



# VALUE ADDED WHEAT CRC PROJECT REPORT

Project 2.1.9 - Gluten structure and modification for ingredient use

## Gluten Uses and Food Industry Needs

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## SUMMARY

Wheat gluten, manufactured by washing starch from dough, is used in a wide range of products, especially for the food industry and also beyond food applications. In these many applications, gluten is used in various forms, as vital gluten (retaining its viscoelastic properties), as non-vital gluten (for which heat treatment has reduced rheological qualities), and in various modified forms (following modification by enzymic, chemical and/or physical treatments). Despite the breadth of uses of gluten in these forms, there is still scope to expand gluten utilisation, taking advantage of its modest cost and of the functional properties of gluten and modified glutes.

This report reviews the production and modification of gluten, and its current status as a food additive. Secondly, the broad needs of industry are reviewed with respect to the ingredients and the functional properties required. Finally, opportunities are explored for matching ingredient needs to the properties of existing and potential gluten preparations. In this way, research opportunities for gluten treatment have been identified. These include improved methods for solubilising gluten, for conventional and emerging food uses, processing treatments to modify the structure of gluten, interactions of gluten preparations with other food polymers, and fractionation of gluten into its constituent proteins, thus leading to diversified uses.

At the outset of this project (VAW CRC 2.1.9), there was the specific need to examine the "potential of altered processing of gluten to overcome problems associated with lipids, colour or odour." Several approaches to achieve this aim have been identified in the project's research. These are included below in the list of approaches to be pursued in on-going research:

- Methods based on the addition of specific salts during processing of gluten as a mean of improving the rheological quality, colour and odour of the resulting gluten, and to improve the consistency of gluten products and diversify their uses;
- Further methods for reducing gluten colour and lipid content, and improving flavour, including the use of enzymic treatments;
- Pursue experiments with chemical and enzymic reagents to increase recovery and nutritional quality of gluten, especially following up on transglutaminase use, possibly in combination with salt washing;
- Study the use of gluten-based ingredients as encapsulants;
- Investigate partial fractionation of isolated gluten to produce gliadin-enriched gluten with improved extensibility.

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# **Gluten Uses and Food Industry Needs**

## **A. Introduction**

### **1. The Nature of Gluten**

#### ***What is Gluten?***

Gluten may be described as 'the sticky, viscous residue remaining after removal of starch from dough'. This definition could include 'corn gluten', the protein residue from isolation of starch from corn. However, this material is quite different from wheat gluten, the residue left from the production of starch from wheat-flour dough. In the commercial situation, the term 'gluten' usually refers to wheat gluten. However, for people with food intolerance to cereals, especially coeliac disease, 'gluten' includes the equivalent group of storage proteins from the grains of rye, triticale, barley and possibly oats. Thus, the term 'gluten-free foods' refers to food products free from these cereal proteins, or whose cereal-protein content is less than a defined amount (usually 200 ppm). In this report, only the properties and uses of wheat gluten are discussed.

Gluten is thus primarily defined as the 'cohesive, visco-elastic proteinaceous material prepared as a by-product of the isolation of starch from wheat flour'. A more biological definition would include the origins of the gluten-protein complex as being derived from the 'storage proteins of the wheat grain'. Both definitions are correct but neither tells the whole story. For the purposes of this report, gluten is the commodity isolated on a commercial scale and sold for a variety of purposes in many countries of the world. In particular, this report deals with the dried form of the product ('Vital wheat gluten') from which the functional properties, characteristic of gluten, may be regenerated by rehydration. Just as important for this report are the many products derived from this primary product by various forms of modification, thereby suiting them to a wide range of value-added uses.

#### ***The Composition of Commercial Dry Gluten***

Although sold as a protein, gluten contains more than just protein. As a commodity, dry gluten usually contains approximately 75% protein, up to 8% moisture, and varying amounts of starch, lipid and fibre. The starch and fibre become entrapped in the cohesive matrix of the protein and become more difficult to remove as the protein content increases. The amount of starch varies, and more extensive washing can reduce the starch and fiber content and increase the protein content. The extra water needed for this creates its own problems by producing a larger amount of effluent from the process, and increasing the biological oxygen demand (BOD) of that effluent. Consequently, gluten of higher protein content is only produced as a special order and at a premium price.

Most of the lipid content of the flour becomes associated with the protein during the washing process. The gluten proteins are largely hydrophobic in nature and the lipids bind to the hydrophobic areas of the protein as they are repelled by the water used in the washing. Lipids are strongly bound to gluten proteins and are removed with much more difficulty than they are removed from the original flour. The lipid content of gluten is primarily determined by the lipid content of the flour from which it came, and is unaffected by additional washing.

The protein that makes up gluten is actually a complex mixture of proteins, containing many, probably several hundred, polypeptides, many of them being disulfide-cross linked to one another. A typical amino-acid analysis of the complex mixture is shown in Table 1. The individual proteins are divided into two main classes – monomeric and polymeric. These terms can be confusing in that any protein is a polymer of amino acids. In gluten, monomeric refers to individual, discrete polypeptide species, while polymeric refers to chains formed from individual monomeric proteins by cross-linking them with disulfide bonds of cystine residues in adjoining chains. The monomeric proteins are often called gliadin and the polymeric ones are called glutenin. It should be noted, that some of the glutenin subunits do exist in gluten in the monomeric form.

**Table 1. Amino acid composition of commercial vital wheat gluten.**

Amino acid	Content <sup>1</sup>	Amino acid	Content <sup>1</sup>
Alanine	3.0	Lysine	2.2
Arginine	4.3	Methionine	2.1
Aspartic acid <sup>2</sup>	4.8	Phenylalanine	7.3
Cystine	2.6	Proline	14.6
Glutamic acid <sup>2</sup>	39.0	Serine	5.6
Glycine	4.6	Threonine	3.1
Histidine	2.7	Tyrosine	4.3
Isoleucine	4.4	Valine	4.6
Leucine	8.4		

<sup>1</sup> Values expressed as g amino acid/100g protein

<sup>2</sup> Glutamic acid and aspartic acid are predominantly in the amidated form, with about 90% existing as glutamine and asparagine, respectively.

## **2. The Origins of Dry Gluten as a Commodity**

Gluten was first prepared from flour almost 300 years ago by an Italian named Beccari who conducted a simple water-washing experiment with wheat flour. This discovery, which can be easily reproduced in the home kitchen, has become the basis of a major cereal industry, utilising millions of tonnes of wheat annually in North America, Europe and Australia. The present commercial process is basically an efficient repetition of Beccari's experiment.

### ***The Early Days of Starch Washing***

Bakers, knowing the value of the gluten component of dough, have added gluten to dough for decades. Although the enrichment of bakers' flour with gluten has been common practice in bread manufacture since the second half of the twentieth century, the purification of starch from wheat flour has been practiced for a much longer time. For example, Isaac Reckitt was washing starch from wheat in 1840 at a site in Hull, England (Wrigley, 2002). The procedure, described in 1912, involved 'making a mash of the wheat after soaking in water for

many days, and when soft enough, putting the mash through sieves, the wheat starch passing through, leaving the bran in the sieves to be cast aside for pigs (Anon, 1912). The gluten was afterwards separated from the starch by washing'. The gluten was also discarded for animal feed. The Hull site has continued as a major operations center for the greatly diversified multinational Reckitt-Benckiser. However, the gluten fraction was discarded for some decades before its industrial potential was realised, and before procedures were developed to isolate the gluten as a functional mass, retaining its intrinsic properties for enhancing baking quality. There are at least two independent accounts of the development of gluten manufacture for commercial exploitation.

### ***The Australian – New Zealand Development of Vital Dry Gluten***

One of these stories begins in Wellington, New Zealand in the 1930s, where a pastry cook, Harry Maltwood Williams, discovered a method of extracting gluten from flour by fermentation, water-washing and the late addition of salt (Wrigley, 2000). In association with James Tucker (an advertising agent in Wellington) and Frank Hunter (Managing Director of Stacey & Hawker Ltd, bread manufacturers of Christchurch), experiments with the process produced bread of increased volume and improved texture. The name "Procera" was devised for the product. The process was patented worldwide and a company was formed - the Procera Bread Process (NS) Limited. Initially, funds were generated locally in New Zealand by licensing the use of the patent, permitting the extension of this approach to the larger market of Australia. Harold Bellingham and Reg Bartle went to Australia for this purpose. The latter, an airplane pilot, travelled in his own plane to visit country bakeries, resulting in introductory telegrams such as 'Arriving Kempsey race course at 3 pm. Bartle, Procera'.

In 1932, James Tucker was employed to perform these functions in Australia, marketing the process for producing a 'High Top' loaf. Part of this marketing package was a band of paper to go around each loaf. The paper strip, provided by the Procera company, included the payment of a royalty charge of 0.1 penny per loaf by the baker. The resulting one-pound loaf sold readily for two pence at a time when a two-pound loaf cost 2.5 pence. This marketing method amounted to the modern practice of franchising. Indeed, when the 'Trade Mark Users' Act' was enacted in 1948, Procera Australia Pty Ltd became the first major franchising company to operate in Australia. The process was also introduced to Britain, where it was known as the 'Procea' method.

In the meantime, Nigel Love, of N.B.Love Mills, Sydney, had developed a similar product, which he trade marked as 'Promax' bread. This was made from a high-protein flour, and it gave results similar to the Procera bread process. As the use of the Procera method was limited to one baker in each town or region, the Promax method spread quickly with the remaining bakers.

