Diet. 84, 19-73.
- Di Sabatino, A., Corazza, G.R., 2012. Noncoeliac Gluten Sensitivity: Sense or Sensibility? Ann. Intern. Med. 156, 309-311.
- DiGiacomo, D.V., Tennyson, C.A., Green, P.H., Demmer, R.T., 2013. Prevalence of gluten-free diet adherence among individuals without coeliac disease in the USA: Results from the continuous national health and nutrition examination survey 2009-2010. Scand. J. Gastroenterol. 48, 921-925.
- Endo, T.R., Gill, B.S., 1996. The deletion stocks of common wheat. J. Hered. 87, 295-307.
- ESPGHAN-Agostini et al: Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2008; 46: 99-110 : http://www.espghan.org/guidelines/nutrition/
- Gil-Humanes, J., Pistón, F., Altamirano-Fortoul, R., Real, A., Comino, I., Sousa, C., Rosell, C.M., Barro, F., 2014a. Reduced-Gliadin Wheat Bread: An Alternative to the Gluten-Free Diet for Consumers Suffering Gluten-Related Pathologies. PLoS ONE 9, e90898.
- Gil-Humanes, J., Pistón, F., Barro, F., Rosell, C.M., 2014b. The Shutdown of Coeliac Disease-Related Gliadin Epitopes in Bread Wheat by RNAi Provides Flours with Increased Stability and Better Tolerance to Over-Mixing. PLoS ONE 9, e91931.
- Gil-Humanes, J., Pistón, F., Hernando, A., Alvarez, J.B., Shewry, P.R., Barro, F., 2008. Silencing of γ-gliadins by RNA interference (RNAi) in bread wheat. J. Cereal Sci. 48, 565-568.
- Gil-Humanes, J., Pistón, F., Rosell, C.M., Barro, F., 2012. Significant down-regulation of γ-gliadins has minor effect on gluten and starch properties of bread wheat. J. Cereal Sci. 56, 161-170.
- Gil-Humanes, J., Piston, F., Tollefsen, S., Sollid, L.M., Barro, F., 2010. Effective shutdown in the expression of coeliac disease-related wheat gliadin T-cell epitopes by RNA interference. Proc. Natl. Acad. Sci. 107, 17023-17028.
- Gilissen, L.J.W.J., van den Broeck, H.C., Londono, D.M., Salentijn, E.M.J., Koning, F., van der Meer, I.M., Smulders, M.J.M., 2012. Food-related strategies towards reduction of gluten intolerance and gluten sensitivity. In: Koehler, P. (Ed.) Proceedings of the 25th meeting of the Working Group on Prolamin Analysis and Toxicity. Fellbach, Germany, pp. 29-35.
- Gilissen, L.J.W.J., van der Meer, I.M., Smulders, M.J.M., 2014. Reducing the incidence of allergy and intolerance to cereals. J. Cereal Sci. 59, 337-353.
- Greco, L., Gobbetti, M., Auricchio, R., Di Mase, R., Landolfo, F., Paparo, F., Di Cagno, R., De Angelis, M., Rizzello, C.G., Cassone, A., Terrone, G., Timpone, L., D'Aniello, M., Maglio, M., Troncone, R., Auricchio, S., 2011. Safety for Patients With Coeliac Disease of Baked Goods Made of Wheat Flour Hydrolyzed During Food Processing. Clin. Gastroenterol. Hepatol. 9, 24-29.