### ***Origins of the Dried-Gluten Industry***

The term 'starch-reduced' was used for the Procera method because the starch was literally thrown away. This in itself produced an ongoing problem, namely, the disposal of the starchy effluent produced by the protein-enriching step of washing out excess starch. Municipal councils complained of blocked drains and sewage difficulties. These problems were the basis of the development of an industrial-scale process to provide the 'protein-enriched' ingredient for the Procera method in dried form, essentially as dehydrated gluten, at

the same time permitting the recovery of the starch that was washed out, so that it could also be dried and used in foods.

This initiative was initially taken in 1938 by Basil Regan of Fielders Mills in Tamworth, northern New South Wales, Australia, and later by Mr J.L.Raith of the NSW Ycast Company. The problem of drying the wet gluten and retaining its 'vital' nature were tackled by Leonard Winch at Fielders in Tamworth, resulting in a process (the 'ring' or 'flash' drier) of chopping the wet gluten, rolling the pieces in flour and subjecting these to heat drying. From these beginnings in the bread industry has developed the significant export commodity of dried gluten for Australia, and a world-wide industry for the production of dried gluten as a major trade commodity.

### ***Origins of Vital Dry Gluten in North America***

The North American origins start with the industrial need for wheat starch, not gluten (Dubois, 1996). The scene is a flour mill, owned by the Jenks family on the shores of Lake Huron, Michigan. In the early 1900s, it had become unprofitable for them to produce flour for shipping to Detroit, due to competition from larger mills in the Midwest. Accordingly, George C. Jenks and his chemist friend, Billy Rossman, sought new outlets for their flour. They developed a process to separate out the starch. It involved making a slack flour-water dough and separating starch from the gluten by gentle water washing. Initially, the gluten was discarded in favor of the starch, which sold well as laundry starch for skirts and shirts.

In the 1920s, a limited outlet for the heat-dried gluten (termed 'gum gluten') was found in the manufacture of low-carbohydrate bread for diabetics. Later outlets for the gum gluten included the production of monosodium glutamate (in the 1940s) by acid hydrolysis. In addition, the gum gluten found significant use with its incorporation in Kelloggs 'Special K' (Thompson and Raymer, 1958).

Until this stage of development, Jenks and Rossman had used a harsh drying process that devitalised the functional properties of the gluten, rendering it largely useless as an ingredient to enhance baking quality. However, in the late 1950s, the Huron Milling Company adopted the 'new type of drying process used in Australia' (quoted from Dubois, 1996), based on the 'ring' or 'flash' drying process. The resulting dry gluten retained its functional properties. When added to a bread-flour dough, the gluten produced improved loaf volume and better crumb texture. Within a year, the vital dry gluten was being used commercially in a variety of breads. However, at the time, there was little success in attempts to blend the dry gluten with wheat flour at the mill to enhance protein quality. Nevertheless, this has more recently become common practice, especially in Europe where gluten fortification has become essential for many low-protein flours (Spooner, 1995). From these origins, dry gluten has become an important commodity, significant in international trade, mainly as an additive to fortify flour for bread manufacture, but also for the wide range of ingredient uses described in this report.



## **B. The Gluten Industry at Present**

### **3. Current Practices of Gluten Manufacture**

The principle of gluten washing still remains similar to that of Beccari hundreds of years ago. Most commercial operations now use variations of either the Batter Process or the Martin Process (Knight, 1965; Bergthaller et al., 2004).

#### ***The Martin Process***

In this method, a wheat flour dough is washed with water while it passes through a tumbling cylindrical agitator. The work applied to the dough is not dissimilar to the effect of kneading a dough under water in that starch comes out of the dough, while the protein content increases in the remaining dough. The tumbling action moves the dough along the cylinder and the starch passes through small holes in the wall while the protein mass remains inside, receiving further washing until it tumbles out at the end.

#### ***The Batter Process***

In this process, a thick suspension or batter of flour is stirred slowly in a tank for several hours, during which time the starch separates from the protein. The mixture is then passed through a fine sieve, which allows the starch granules to pass through, while the curds of gluten are retained on the screen. This gluten is then washed with water to remove further starch in a similar manner to the Martin process. The gluten is then dried.

The Martin Process is a continuous process, while the nature of the Batter Process makes it more suited to batch operation.

#### ***Other Processes***

Most commercial operators use one or other of the above methods with modifications, but there have been many other processes developed for the commercial production of gluten. While most have not made it past the laboratory curiosity stage, others, for example the Alfa-Laval Raisio process, have been applied in full-scale production facilities. The basis of these other processes varies and some use centrifugal techniques, which may involve either conventional industrial centrifuges or hydro-cyclones to separate the starch from the protein. Many operators use hydro-cyclones as the principal way of cleaning the starch, and, in some cases, for the actual separation of the starch and gluten.

Many of the newer methods utilise whole grain as the raw material, avoiding the production of flour in a dry-milling step. This allows a more complete isolation of the starch fraction from the wheat, but cleaning the protein and starch of residual bran is a major disadvantage of these types of methods. Improved milling processes have reduced the amount of endosperm remaining in the bran and offal fractions in conventional milling, so there is little advantage to be gained in wet-milling for starch and gluten recovery. There is also the need to dry the bran unless it can be processed on the spot or at least locally, and this cost will usually exceed the economic benefit of improved starch yield. Thus, despite these newer methods,

modifications of the traditional processes have remained the preferred choice for almost all the gluten produced worldwide.

### ***Drying Gluten***

The functional properties of gluten are very susceptible to heat when wet, and relatively low temperatures destroy the cohesive, visco-elastic properties ('vitality'), which make it unique among food proteins. Attempts to dry gluten while retaining these properties were unsuccessful until the application of the ring drier to gluten in the first half of the 20<sup>th</sup> century. This process has been the basis of gluten drying since then. The principle is simple - wet gluten, whose moisture content is around 70%, is mixed with sufficient dry gluten to reduce the moisture to about 20%. This lowered-moisture material is comminuted and subjected to flash drying in a ring drier. A portion of the dried gluten is removed and packaged while the rest is returned to the drying cycle to reduce the moisture content of more wet gluten. The procedure is still very sensitive to excessive heat, but with careful control of the temperature, a vital wheat gluten is produced.

An alternative way of drying to prepare a vital gluten is to disperse the gluten in aqueous ammonia or acetic acid and then spray-dry this dispersion. The resulting product retains the visco-elastic properties of gluten, and it may be used for most of the same purposes as normal vital gluten. The cost of this drying procedure, together with environmental concerns, limit its application except for special reasons.

Another dry-gluten product is known as 'devital gluten'. More severe temperatures are intentionally used in its drying, often by drum drying. As a result, this material has lost the characteristic cohesive, visco-elastic properties of native gluten, but it retains the insolubility and water-binding capacity of vital gluten. Devital gluten is commonly used where the cohesiveness of vital gluten can actually be a disadvantage.

### ***Waste Products***

The amount of water required for each tonne of flour varies according to the operator. All processes have a significant waste stream, which consists of the wash water plus soluble protein, damaged starch and sugars, plus some fibre. Disposal procedures for this waste vary, depending on the manufacturer, and methods include fermentation to produce ethanol or methane, isolation by drying for use in animal feed, and discharge into the sewerage system. This last option has become less common as environmental concerns grow worldwide.

## **4. Current Uses and Properties of Commercial Gluten**

### ***Properties of Gluten***

The most important properties of gluten in traditional applications are its lack of solubility in water and its rheological functionality. By the nature of its preparation, gluten is a protein that is insoluble in water. While there are small amounts of water-soluble proteins trapped in the gluten matrix, these are essentially not extractable into water under normal conditions. Despite its insolubility and its hydrophobic nature, gluten absorbs approximately twice its dry weight of water to form a hydrated visco-elastic mass. This material is effectively the same as the wet gluten first isolated from flour. In the case of commercially prepared

gluten, drying conditions may cause some deterioration of the functional properties, but gluten prepared in the laboratory shows no change in its properties after freeze-drying and rehydration.

Two major protein classes make up gluten, namely, gliadin and glutenin. Both are insoluble in water. Gliadin can be solubilised in 70% aqueous ethanol, one of the steps of the Osborne fractionation of wheat proteins, and the residue after this extraction is considered to be glutenin. Isolation of the dissolved material, however, yields a protein which has lost most of its functional properties.

Both gliadin and glutenin may be solubilised to a certain degree by the use of acidic conditions. By careful control of the pH, gluten may be separated into a number of fractions. Reprecipitation by pH adjustment, or by drying the acidic solution directly, gives products which maintain their functional properties. This can be shown by reconstituting flour which is carried out by recombining the starch, the isolated protein fractions and the water-soluble components prepared during the extraction of gluten. Doughs prepared by careful reconstitution show little change in dough strength from that of the original flours.

Its rheological properties are the basis of the functional uses of vital gluten. It is these properties that give wheat flour doughs the characteristics that allow the production of breads, cakes, biscuits and noodles. Thus, gluten can be considered to be like a dough in which the diluting effect of starch is no longer present. In the wet state, the protein molecules form a cohesive matrix which, in dough, also holds the starch granules within it. This matrix is also elastic, allowing it to stretch and expand. In aerated doughs, this elasticity permits the expansion of gas bubbles, which produce the texture of bread and cakes. If the gluten matrix is too weak, or the protein content is too low to form an effective matrix, the bubbles expand beyond the elastic limit and burst, reducing the overall volume of a baked product. In such cases, fortification with added gluten is essential for the satisfactory production of bread.

### ***Allergenicity of gluten***

Foods can provoke adverse reactions that produce different clinical disorder symptoms, at times very severe, depending on an individual's susceptibility to a certain food. Coeliac disease, is a well-known permanent food intolerance to wheat gluten (or related proteins in rye and barley), and is characterised by inflammation of the small intestine which adversely affects the absorption of water and nutrients causing, in some cases, malnutrition. The reaction is a triggered by ingestion of prolamin storage proteins (LMW glutenin,  $\alpha$ - and  $\gamma$ -gliadin), major part of gluten proteins, which results an inappropriate T-cell-mediated immune response against ingested proteins. The only treatment is a strict diet avoiding all products containing gluten.

Allergenicity of gluten is not an issue when gluten is used or added to wheat grain/flour based food products since wheat proteins are likely to be present in the products already. However, there is a concern about the effects of gluten on people with coeliac disease and others with wheat-protein allergies, particularly as the incorporation of gluten into foods that not traditionally contain wheat proteins, is increasing.

Many researchers have been trying to understand the mechanism of the coeliac disease and to reduce allergenicity of the proteins by modifying the allergen structure in such a way that the allergenic epitopes are no longer recognised by the immune system. Until now, the technological approach to decrease allergenicity has largely been empirical. The main reason for this is a lack of detailed knowledge on integral allergen and epitope structure and their genetic background, which hampers the design of more rational, generic strategies for processing.

Several potential approaches have been suggested in an attempt to reduce the allergenicity and/or toxicity of gluten. The use of acidic oxidative potential water (an electrolysed strong acid solution containing active oxygen species) has shown the ability of breaking down gluten proteins, thereby enhancing digestibility and lowering allergenicity of wheat proteins (Matsumoto, 2002). Enzymic processing may also be used to aid the removal of allergenicity. The allergenicity of wheat flour prolamins can be decreased by treatment with the protease bromelain, which cleaves the prolamin IgE-epitope Gln-Gln-Gln-Pro-Pro near the proline residues (Tanabe *et al.*, 1996). The addition of thioredoxin-H reduces intramolecular disulfide bonds in prolamins, before its use in breadmaking, showed a significantly reduced gliadin immunoreactivity in common wheat, TH modification of gliadin might be applied for decreasing immunoreactive properties of wheat storage proteins (Waga *et al.*, 2003). The deamidation process has also been reported to reduce IgE-binding of gluten allergenic protein by modifying glutamine residues in the epitope.

Scientific knowledge about coeliac disease, including knowledge about the proteins that cause the problem and the grains that contain these proteins, is still very much incomplete and is being continuously acquired. Any modification for the application of gluten, particularly in non-cereal based food products, should consider the impact of the modification on either decreasing or increasing the toxicity of gluten proteins to those individual sufferers.

### ***How is Vital Gluten Used?***

The most common usage of gluten in western countries has traditionally been, and continues to be, in baked goods of various types. However, with an increasing awareness of wheat gluten's unique structural and functional properties has come an expanding diversity of applications. The uses of gluten worldwide vary from country to country as shown in Table 2. Gluten is used in other countries in a variety of ways.

The second largest use is in pet foods. Here gluten is added as a protein source to improve the nutritional quality of the pet food. Its hydration and lipid-binding properties also assist in improving the overall properties of the product. A growing market is as an ingredient in aquaculture feed, where its cohesive properties hold the feed together when it is put into water. In addition, it is used in a wide range of food products, including breakfast cereals, meats, cheeses, and snack foods, for seafood analogs and texturised foods.

Non-food applications include pet foods, aquaculture feed, biodegradable plastics, films, coatings, adhesives, inks, cosmetics and pharmaceuticals.

Gluten has been modified in various ways to produce a wide range of food ingredients, providing water-binding and emulsifying properties for meat products, nutritional advantages for sports drinks and medical supplements, dairy analogues such as coffee whitener and calf milk, emulsifier for powdered shortenings, and milk replacement in bakery applications.

**Table 2. Usage of gluten in different regions (as percentage of total usage for region).**

Uses	North America	European Union	Australia	Japan	Total world
Baking	83	17	54	30	63
Flour fortification	1	66	9	-	14
Pet food	12	13	13	-	8
Meats	1	-	9	25*	5
Breakfast cereals	1	-	12	-	2
Noodles	-	-	-	10	-
Sausages	-	-	-	12	-
Other	2	4	3	23	8

- Includes gluten used for synthetic fish products.

### ***Gluten in Baked Goods***

Wheat-based products are a major commercial use of vital gluten, in which the gluten is used to fortify flours of lower-than-desirable protein content. Increasing the protein content of a flour by adding vital gluten improves the quality of the flour to be equivalent to one with a higher protein content. This fortification may be necessary because the flour has a naturally low protein and a higher protein content is needed to make quality products, or because the addition of gluten provides a particular property sought in the food by improving the quality of the protein. This practice has become increasingly common in parts of Europe, where gluten fortification of low-protein bread flours offers an attractive alternative to blending with expensive, imported high-protein wheats to satisfy functional performance requirements. Bakers also use gluten to fortify their basic flours at different levels to obtain desired performance for the production of specialty breads and different types of bakery goods. This minimises flour inventories and avoids storage of high-protein flours.

Vital wheat gluten's unique viscoelastic properties improve dough strength, mixing tolerance, and handling properties. Its film-foaming ability provides gas retention and controlled expansion for improved volume, uniformity, and texture; its thermosetting properties contribute necessary structural rigidity and bite characteristics; and its water absorption capacity improves baked product yield, softness, and shelf-life. The level of gluten used can be quite specific, depending on the particular applications and the required texture and shelf-life of bakery products (Table 3). For example, addition of about 1% of gluten to flour reduces pretzel breakage in the finished product, but the addition of too much gluten may result in pretzels that are too hard to eat. Gluten is used at approximately 2% in pre-sliced hamburger and hot-dog buns to improve the strength of the hinge and provide desirable crust characteristics when buns are stored in a steamer. It can also be used to strengthen pizza crust, making it possible to produce both thin and thick crusts from the same flour. The incorporation of gluten provides crust body and chewiness and reduces moisture transfer from the sauce to the crust.

**Table 3. Use of gluten in bakery foods** Adapted from Maningat *et al.* (1994).

<b>Bakery Products</b>	<b>Use level (% gluten content on a flour basis)</b>
Pizza crust	1-2
Vienna bread, hamburger buns, hard rolls, bran bread, brown soft rolls	2-3
Frozen doughs	2-5
Raisin bread	3-4
Wheat bread with bran	3-4
Salad rolls	4
Multi-grain bread	4-5
High protein bread, bagels	5-6
Whole meal fiber-increased bread	5-7
Whole wheat bread from flaked wheat	10
High-fibre, reduced-calorie bread	8-12
Bread with low slice weight	30

#### ***Gluten in Non-Bakery Food Products and Animal Feeds***

The desired property of gluten in its ability to bind fat and water while at the same time increasing the protein content makes gluten attractive to various types of application in meat, fish, and poultry products. Gluten improves the utilisation of beef, pork and lamb meats by a restructuring process, which converts less desirable fresh meat cuts into more palatable steak-type products. Gluten has also proven as a satisfactory binder for turkey-meat pieces because of its ability to produce intact loaves with good slicing qualities. In processed-meat products, gluten is an excellent binder in poultry rolls, canned “integral” hams, and other non-specific loaf-type products, where it also improves slicing characteristics and minimises cooking losses during processing.

A major use in non-bakery foods is as a meat replacement in vegetarian foods, and in the production of artificial forms of expensive foods such as seafood and crab analogues (Table 4). Due to the growing concern for health and food safety, an increasing number of consumers are looking for meatless alternatives. Pure wet wheat gluten can be seasoned, shaped, and cooked into meatball and steaks. Gluten can also acts as a binder and provides a meat-like structure in veggie burgers. Texturised wheat gluten has been developed by extrusion technology to mimic the mouth-feel, chew, and taste of meat. “Meat” products created by this process are suited to ready-to-eat entrees, as sandwich fillings, or for pizza and salad toppings.

Gluten’s viscoelastic properties can be used in preparing synthetic cheese with the characteristic texture and eating quality of natural cheese. Gluten used alone or in combination with soy protein, has been used to replace approximately 30% of the more expensive sodium caseinate used in imitation-cheese products.

**Table 4. Non-bakery foods and animal feed applications of gluten.** Adapted from Maningat *et al.* (1994).

<b>Non-Bakery Products and Animal Feed</b>	<b>% Gluten content, product basis</b>
Seafood analogues	1.3
Crab analogues	2.1
Artificial caviar	1-30
Frankfurters	3.2
Sausage analogue	8
Meat-like sausage	16.7
Meat-like balls and hamburger	10.6
Restructured beef steaks	3.6
Imitation cheese	5.8-6.3
Synthetic cheese	14.2
Meringue	15.1
High-protein snack	1-50
High-protein pasta	1.6
Breakfast cereals	Not available
Fruit filling for nutritional bar	Not available
Flour tortillas	1-4
Extruded/fibrous wheat gluten products	20-23
Light-coloured seasoning liquid	>25
Pet foods	3-28.2
Aquaculture diet	5-10
Calf-milk replacer	15-30
Piglet food	Not available
Fat-filled powders for animal feed	Not available

Wheat gluten-fortified breakfast cereals have been widely accepted by consumers because they are very flavourful and nutritious, especially when consumed with milk. Kellogg's Special K cereal is perhaps the most familiar example in this product category. In extruded snacks, wheat gluten provides nutritional value, crispness, and desired texture. Gluten has also been mixed with fruit puree to be used as a fruit filling for nutritional bars. Addition of gluten to corn tortillas improves its rollability and pliability.