- Gupta, P.K., Mir, R.R., Mohan, A., Kumar, J., 2008. Wheat genomics: Present status and future prospects. Int. J. Plant Genom. vol. 2008.
- Hischenhuber, C., Crevel, R., Jarry, B., MaKi, M., Moneret-Vautrin, D.A., Romano, A., Troncone, R., Ward, R., 2006. Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. Aliment. Pharmacol. Ther. 23, 559-575.
- Ivarsson A, Myléus A, Norström F, van der Pals M, Rosén A, Högberg L, et al. Prevalence of childhood coeliac disease and changes in infant feeding. Pediatrics. 2013;131:e687-94.
- Junker, Y., Zeissig, S., Kim, S.-J., Barisani, D., Wieser, H., Leffler, D.A., Zevallos, V., Libermann, T.A., Dillon, S., Freitag, T.L., Kelly, C.P., Schuppan, D., 2012. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J. Exp. Med. 209, 2395-2408.
- Kasarda, D.D., 2013. Can an increase in coeliac disease be attributed to an increase in the gluten content of wheat as a consequence of wheat breeding? A perspective. J. Agric. Food Chem. 61, 1155-1159.
- Kotaniemi-Syrjänen, A., Palosuo, K., Jartti, T., Kuitunen, M., Pelkonen, A.S., Mäkelä, M.J., 2010. The prognosis of wheat hypersensitivity in children. Pediatr. Allergy Immunol. 21, e421-e428.
- Lange, W., Jochemsen, G., 1992. Use of the gene pools of Triticum turgidum ssp. dicoccoides and Aegilops squarrosa for the breeding of common wheat (T. aestivum), through chromosome-doubled hybrids. Euphytica 59, 213-220.
- Londono, D.M., Smulders, M.J.M., Visser, R.G.F., Gilissen, L.J.W.J., Hamer, R.J., 2014. Development of a standard test for dough-making properties of oat cultivars. J. Cereal Sci. 59, 56-61.
- Londono, D.M., van't Westende, W.P.C., Goryunova, S., Salentijn, E.M.J., van den Broeck, H.C., van der Meer, I.M., Visser, R.G.F., Gilissen, L.J.W.J., Smulders, M.J.M., 2013. Avenin diversity analysis of the genus Avena (oat). Relevance for people with coeliac disease. J. Cereal Sci. 58, 170-177
- Ludvigsson J.F. and Fasano A. Timing of Introduction of Gluten and Celiac Disease Risk. Ann Nutr Metab 2012;60 (suppl 2):22–29
- Ludvigsson, J.F., Leffler, D.A., Bai, J.C., Biagi, F., Fasano, A., Green, P.H.R., Hadjivassiliou, M., Kaukinen, K., Kelly, C.P., Leonard, J.N., Lundin, K.E.A., Murray, J.A., Sanders, D.S., Walker, M.M., Zingone, F., Ciacci, C., 2013. The Oslo definitions for coeliac disease and related terms. Gut 62, 43-52.
- MacRitchie, F., Lafiandra, D., 2001. Use of Near-Isogenic Wheat Lines to Determine Protein Composition-Functionality Relationships. Cereal Chem. 78, 501-506.
- Marsh, M.N., 1992. Gluten, major histocompatibility complex, and the small intestine: A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('coeliac sprue'). Gastroenterology 102, 330-354.

- Mazzarella, G., Salvati, V.M., Iaquinto, G., Stefanile, R., Capobianco, F., Luongo, D., Bergamo, P., Maurano, F., Giardullo, N., Malamisura, B., Rossi, M., 2012. Reintroduction of Gluten Following Flour Transamidation in Adult Coeliac Patients: A Randomized, Controlled Clinical Study. Clin. Dev. Immunol. 2012, 10.
- Mazzeo, M.F., Bonavita, R., Maurano, F., Bergamo, P., Siciliano, R.A., Rossi, M., 2013. Biochemical modifications of gliadins induced by microbial transglutaminase on wheat flour. Biochim. Biophys. Acta 1830, 5166-5174.
- Molberg, Ø., Uhlen, A.K., Jensen, T., Flæte, N.S., Fleckenstein, B., Arentz-Hansen, H., Raki, M., Lundin, K.E.A., Sollid, L.M., 2005. Mapping of gluten T-cell epitopes in the bread wheat ancestors: Implications for coeliac disease. Gastroenterology 128, 393-401.
- Mustalahti K, Catassi C, Reunanen A, et al. The prevalence of CD in Europe: results of a centralized, international mass screening project. Ann Med 2010; 42: 587–95.
- Nijeboer, P., Mulder, C.J.J., Bouma, G., 2013. Non-coeliac gluten sensitivity: Hype, or new epidemic? Nederlands Tijdschrift voor Geneeskunde 157.
- Pastorello, E.A., Farioli, L., Conti, A., Pravettoni, V., Bonomi, S., Iametti, S., Fortunato, D., Scibilia, J., Bindslev-Jensen, C., Ballmer-Weber, B., Robino, A.M., Ortolani, C., 2007. Wheat IgE-Mediated Food Allergy in European Patients: α-Amylase Inhibitors, Lipid Transfer Proteins and Low-Molecular-Weight Glutenins. Int. Arch. Allergy Immunol. 144, 10-22.
- Peumans, W.J.; Van Damme, E.J. Prevalence, Biological activity and genetic manipulation of lectins in foods. Trends Food Sci. Technol. 1996, 7,132-138
- Pistón, F., Gil-Humanes, J., Rodríguez-Quijano, M., Barro, F., 2011. Down-Regulating γ-Gliadins in Bread Wheat Leads to Non-Specific Increases in Other Gluten Proteins and Has No Major Effect on Dough Gluten Strength. PLoS ONE 6, e24754.
- Prandi, B., Faccini, A., Tedeschi, T., Galaverna, G., Sforza, S., 2013. LC/MS analysis of proteolytic peptides in wheat extracts for determining the content of the allergen amylase/trypsin inhibitor CM3: Influence of growing area and variety. Food Chem. 140, 141-146.
- Rewers M. 2005. Epidemiology of coeliac disease: what are the prevalence, incidence, and progression of coeliac disease? Gastroenterology 128:S47–51.
- Reilly, N., Green, P., Epidemiology and clinical presentations of coeliac disease, Semin Immunopathol. 34 (2012) 473–478.
- Richtlijn coeliac disease en dermatitis herpetiformis uit 2007 (http://www.glutenvrij.nl/uploaded/FILES/02_NCVinfo/rl_coeliac disease_08.pdf)
- Richtlijn voor de vezelconsumptie, Gezondheidsraad 2006
- Rizzello, C.G., De Angelis, M., Di Cagno, R., Camarca, A., Silano, M., Losito, I., De Vincenzi, M., De Bari, M.D., Palmisano, F., Maurano, F., Gianfrani, C., Gobbetti, M., 2007. Highly Efficient Gluten Degradation by Lactobacilli and Fungal Proteases during Food