Gluten is also used in the preparation of soy-sauce extenders, and the manufacture of mono-sodium glutamate. The high glutamine content of gluten makes it an ideal starting material for this latter product. Soy sauce made using gluten has light colour, slow browning rate, excellent flavour and good body over traditional soy sauce. Due to the scare of the bovine spongiform encephalopathy (BSE) disease, replacement of gelatin by other food proteins has found applications for gluten recently in food products, such as chew candy or fruit chew (Patent, EP969611), or as a clarifying agent of musts and white wines (Marchal *et al.*, 2002).

The pet food industry is the second largest user of wheat gluten. The usage may be in preparing simulated meat for canned pet food or in both canned and intermediate moisture-type products, where its water absorption and fat-binding properties can improve yields and quality. Gluten not only binds chunks of raw and cooked meat together, but also absorbs the natural juices of meat that would otherwise be lost during the cooking process. Because of its relative low costs, gluten can also significantly contribute to the nutritional requirements of pet food.

Another increasing use of gluten is in aquaculture feed, as the farming of aquatic species is rapidly expanding. Its adhesive properties provide the binding needed for the pellet or granule forms of feed commonly used, its water insolubility reduces pellet breakdown, its viscoelastic properties can provide a chewy texture preferable to an extremely hard pellet, it lends itself to extrusion and air incorporation, depending on whether surface- or bottom-feeding is desired, and again, it provides nutritional value at low cost.

Gluten and modified glutens have been also used in calf-milk replacements. Because of the variability in price of skimmed milk powder, some vegetable proteins are being considered as alternative sources of protein to provide the amino-acid requirement for young calves. Gluten has been reported to be used as feed and fat-filled powders for feeding piglets (e.g., Amytex and Solpro from Tate & Lyle).

The thermoplasticity and good film-forming properties of wheat gluten may be used to produce natural adhesives. Through the controlled hydrolysis, breaking of sulfide links and the use of plasticisers, the properties of the adhesives can be modulated (Research at Institute for Agrobiotechnology, Tulln, Austria). Recently in a completed EU FAIR project, wheat gluten was used as biopolymer for the production of renewable and biodegradable material. Biomaterials of varying mechanical properties were prepared from industrial gluten (native or deamidated) and from gliadin- and glutenin-enriched fractions using either an aqueous casting procedure or thermo-moulding. The differences of mechanical properties induced by the process of film preparation were larger than those arising from variations of protein composition and properties (except for films cast from water dispersion) due to the wheat genotype, including durum wheat. It was therefore concluded that there is no need for specific breeding as far as uses of wheat proteins in non-food film material are concerned. Hence wheat unsuitable for bread- or pasta-making or low-quality gluten could be used without trouble for preparing plastic films.

### ***Other Non-food Uses***

The properties that make gluten suitable for food use also make it ideal for use in certain types of industrial products. Table 5 lists possible applications of gluten in non-food uses. The list includes some products that are already commercialised, as well as ones tried by researchers to demonstrate the potential of gluten in non-food uses.

Gluten's adhesive properties make it useful in pressure-sensitive medical bandages and adhesive tapes. Its reactivity make it useful for binding heavy metals in industrial processes, removing ink from waste paper, or solidifying waste oils. Peptides from gluten are useful in cosmetics, lotions, and hair preparations such as skin-care products (for firming and moisturising skin from Rachel Perry, Croda, MGP), biodegradable resins (Polytriticum<sup>®</sup>) and other personal care products (Foam Pro L for hair, facial cleansers from MGP Ingredients). Gluten's hydrophobic and (in)solubility properties permit slow-release encapsulation of flavours, colours, medicines, pest- or weed-control agents. Gluten can also be beneficially incorporated into building materials, such as light-weight, frost-resistant concrete. Cross-linked gluten can be used for extracting/recovering heavy metals in solution.



**Table 5. Selected novel applications of wheat gluten.** Adapted from Bietz and Lookhart (1996).

<b>Application</b>	<b>Description</b>	<b>Use level (% gluten in product)</b>
Edible films prolong chewing gum shelf life (Meyers, 1995)	Gluten can polymerise into films and plastics that can serve as moisture or oxygen barriers, useful as coatings on foods or other products.	50
Derivatives of gluten peptides useful as plasticizers (Aranyi <i>et al.</i> , 1970)	Peptides from gluten are chemically modified and used to plasticise polymers from which biodegradable laundry detergent envelopes are formed.	
Thin-walled medical goods (Herold, 1990)	Biodegradable, elastic products such as surgical gloves, catheters, bandages, and condoms prepared from collagens containing 2-50% gluten by extrusion or coagulation.	2 – 50
Biodegradable cereal-based thermoplastics (Lim and Jane, 1994)	Cereal grains treated with organic solvent, and starch and proteins (e.g., gluten) chemically crosslinked. Resulting material molded or extruded into biodegradable, water-resistant articles having high physical strength.	60
Shaped food containers (Aung, 1994)	Flour, starch, and water converted by heat and pressure into rigid, stable shaped food containers. Materials such as gluten can be added that, upon heat and pressure, form a water-repellent coating.	1 – 2
Detergents (Tsuda <i>et al.</i> , 1989)	Partially hydrolysed wheat gluten incorporated into superior detergent for washing dishes, fruits, etc.	
Heavy metal recovery (Winter, 1982)	Heavy metal ions may be selectively extracted from solution through contact with cross-linked vegetable proteins, such as those from wheat.	
De-inking agents for repulping waste paper (Kao Corp., 1984)	Adding wheat gluten when repulping newspaper gives a whiter product than obtained without added gluten.	0.05 - 1.2
Solidification of waste oils (Nagasaka <i>et al.</i> , 1988)	Gluten blended with guar gum, bentonite, and soap powder to form agent for solidification of domestic waste oil.	
Pressure-sensitive adhesive tapes (Aranyi <i>et al.</i> , 1971, Pawelchak <i>et al.</i> , 1981)	Gluten or gluten-derived peptides can be incorporated into pressure-sensitive tapes, including ones suitable for medical use.	50
Reinforced rubber compositions (Yokohama Rubber Co., 1981)	Rubber containing 0.5-10% gluten vulcanised in contact with steel cords to improve rubber cord adhesion.	0.5 – 10

**Table 5 continues.**

Cosmetics (Matsui <i>et al.</i> , 1987)	Gluten-derived peptides (MW 3,000 – 5,000) are water-soluble moisturizing agents suitable for use in cosmetic lotions, creams, and hair products.	
Frost-resistant concrete (Gustafsson <i>et al.</i> , 1986, 1991)	Gluten can be used to entrain air in concrete, thus making it resistant to frost-cracking.	10
Gluten-encapsulated materials (Noznick and Tatter, 1967)	Gluten can encapsulate flavours, colours, or medicines, providing slow-release materials.	
Agricultural pest control granules (Quimby <i>et al.</i> , 1994)	Bacteria, fungi, or other pest- or weed-control agents can be encapsulated in gluten granules that are then coated with oil to slow drying and maintain vitality. In the presence of light and water, active ingredients are slowly released.	
Paper sizing (Kempf <i>et al.</i> , 1989, Kersting and Kempf, 1989)	Gluten may be cyanoethylated, deamidated, or modified with acetic, succinic, or maleic anhydride to produce excellent cobinders for paper sizing.	4 – 5
Biodegradable hydrating cat litter (Kiebke, 1994)	Cat litter incorporates wheat gluten, which encapsulates urine into a permanently hard clump.	
Biodegradable cellulosic material (Wyatt and Wyatt, 1994)	Shock-absorbing biodegradable 'peanut' packing materials can be extruded from cellulose-containing materials to which bonding agents such as gluten are added.	
Pre-emergence weed control (Christians <i>et al.</i> , 1994)	Hydrolysates of gluten can be used as growth regulators to inhibit root formation of germinating weeds among field crops.	
Manufacture of ceramics (Hayes and Roberts, 1994)	A substrate that will vaporize at high temperature is coated with gluten, ceramic material, and water. At high temperature, the substrate burns out, causing the coating to sinter.	10
Cigarette filters (Takenaka <i>et al.</i> , 1969)	Cigarette filters prepared from gluten and wheat flour have high adsorptivity for gases and give excellent smoking taste.	25
Dry-cell batteries (Matsushita Electric Ind. Co., 1969)	Mucilage containing 10-35% gluten is pasted onto a fibre-based separator for use in dry-cell batteries.	10 – 35
Non-scratching cleanser pastes (Borchert and Grossmann, ????)	Gluten hydrolysate incorporated with scouring agents into non-scratching paste dishwashing and lavatory cleaners.	

The use of gluten in films has also been tried. Production of gluten films with satisfactory properties could provide a new biodegradable film for widespread use. Gluten has the ability to provide edible films that protect food or food components from interactions with the environment as they can serve as barriers to mass transfer (e.g., oxygen, water vapour, moisture, aroma, lipids). Gluten-based films may be cast from solutions of gluten in ammonia

or alcohol. The properties of wheat gluten film can be altered by the pH, heat treatment and solvent concentration of the film-forming solution. This will require the matching of the functionality of the gluten film to its intended application. This requires an understanding of the relative importance of the various functionalities of films, such as moisture and oxygen barrier properties, and its durability and cohesiveness for the target application. There are wheat-protein isolates available that may be prepared in water solution and used as edible coatings for products (e.g. pizza crust). The ability of these films to act as moisture and fat barriers improves the crispness of the coated products.

## **5. Current Procedures for Modification of Gluten**

Gluten is a protein intermediate in price between low-value commodities suitable only as animal feed without further processing and high-value materials, such as casein and soy isolates. This gives significant scope for modification of its properties for value addition. Various threatened surpluses in the market for gluten have turned the attention of manufacturers to ways of converting gluten into products with vastly different properties.

Gluten, like many other protein ingredients, has multi-functional properties. However, while a range of functionalities allows an ingredient to be used in a range of applications, not all of the functionalities inherent in gluten or engineered into modified ingredients are needed in a single application. For an ingredient to be the ingredient of choice for a particular application, the functionality of the ingredient has to be matched with the attributes required of the ingredient in the target application. While the inherent functionality of a gluten gives an indication of its potential functionality in an application, it should be realised that when an ingredient is added to a food, the expressed functionality of the ingredient may be modified due to changes in the molecular environment of the ingredient (e.g., pH, presence of salts, sugars, salts), the interactions of the ingredient with other components in the formulated food and the processing treatment applied in the manufacture of the final product.

It is essential for an ingredient supplier to be aware of how and the extent to which his ingredient can be modified by conventional chemical, physical and enzymic means, as well as the effects of newer processing technologies (e.g., ultrasonics, high pressure) on the properties of the ingredient.

### ***Chemical Modification***

The main modification applied to gluten is solubilisation. Gluten is soluble, or at least dispersible, in a variety of solvents including urea solutions, lactic acid, soaps and detergents (with or without urea), acetic acid, hydrochloric acid, sodium hydroxide, 70% ethanol, and 2-chloroethanol. Many of these are incompatible with food products, but for non-food purposes, there are few limitations other than cost, safety and environmental concerns.

Solubilisation by deamidation is the major method of modification currently applied. This may be achieved with either acid or alkali. Approximately 90% of the glutamic acid in gluten is in the form of glutamine (Table 1). Removal of the amide group of these residues to form the corresponding carboxylic acid changes the potential ionic charge on the protein, thus increasing its solubility above a certain pH. In acidic deamidation, there is also a degree of peptide hydrolysis to form lower-molecular-weight polypeptides, which are also usually more soluble than larger ones.

In alkali solutions, peptide hydrolysis does not usually occur as readily as in acid. However, there is the possibility of alkaline attack on the disulfide bonds of cystine, with the possibility of creating cross-links due to the formation of lysinoalanine. Use of temperatures close to ambient has shown no formation of lysinoalanine. The reaction mixtures of acid or alkali deamidation require neutralisation before the products can be used for their final purpose, a step that produces significant amounts of salts which usually need to be removed. This can be done by iso-electric precipitation, that is, adjustment of the pH to the iso-electric point at which proteins are least soluble as they have no net charge. A procedure that uses acidic or basic proteins to neutralise the alkali or acid, respectively, has been reported. No inorganic salt is formed in this process, but the overall proportion of gluten in the final product is very much reduced. Deamidated gluten is easily dispersible, making it suitable for use in foods for emulsification or foam stabilisation. It has been used in meat products, non-dairy coffee whiteners, beverages and milk puddings. No benefits have been reported for the use of deamidated gluten in bread doughs.

Gluten may be treated with sulfuric acid, phosphoric acid or chlorosulfonic acid to prepare products that bind greatly increased amounts of water. There have been reports that some of these products bind up to 200 times their own weight of water. Other chemical modifications include acylation with carboxylic acid anhydrides. In particular, treatment of gluten with succinic anhydride increases its solubility at pH 7 (close to the point of minimum solubility of native gluten) but decreases its solubility at pH 3 where it is normally quite soluble.

### ***Enzymic Modification***

Hydrolysis of the peptide bonds by enzymes also increases the solubility of gluten. This is achieved by reducing the molecular weight of the polypeptide chains. A number of commercially available enzymes have been used for this purpose, including papain, bromelain, subtilisin, trypsin and pronase. These enzymically solubilised preparations of gluten have many of the properties of chemically deamidated gluten, such as foam stability and emulsion formation.

Unlike deamidated gluten, enzyme-solubilised gluten has beneficial effects on dough properties. The addition at levels of 1-2% enzyme-solubilised gluten reduced dough-mixing times by amounts similar to those achieved by chemicals such as cysteine and ascorbic acid, which are often added commercially to give improved loaf volumes.

Many of the reports of enzyme-solubilised gluten refer to it having a bitter taste. This is believed to arise from the formation of small peptides that have been identified with bitter flavours in other proteins. Thus, treatment with enzymes needs to be carefully controlled to minimise the formation of these small peptides.

### ***Physical Modification***

A number of approaches have been applied to gluten to alter its properties by physical means. The product known as devital gluten is prepared from gluten by heat treatment. This product lacks the cohesive viscoelastic nature of vital wheat gluten, but retains its water binding character. Other physical modifications include texturisation by extrusion, high pressure processing and UV irradiation. Extrusion technology is used widely for producing fibrous structure of gluten to simulate meat fibers. Alignment of wheat protein molecules

during the extrusion process results in the formation of thin filaments or microfibrils, which assemble further to form a macroscopic fibrous structure. Hydration of the fibrous strands gives the laminated, fleshy appearance of texturized wheat gluten. High pressure has been found to change the gluten to either more liquid-like at relatively low pressure (200 MPa), or more solid-like as the pressure increased to 800 MPa. There was evidence of the weakening of noncovalent bonds at mild treatment conditions, but further chemical cross-links occurred with increasing severity of treatment. Films cast from ethanol solutions of gluten are irradiated and the tensile strength of the film is enhanced, presumably the formation of crosslinks between the protein chains.

## **C. Industry Needs for Ingredients**

### **6. Functional Ingredients for the Food Industry**

The functional properties that are required of ingredients are dependent on the target application and influenced by desired product properties. Taste and safety of the ingredient are also essential attributes.

The food industry is always seeking new cost-effective functional ingredients that impart desirable properties to a range of manufactured food products. Food ingredients serve a number of functions in food. These can broadly be classified as the following, depending on their function in the food:

- (a) Nutritional functionality – where the ingredient adds to the nutritive value,
- (b) Physical functionality – where the ingredient contributes to textural and sensory properties, and
- (c) Physiological functionality – where the ingredient has a bio-modulating function (i.e., it is bioactive).

In terms of its nutritional value, gluten (or wheat protein) is considered to be poorer than proteins from animal sources, primarily because it has insufficient amounts of two essential amino acids: lysine, and threonine. However, gluten does contain high levels of the amino acid glutamine, which is essential for strengthening muscle/body building. Therefore, the utilisation of gluten proteins is mainly due to its physical functionality and its relatively low cost.

### ***Physical Functional Properties of Protein Ingredients***

Functional properties of proteins are those physicochemical properties of proteins, which affect their behaviour in food systems during preparation, processing, storage, and consumption, and contribute to the quality and sensory attributes of food systems. The most important functional properties of proteins in food applications include protein solubility, swelling, water-retention capacity, gelling capacity, foaming and emulsifying properties and fat-binding properties. Functional properties of proteins as food components are affected by molecular weight and shape of protein molecules, the primary structure and its diversity, the conformational structure affected by covalent and/or non-covalent bonds, and charge distribution on the protein molecules. Functional properties of proteins in food systems also depend on the protein interactions with other proteins, lipids, carbohydrates, water, ions and

flavours. The preparation and processing of proteins for use as ingredients in different food formulations influences their functionality in foods.

### ***Matching the Functional Properties of Proteins to Food Products***

In exploring and developing new sources of food proteins, it is important to control their functional properties as these have a major influence on the attributes of the a food. In addition to the provision of essential amino acids, the success of new protein applications relies on them possessing desirable functional properties and acceptable sensory characteristics. Therefore, the development of new processes for manufacturing protein ingredients should be carried out in order to provide protein products with improved functional and sensory properties.

Functional properties of proteins, in particular food systems, are also affected by processing aids and conditions. The factors such as pH of medium, temperature of treatment, ionic strength, moisture content, oxidation/reduction potential, shear stress and others, are critical for the proper use of food protein ingredients. On the other hand, these factors can also be utilised to modify and control functional properties of proteins.

Although protein-based ingredients are used in many of applications listed in Table 6, other non-protein ingredients, particularly polysaccharides, can also be used to impart many of these desired physical properties to the manufactured food. Thus gluten will not only compete with other protein ingredients such as milk, soy and meat proteins that are used in food applications, but will need to be competitive in terms of physical functionality and cost-effectiveness, compared to ingredients from non-protein sources.

**Table 6. Functional properties of protein ingredients used in selected food systems.**

<b>Food Product</b>	<b>Functional Properties</b>
Beverages	Solubility, hydration, pH dependent
Soups, gravies	Thickening, water binding, emulsification
Restructured meats, sausages	Water holding, gelation, cohesion-adhesion, elasticity, fat absorption
Meat and seafood analogues, simulated meats	Water holding, Flavor-binding, cohesion-adhesion, texture simulation
Yoghurt	Water holding, viscosity building, gelation
Cheese	Water holding, gelation, emulsification
Ice-cream	Water holding, aeration, foam stabilisation, emulsification
Baked goods	Elasticity, foam stabilisation, emulsification
Confectionery	Water holding, viscosity building, gelation, emulsification
Mousse	Water holding, foaming, emulsification
Spreads	Water holding, viscosity building, emulsification,
Pet foods	Water holding, viscosity building, gelation, emulsification
Coffee creamer	Emulsification, heat stability

While the insoluble nature of gluten is a desirable attribute in traditional applications of this ingredient in bread and baked products, where it is essential for their structural properties, its insolubility in water limits its usefulness in many other applications. This is because solubility is often a pre-requisite for good hydration and water holding, viscosity building, gelling, foaming capacity and foam stabilisation and emulsification properties that are desirable in protein-ingredient applications. In addition, when an ingredient is used in a liquid system that undergoes heat processing, it is necessary for the ingredient to have adequate stability to heat to allow it to be heat-processed without coagulation or excessive thickening.