Processing: New Perspectives for Coeliac Disease. Appl Environ Microbiol 73, 4499-4507.

- Rubio-Tapia, A., Ludvigsson, J.F., Brantner, T.L., Murray, J.A., Everhart, J.E., 2012. The Prevalence of Coeliac Disease in the United States. Amercan Journal of Gastroenterology 107, 1538-1544.
- Salentijn, E., Esselink, D., Goryunova, S., van der Meer, I., Gilissen, L., Smulders, M., 2013. Quantitative and qualitative differences in coeliac disease epitopes among durum wheat varieties identified through deep RNA-amplicon sequencing. BMC Genomics 14, 905.
- Salentijn, E.M.J., Goryunova, S., Bas, N., van der Meer, I.M., van den Broeck, H.C., Bastien, T., Gilissen, L.J.W.J., Smulders, M.J.M., 2009. Tetraploid and hexaploid wheat varieties reveal large differences in expression of alpha-gliadins from homoeologous Gli-2 loci. BMC Genomics 10, 48.
- Sapone, A., Bai, J., Ciacci, C., Dolinsek, J., Green, P., Hadjivassiliou, M., Kaukinen, K., Rostami, K., Sander, D., Schumann, M., Ullrich, R., Villalta, D., Volta, U., Catassi, C., Fasano, A., 2012. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Medicine 10, 13.
- Sapone, A., Lammers, K., Casolaro, V., Cammarota, M., Giuliano, M., De Rosa, M., Stefanile, R., Mazzarella, G., Tolone, C., Russo, M., Esposito, P., Ferraraccio, F., Carteni, M., Riegler, G., de Magistris, L., Fasano, A., 2011. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: coeliac disease and gluten sensitivity. BMC Medicine 9, 23.
- Sicherer, S.H., 2001. Clinical implications of cross-reactive food allergens. J. Allergy Clin. Immunol. 108, 881-890.
- Sollid, L., Qiao, S.-W., Anderson, R., Gianfrani, C., Koning, F., 2012. Nomenclature and listing of coeliac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. Immunogenics 64, 455-460.
- Spaenij-Dekking, L., Kooy-Winkelaar, Y., van Veelen, P., Drijfhout, J.W., Jonker, H., van Soest, L., Smulders, M.J.M., Bosch, D., Gilissen, L.J.W.J., Koning, F., 2005. Natural variation in toxicity of wheat: potential for selection of nontoxic varieties for coeliac disease patients. Gastroenterology 129, 797-806.
- Staudacher, H.M., Whelan, K., Irving, P.M., Lomer, M.C.E., 2011. Comparison of symptom response following advice for a diet low in fermentable carbohydrates (FODMAP) versus standard dietary advice in patients with irritable bowel syndrome. J. Hum. Nutr. Diet. 24, 487-495.
- Stein, J., Schuppan, D., 2014. Coeliac Disease - New Pathophysiological Findings and Their Implications for Therapy. Viszeralmedizin 30, 156-165.
- Tatham, A.S., Shewry, P.R., 2008. Allergens to wheat and related cereals. Clin. Exp. Allergy 38, 1712-1726.