### ***Success Stories with other Protein Ingredients***

The major success stories with protein ingredients are those related to the dairy-protein and soy-protein industries, where fractionation and processing modifications have been the main methods used for diversifying the applications of these protein products.

Milk-protein containing products (e.g., skim milk powder with ~35% protein, whole milk powder with ~26% protein) have traditionally been in a variety of dairy and other food products. Milk has also been fractionated into a range of protein products to diversify the applications of milk-based ingredients. Protein-based ingredients that have on the market for a long time include the caseinates (~90% protein) and whey-protein concentrates with varying protein contents (35-80% protein). Newer protein products have included whey-protein isolates (with ~90% protein), milk-protein concentrates (up to ~85% protein), major whey-protein fractions (e.g., beta-lactoglobulin) as well as minor proteins with bioactive properties (e.g., lactoferrin and lactoperoxidase). The production of these newer products has been made possible through improved fractionation technology. In addition, a range of hydrolysed whey protein and casein products with different degrees of hydrolysis are on the market. They have been produced for applications in sport-nutrition products to obtain differentiated properties.

Soy proteins also offer an excellent example of how value has been added to a plant protein for use in food and non-food products. Like the wheat gluten/starch industry, the initial growth of the soybean industry was primarily driven by oil production rather than its protein products. As scientists, food technologists and consumers became increasingly aware of the high nutritional values of soy proteins as a plant protein source and the potential for use new advances in processing technology for oil production with little or no adverse effect on soy protein, the growth of utilising soy proteins as food ingredients has increased steadily in the last decade or so. Nowadays, soybean-protein production, i.e., use of soybean meal, adds more value to soybeans than the oil.

Soy proteins have widely been used as nutritional substitutes in various food applications in promoting its health benefits which is the driving force for consumers. However, the challenge of using soy proteins as functional ingredients for a particular food application is actually dependent on the physico-chemical properties of soy proteins that are in turn governed by structural and conformation attributes of proteins. A great deal of effort has directed into the development of soy-protein products to vary its physical functional properties to suit particular food applications. As a result, soy-protein products have widely been used and accepted as food ingredients to enhance the value of finished food products. Commercial soy-protein products are available including defatted soy flakes, soy meal, soy flour and grits, soy concentrates, soy isolates, texturised soy proteins, etc.

Soy flours are widely used in bakery products and cereals (to supplement the nutritional value of wheat proteins due to the high lysine content of soy protein). Soy concentrates and isolates are used in a variety of meat and dairy products, because of their high solubility and

high protein content, soy isolates are also used in infant formulas, beverages, and as an amino-acid source to substitute for casein, egg white and meat.

Unlike whey and soy proteins, gluten or wheat proteins are not high in biological value and have not been widely researched for nutritional advantages. Gluten, on the other hand, perhaps has been disadvantaged slightly due to its link with the coeliac disease. Extensive research and education of consumers is needed to fully understand the value of gluten protein in terms of its possible or particular nutritional-health benefits or defects. However, gluten does possess functional attributes that enhance product quality in foods beyond baked goods, thus soy protein should serve as a model for gluten utilisation in some ways, such as changing or enhancing particular physical functional properties to suit or enhance performance in a particular food system. Again, in order to fully utilise this low-cost plant protein, the physico-chemical properties of gluten need to be understood and tailored to suit its intended purpose.

### ***Meeting the Needs of the Wider Food Industry***

In broad terms, protein ingredients can provide or add value to food systems due to their ability to contribute to nutritional and functional properties of food in a cost-effective manner. Compared with milk- or soy-protein products, gluten has a lesser biological value, but it has economic benefits over and above the more expensive milk- or soy-protein products. The functional properties of wheat gluten, which other products cannot duplicate, give it a unique place among the various protein products. However, gluten, modified gluten and its fractions need to compete on price and fitness-for-purpose with other protein ingredients, if wheat proteins are to be successful in a wider food market. This can be achieved by understanding customer needs and further exploring opportunities which may lead to enhanced nutritional and physical functionality as well as the health benefits of wheat proteins.

Modification of gluten by chemical and enzymic methods has traditionally been used to improve its solubility and to alter its inherent functionality, thus widening the use of gluten. A range of soluble wheat proteins is now available on the market and these are finding applications in foods where the more expensive milk and soy proteins have been used. Fractionation of wheat gluten proteins to its constituents, gliadin and glutenin, has recently been used to make available a new range of wheat protein based ingredients. Gliadin has excellent film-forming properties and this functionality can be exploited in applications where surface properties are important. Glutenin, because of its elastic properties, can help to strengthen doughs. Another wheat protein-based ingredient available is a textured wheat protein, which has a fibrous texture. It has applications in textured-meat products such as meat analogues where it competes with textured soy proteins. There is also a new water-soluble hydrolysed wheat protein, which delivers high levels of glutamine. It has been developed for beverages and nutrition bars. These are good examples of how innovation and advances in science and technology have gradually found wider food applications for gluten. They can continue to do so.



## D. New Opportunities to Meet Industry Needs

### 7. Desirable Properties for Gluten Products

Consultation with industry indicated that a range of properties were desirable properties in native or modified gluten products. These are summarised in Table 7, together with likely means of testing and achieving these properties. This list should serve as a guide for future development of gluten and gluten modification.

**Table 7. Desirable properties for native or modified gluten products.**

Property desired	Commercial importance*	Means of testing property	Likely means of achieving property
<b>Sensory:</b> Pale colour Low odour Bland taste	++ + +++	Colour L/a/b values, GC-olfactory, sensory	Removal of lipids, reduction of polyphenol oxidase (PPO) activities
<b>Health:</b> Low likelihood of allergy & intolerance Nutrition (glutamine source)	+ +++	Amino acid content and/or sequencing	Hydrolysis, modification (targeted sites), use of transglutaminase
<b>Texture:</b> Extensibility Freeze-thaw stability Frozen dough property Extrudability	+++ +++ ++ ++	Small and large deformation rheological measurements	Chemical/enzymic/physical modification, removal of lipids, corporation of salt in gluten process; raw- material selection.
<b>Interaction with water:</b> Solubility in water Dispersibility Water-holding Gel forming Humidity resistance	+++ +++ +++ ++ +++	Solubility test as function of pH, water sorption	Deamidation, enzymic modification, hydrolysis, interaction species
<b>Surface properties:</b> Foaming Emulsification Film-formation Encapsulation Adhesion	++ +++ ++ +++ ++	Foaming/emulsion capacity and stability, particle sizing, film casting	Improving gluten solubility in water

\* '+++' – very important; '++' – medium; and '+' – less important.

## 8. Possible Means of Achieving Desirable Outcomes

### *Selection of Raw Materials*

The raw material for the preparation of gluten and wheat starch is wheat flour milled from wheat grain. For economic reasons, the starch/gluten industry general uses poorer quality wheat (e.g., weather damaged) compared to that used for bakery applications. They are often mixed varieties of all sorts, which are sold at a lower price either as animal feeds, or used as the feed material for gluten/starch manufacture as long as their protein levels are reasonably high. In addition, in order to maximize protein production and reduce the costs further, a high-extraction flour, typically above 80%, is used for gluten/starch production, instead of a standard extraction (around 78%) flour normally used for white bakery goods. The practice has several drawbacks, namely, inconsistency in gluten functional qualities generically caused by varieties; higher level of enzymes due to the use of high proportion of flour from the reduction streams (Rani *et al.*, 2001). It is known that the brown component of pasta or noodle darkening is due to peroxidase and polyphenoloxidase (PPO) activities. Lipoxigenase is able to oxidize fatty acids leading to the development of off-flavour volatile substances although lipoxigenase is also known to exhibit a gluten-strengthening effect and oxidize carotenoid and chlorophyll pigments in flour resulting in a bleaching action, which may improve gluten quality.

Selection of more appropriate wheat varieties (e.g., low PPO) and/or selection of the most appropriate flour mill streams (e.g., low extraction rate) to reduce the levels of enzymes is desirable as the starting material as these will result in gluten with improved appearance, colour and flavour. With the use of known wheat varieties, a more consistent gluten product with specified rheological properties may be attained. Although this would be the most straightforward approach i.e., without altering any process conditions, to produce consistent high-quality gluten, the high cost associated with the segregation of the raw material and flour mill-stream selections needs to be assessed against other processing methods to make this approach worthwhile.

### *Modification of Gluten Properties*

The current procedures for modification of gluten have included the use of chemical, enzymic and physical means have been discussed in Section 5. Deamidated gluten by mild acid hydrolysis is an established commercial product. Several enzyme modified gluten products are also available commercially.

During research on gluten modification in the CSIRO Wheat Research Unit (1972 to 1987), attempts were made to solubilise gluten by acid and alkaline hydrolysis (Batey and Gras, 1981b, 1984) or by enzymic hydrolysis (Batey, 1986). Good solubility could be achieved, presumably by deamidation of the glutamine residues by either acid or alkali. High temperatures (>90°C) with alkaline hydrolysis also attacked the disulfides of cystine, resulting in sulfur containing compounds which gave the product an unpleasant odour on acidification. Reaction at lower temperatures (40°C) did not appear to attack the cystine as there were no sulfurous odours. In addition, reaction at this temperature did not result in the formation of lysinoalanine, a toxic amino acid which can be formed from cystine when treated with alkali.

The acid or alkali hydrolysed product contained a large amount of salt on neutralization. Neutralisation with an acidic protein (for alkaline hydrolysis) or basic protein (for acid hydrolysis) enabled a salt free product to be prepared (Batey and Gras, 1981a, 1983). There was no readily available basic protein, but using casein was found to be a satisfactory

way of neutralizing alkaline hydrolysates of gluten. This product contained almost no salt, and had a very good amino acid balance, better than either gluten or casein alone.

Other possibly chemical modification, such as phosphorylation, or succinylation, has also been claimed to improve gluten solubility and foaming, emulsification properties. It may be possible to bleach gluten colour chemically. Benzoyl peroxide has been used widely in the past for bleaching flour colour, but it is no longer used in Australia. This may be exploited to treat the flour prior to gluten/starch production, but some overseas markets, e.g., Japan, would not permit its use for imported gluten.

Enzymic hydrolysis with Alcalase® produced a material with good solubility, and with reasonable foaming properties (Batey, 1986). This material could be added to wheat flour doughs to improve the mixing characteristics of the flour (Asp *et al.*, 1986).

Transglutaminase (TG), an enzyme able to cross-link proteins through the formation of inter- or intra-molecular peptide bonds between glutamine and lysine residues, may be useful for modifying gluten and/or increasing the yield. It has been shown that the addition of covalent bonds by transglutaminase treatment modifies the gluten network properties (Larre *et al.*, 2000), despite the low lysine content in gluten proteins. The enzyme has also been used in conjunction with protease or acid hydrolysis to improve gluten emulsification and foaming properties (Fadil *et al.*, 1996) by re-polymerizing hydrolysed gluten peptides. A recent study (Rosell *et al.*, 2003) showed that the addition of TG to wheat flour increases the amount of wet gluten, but did not improve gluten quality as measured by the gluten index.

Through extrusion technology, gluten may be used in texturised vegetable-protein products such as meat analogs and snack-food bars. Recent research (Miraglio, 2003) showed that vegetarian chicken-style products made with texturised gluten have good sensory and texture qualities compared to similar products made with texturised soy concentrate. This is an area where gluten may have great potential in competing with soy proteins.

Table 8 lists selected approaches reported in the scientific literature that have been used to modify gluten in order to match the needs of the food industry. Some of the modification approaches used are still only in the research stage, and not yet adopted by the industry.

Other approaches that could be further explored are:

- Fermentation – using methods similar to that applied in the soy industry (e.g., in the production of tempeh).
- Texturising and extrusion- using methods similar to soy for the production of texturised vegetable proteins.
- Extruded snack-food products – this could potential be done with a commercial partner (e.g., Sanitarium).
- Modification of wet *versus* dry gluten – examining the relative merits of treating gluten before or after drying.
- Enzymic modification - using a range of enzymes
  - to modify protein with peptidases, including the bug-enzyme protease of Sivri and Wrigley (2002),
  - to remove lipids with lipases,
  - to remove pentosans with xylanases, pentosanases (also prior to fermentation of starch slurry for ethanol production),
  - to remove starch by treatment with amylases or pullulanase.

**Table 8. Potential modification of gluten to diversify its functionality and uses.**

<b>Modification</b>	<b>Functional Properties</b>	<b>Reference</b>
<b>Chemical / Enzymic</b>		
Deamidation	Deamidation increases solubility of gluten. Emulsifying and foaming properties are improved Deamidation of gluten by acid hydrolysis (without reducing molecular size) increases emulsifying and foaming properties Deamidation of gluten alters its gelation properties	Popineau <i>et al.</i> , 1988 Matsudomi <i>et al.</i> , 1981 Friedle and Howell, 1996
Deamidation and hydrolysis	A deamidation-hydrolysis sequence of treatments improves emulsifying properties whereas the inverse sequence results in improved foaming properties	Mimouni <i>et al.</i> , 1994
Hydrolysis	Emulsifying and foaming properties of gluten at acidic pH decrease with increasing degree of hydrolysis in the range 0-5% Solubility of gluten is increased by treatment of gluten with 0.25M NaOH Treatment with acid solubilises gluten and improves emulsifying activity	Linares <i>et al.</i> , 2000 Batey and Gras, 1981b Wu, 1976
Enzyme Hydrolysis	Hydrolysis of gluten by neutrase improves solubility, foaming and emulsifying Treatment of gluten with various enzymes (alcalase, pepsin or papain) shows that foaming properties are obtained after mild treatment and emulsifying properties were enhanced after more thorough hydrolysis	Qi <i>et al.</i> , 2001 Mimouni <i>et al.</i> , 1999
Phosphorylation	Solubility, emulsifying capacity and foaming capacity are increased on phosphorylation of gluten	Li and Yi, 2002
Succinylation	Succinylation improves solubility, foaming and emulsifying activity of gluten	Wang and Wu, 2002
Transglutamination	Treatment of gluten with transglutaminase from <i>Streptovercillium</i> sp reinforces network formation and make it less sensitive to thermal processing	Larre <i>et al.</i> , 2000
<b>Physical</b>		
High pressure	High pressure processing of hydrated gluten affects its viscoelastic properties	Apichartsrangkonn <i>et al.</i> , 1999
Irradiation	UV irradiation of gluten films increased film tensile strength	Rhim <i>et al.</i> , 1999
Texturisation	Texturisation of gluten and its use in meat extensions and vegetarian foods	Maningat <i>et al.</i> , 1999
<b>Gluten Interacted with components (Hybrid Products)</b>		
Caseinate	A gluten-caseinate co-precipitate with similar functional properties of casein is made by using an acid-treated gluten solution to acidify milk	Walker and Connolly, 1984
Chitosan	Conjugation of gluten peptides with chitosan results in good emulsifying and antimicrobial activity	Babiker, 2002
Dextran	Conjugation of pronase-treated gluten with dextran under dry heating conditions improves solubility and emulsifying properties	Kato <i>et al.</i> , 1991
Whey protein concentrate	Gluten increases sensitivity of whey protein concentrate to heat and firmness of whey protein concentrate gels	Lupano, 2000

- Treatments to improve the colour of gluten – using glucose oxidase to scavenge oxygen, use of lipoxygenase or peroxidase to bleach colour of gluten, or by employing methods for inhibition of polyphenol oxidase.
- Treatment with GMO (genetically modified organisms) enzymes.
- Heat treatment – to gelatinise starch, for its removal from crude gluten or from a flour slurry.
- Physical modification – using treatments such as sonication and heat to alter gluten properties.
- Combination with other food proteins – manufacture of hybrid products with other grains, milk and meat by-products.
- Film formation – During research on gluten modification in the CSIRO Wheat Research Unit (1972 to 1987), attempts were made to cast films from solutions of gluten in ammonia by a visiting scientist. Satisfactory films could be made, but this work was not followed up.
- Solubilisation of gluten – Suspension of gluten in 15% ethanol at 80°C, followed by spray drying produced a solid gluten product that was functional. Alternatively, the gluten could be treated after centrifugation. Details are provided in the Newport Scientific patent (1996) and by Turner *et al.* (1996).

## **E. Best Bets for Value-Addition for Gluten**

### **9. Promising Leads to Pursue**

#### ***The Future for Gluten***

Production of gluten is still driven by the need for starch. Thus, it will always be produced while wheat is a major source of starch. There is the risk that demand for starch will grow faster than the demand for gluten, but to date, this has not happened despite dire predictions on more than one occasion. Although its absolute price has not changed significantly over many years, by becoming relatively cheaper it has found its way into more applications for which it was formerly too expensive. This has served to maintain its value while output has greatly increased. Consumer concerns about gluten free foods may limit its application in some areas, but the ubiquity of wheat in many foods ensures that gluten will remain an acceptable additive. The greatest threat to the gluten industry has been and will remain the lower cost of preparing starch from sources other than wheat. Gluten has played a major role in keeping the production of wheat starch economically viable in face of cheaper starch from other sources. This situation is expected to be unchanged in the future. However, advances in new methods for modification of gluten will ensure that it remains a competitive protein ingredient in food and non-food applications.

#### ***On-Going Research Objectives***

At the outset of this project, there was the specific need to examine the “potential of altered processing of gluten to overcome problems associated with lipids, colour or odour”.

For research outcomes to have realistic commercial value, the objectives to aim for should provide:

1. Value addition.
2. Increased competitiveness.
3. Better efficiency.

The above objectives could be achieved by the following approaches:

- Relatively simple changes to existing technologies; e.g., improved gluten-washing procedures.
- Methods to improve quality can achieve better market share and better competitiveness.
- Differentiation of a company's product can be valuable.
- Replacement of more expensive protein ingredients, such as casein and egg products.
- Improved recovery of 'gluten', e.g. by capturing more soluble protein via enzymic or chemical cross-linking.

### ***Plans for On-Going Research***

#### **Methods based on the addition of specific salts during processing of gluten as a mean of improving the rheological quality, colour and odour of the resulting gluten, and to improve the consistency of gluten products and diversify their uses**

It is common knowledge in baking industry that the addition of small amounts of sodium chloride improves/strengthen the dough, and it was also know that sodium chloride has been used in gluten/starch industry for better dough handling during washing. It was found that the gluten obtained subsequently contained less lipid, reduced to about half the level for gluten produced without the addition of sodium chloride in the wash water. Preliminary experiments have indicated that there are a number of alternative salts that may be used to improve and/or alter the rheological properties of gluten, and they may be better choice than sodium chloride from industrial prospective.