- Van Buul, V.J., Brouns, F.J.P.H., 2014, Health effects of wheat lectins: A review. J. Cereal Sci. 59, 112-117.
- Van den Broeck, H.C., de Jong, H.C., Salentijn, E.M.J., Dekking, L., Bosch, D., Hamer, R.J., Gilissen, L.J.W.J., van der Meer, I.M., Smulders, M.J.M., 2010a. Presence of coeliac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of coeliac disease. Theor. Appl. Genet. 121, 1527-1539.
- Van den Broeck, H.C., Gilissen, L.J.W.J., Smulders, M.J.M., van der Meer, I.M., Hamer, R.J., 2011. Dough quality of bread wheat lacking a-gliadins with coeliac disease epitopes and addition of coeliac-safe avenins to improve dough quality. J. Cereal Sci. 53, 206-216.
- Van den Broeck, H.C., Hongbing, C., Lacaze, X., Dusautoir, J.-C., Gilissen, L.J.W.J., Smulders, M.J.M., van der Meer, I.M., 2010b. In search of tetraploid wheat accessions reduced in coeliac disease-related gluten epitopes. Mol. Biosyst. 6, 2206-2213.
- Van den Broeck, H.C., van Herpen, T.J.W.M., Schuit, C., Salentijn, E.M.J., Dekking, L., Bosch, D., Hamer, R.J., Smulders, M.J.M., Gilissen, L.J.W.J., van der Meer, I.M., 2009. Removing coeliac disease-related gluten proteins from bread wheat while retaining technological properties: a study with Chinese Spring deletion lines. BMC Plant Biol. 9, 41.
- Van der Waaij, L.A., Stevens, J., 2014. Het FODMaP-beperkte dieet: effectief bij PDS. Nederlands Tijdschrift voor Geneeskunde 158, 1-6.
- Van Herpen, T.W.J.M., Goryunova, S., van der Schoot, J., Mitreva, M., Salentijn, E.M.J., Vorst, O., Schenk, M., van Veelen, P., Koning, F., van Soest, L., Vosman, B., Bosch, D., Hamer, R.J., Gilissen, L.J.W.J., Smulders, M.J.M., 2006. Alpha-gliadin genes from the A, B, and D genomes of wheat contain different sets of coeliac disease epitopes. BMC Genomics 7, 1.
- Van Riemsdijk, L.E., Pelgrom, P.J.M., van der Goot, A.J., Boom, R.M., Hamer, R.J., 2011. A novel method to prepare gluten-free dough using a meso-structured whey protein particle system. J. Cereal Sci. 53, 133-138.
- Vazquez-Roque, M.I., Camilleri, M., Smyrk, T., Murray, J.A., Marietta, e., O'neill, J., Carlson, P., Lamsam, J., Janzow, D., Eckert, D., Burton, D. and Alan R. Zinsmeister A Controlled Trial of Gluten-Free Diet in Patients With Irritable Bowel Syndrome-Diarrhea: Effects on Bowel Frequency and Intestinal Function. Gastroenterology 2013;144:903–911
- Ward, J.L., Poutanen, K., Gebruers, K., Piironen, V., Lampi, A.-M., Nyström, L., Andersson, A.A.M., Åman, P., Boros, D., Rakszegi, M., Bedő, Z., Shewry, P.R., 2008. The HEALTHGRAIN Cereal Diversity Screen: Concept, Results, and Prospects. J. Agric. Food Chem. 56, 9699-9709.
- Wen, S., Wen, N., Pang, J., Langen, G., Brew-Appiah, R.A.T., Mejias, J.H., Osorio, C., Yang, M., Gemini, R., Moehs, C.P., Zemetra, R.S., Kogel, K.-H., Liu, B., Wang, X., von Wettstein,

D., Rustgi, S., 2012. Structural genes of wheat and barley 5-methylcytosine DNA glycosylases and their potential applications for human health. Proc. Natl. Acad. Sci. 109, 20543-20548.

- www.mlds.nl, vezelrijke voeding
- Zanini, B., Petroboni, B., Not, T., Di Toro, N., Villanacci, V., Lanzarotto, F., Pogna, N., Ricci, C., Lanzini, A., 2013. Search for atoxic cereals: a single blind, cross-over study on the safety of a single dose of Triticum monococcum, in patients with coeliac disease. BMC Gastroenterology 13, 1-5.
- Zörb, C., Becker, D., Hasler, M., Muehling, K.H., Gödde, V., Niehaus, K., Geilfus, C.M., 2013. Silencing of the sulphur rich a-gliadin storage protein family in wheat grains (Triticum aestivum L.) causes no unintended side-effects on other metabolites. Front. Plant Sci. 4.
- Zuidmeer, L., Goldhahn, K., Rona, R.J., Gislason, D., Madsen, C., Summers, C., Sodergren, E., Dahlstrom, J., Lindner, T., Sigurdardottir, S.T., McBride, D., Keil, T., 2008. The prevalence of plant food allergies: A systematic review. J. Allergy Clin. Immunol. 121, 1210-1218.e4.