#### **Further methods for reducing gluten colour and lipid content, and improving flavour, including the use of enzymic treatments**

Lipid, itself or its oxidation products, is a significant source of undesirable gluten colour and flavour. Further research is being carried out at Food Science Australia to understand this, and to exploit new opportunities for gluten/starch industry. In a similar way, enzymes or enzyme inhibitors may be added in wash water to reduce or suppress undesirable effects related to some enzymic reactions. Again, this is an area of great interest to VAW CRC and Food Science Australia.

#### **Pursue experiments with chemical and enzymic reagents to increase recovery and nutritional quality of gluten, especially following up on transglutaminase use, possibly in combination with salt washing**

Wheat proteins contain about 10% water-soluble proteins, i.e. albumin and globulin. They are not gluten forming protein and are usually lost during water washing process.

Transglutaminase may be used to cross-link these water-soluble proteins (higher in lysine than gluten protein) to gluten protein, thereby increasing total protein recovery and altering the nutritional and functional properties of gluten product. It may be possible to combine the use of transglutaminase enzyme with the salt washing process to further manipulate gluten physical properties.

### **Study the use of gluten-based ingredients as encapsulants**

Microencapsulation is a technique which relates to packaging of a component (i.e. the core) within a secondary material (the encapsulant). There is potential for gluten and modified gluten to be used as an encapsulant for ingredients and bioactives for the food and pharmaceutical industry. Gluten products may be improved for the purpose of microencapsulation of ingredients by capitalising on its inherent film-forming and barrier properties. At present, its use in food products is limited because of its insolubility in water. Enhancing the solubility of gluten in water, whilst maintaining its surface and barrier properties, is a strategy that could be explored for tailoring gluten ingredients for the growing encapsulation industry. The achievement of improved encapsulation properties will enable it to compete more effectively with other protein encapsulants (e.g., milk and soy proteins) that are currently being used for this purpose.

### **Investigate partial fractionation of isolated gluten to produce gliadin-enriched gluten with improved extensibility.**

The gliadin fraction of gluten is known to contribute significantly to the extensibility of gluten. Gliadin enrichment of commercial gluten would be expected to extend the desirable attribute of extensibility.

### **Acknowledgements**

Parts of the above text are adapted from Batey (2004) and Wrigley (2000 and 2002).

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### **Relevant websites**

[www.amylum.com/home.asp](http://www.amylum.com/home.asp)

[www.manildra.com.au](http://www.manildra.com.au)

[www.midwestgrain.com](http://www.midwestgrain.com)

## **APPENDIX. Gluten-containing recipes for food and non-food products**

Source: Prof W.Bushuk, University of Manitoba, Winnipeg, Canada (2002).

### **FOOD PRODUCTS**

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#### **BAGEL MIX**

Bread flour	95.2%
Vital wheat gluten (activated)	4.8%
Granulated sugar	3.0%
Salt	2.0%
All-purpose shortening	3.0%
Compressed yeast	2.0%
Water	50.0%

#### **MEAT-LIKE SAUSAGE**

Wheat gluten	340 g
Water	570 g
Ascorbic acid	0.2 g
Acetic acid (5%)	30 mL
Hydrated textured wheat protein	1,000 g
Vegetable oil	100 g
Salt as desired	
Pepper as desired	
Other spices as desired	
Cellulose casing	

## MEAT-LIKE BALLS AND HAMBURGER

Wheat gluten	500 g	
Water	900 g	
Ascorbic acid	1.5 g	
Malic acid (5%)	10 mL	
Hydrated structured cottonseed protein		3,000 g
Vegetable oil	200 g	
Egg albumen	50 g	
Gliadin	50 g	
Spices as desired		

## MERINGUE

Devitalised wheat gluten	50 g
Water	250 g
Tartaric acid	2 g
Glycerine	30 g

## FRANKFURTERS

Beef lean trim (80/20) lean: fat ratio		4,170 g
Regular pork trim (50/50)	2,910 g	
Fat pork trim (20/80)	3,330 g	
Water	3,690 g	
Vital wheat gluten	480 g	
Sodium chloride, 2.25%	306.8 g	
Dextrose, 2%	272.7 g	
Sodium nitrite	1.6 g	
Sodium erythorbate	5.7 g	
Spices	85.1 g	

## CRAB ANALOGUE

Wet wheat gluten (30% solids)	1 kg
Sodium bisulfite	0.2 g
Sodium bicarbonate	2 g
Water	13 litres
Sodium bisulfite	0.02 g
Sodium bicarbonate	0.2 g
Red dye	3.6 mL

## **RESTRUCTURED BEEF STEAKS**

Round beef	56.0%
Flank beef	30.0%
Vital wheat gluten	3.6%
Water	9.4%
Sodium chloride	0.44%
Sodium tripolyphosphate	0.25%
Hydrolysed vegetable protein	0.31%

## **SAUSAGE ANALOGUE**

Vital wheat gluten	8.0%
Textured wheat protein (ground)	8.72%
Egg white solids	4.85%
Non-fat dry milk	1.94%
Sodium caseinate	0.97%
Dextrose	0.97%
Vegetable fat	16.50%
Pork sausage flavour	5.80%
Spices	0.55%
Colour	0.08%
Salt	0.30%
Water	51.32%

## **SYNTHETIC CHEESE**

Wheat gluten	14.2%
Narrow melting range fat	28.5% <i>r</i>
Egg white	4.7%
Gelatin	4.7%
Water	47.7%
Salt	0.2%
Colouring as desired	

## **HIGH-PROTEIN SNACK**

Milk curd	59.2%
Fluid skim milk	9.7%
White com meal	3.0%
Wheat gluten	2.2%
Butter	4.8%
Leavening	0.5%
Potato mash (dry)	20.6%

## **ARTIFICIAL CAVIAR**

Wheat gluten	1-30%
Cattle blood serum	1-15%
Xanthomonas polysaccharide	0.001-0.1 %
Fish additives	
Dye	

## **EXTRUDED/FIBROUS WHEAT GLUTEN PRODUCTS**

### **Recipe A.**

Wet gluten	70%
Ground meat of cod fish	10%
Corn starch	5%
Squid extract	13%
Salt	2%
Squid flavour	0.1 %

### **Recipe B.**

Wet gluten	55%
Vital wheat gluten	5%
Wheat starch	5%
Beef	10%
Beef extract	20%
Salt	3%
Natural colouring matter	1 %
Meat flavour	0.01%

## **NON-FOOD EDIBLE PRODUCTS**

## **PHARMACEUTICAL TABLETS**

### **TABLETS Recipe A.**

Soxasolamine	18-20%
Acetaminophen	55-56%
Wheat gluten	2-4%
Colouring	0.1-0.5%
Moisture	1-2%
Lubricant	0.5-1%
Excipients and disintegrants (adjust to 100%)	

### **TABLETS Recipe B.**

Chlorsoxasone	19-21%
Acetaminophen	48-53%
Wheat gluten	3-5%

Moisture	0.5-2%
Colouring	0.1-0.3%
Lubricant	0.5-1 %
Excipients and disintegrants (adjust to 100%)	

**TABLETS Recipe C.**

Butabarbital sodium	5-10%
Wheat gluten	2-4%
Moisture	1-2%
Lubricant	0.5-1%
Excipients and disintegrants - (adjust to 100%)	

**TABLETS Recipe D.**

Thyroid	4-5%
Liver, desiccated	34-36%
Thiamine mononitrate	0.1-0.2%
Riboflavin	0.1-0.2%
Niacinamide	1-2%
Vitamin B <sub>12</sub>	0.0001-0.0002%
Gluten	3-5%
Moisture	1-2%
Lubricants	3-5%
Excipients (adjust to 100%)	

**CHEWING GUM**

Wheat gluten	50%
Glycerine	15-30%
Water optimum	
Flavouring material as desired	

**EDIBLE FILMS AND COATINGS**

WHEAT GLUTEN	GLYCEROL	WATER	95% ETHANOL	NH <sub>4</sub> OH 6N	SEIN	SOY PROTEIN
5 g	2 g	16 g	24 mL	4 mL	-	-
7 g	2 g	16 g	24 mL	4 mL	-	-
3.5 g	2 g	16 g	24 mL	4 mL	-	-
5 g	2 g	24 g	16 mL	4 mL	-	-
5 g	2 g	---	24 mL	20 mL	-	-
5 g	2 g	16 g	24 mL	1 mL	-	-
2.5g	2 g	16 g	24 mL	4 mL	-	2.5 g
4 g	2 g	16 g	24 mL	4 mL	1.0 g	-

## **PET FOODS**

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### **PET FOOD Recipe A.**

Meats and offals	27%
Fats	7%
Propylene glycol	5%
Emulsifier and stabiliser	0.6%
Sodium chloride	2.4%
Sugars	24%
Maise starch	7%
Defatted soya meal	0-22%
Vital wheat gluten	5-30%

### **PET FOOD Recipe B.**

Vital wheat gluten	29.04%
Water (195°F)	48.39%
Fresh beef blood	21.78%
Sodium nitrite solution (2%)	0.26%
Titanium dioxide	0.53%

### **PET FOOD Recipe C. Flavoured**

Vital wheat gluten	28.15%
Boiling water	46.9%
Reconstituted spray-dried blood	21.11 %
Sodium nitrite solution (2%)	0.25%
Titanium dioxide	0.53%
Commercial beef flavour	0.53%
Hydrolysed vegetable protein	2.19%
Monosodium glutamate	0.34%

### **PET FOOD Recipe D. Chicken Flavoured**

Vital wheat gluten	33.13%
Hot water (195°F)	40.49%
Skim milk	24.85%
Titanium dioxide	0.61%
Commercial chicken flavour	0.92%

### **PET FOOD Recipe E. Natural Meat Flavoured**

Vital wheat gluten	33.21%
Boiling water	55.35%
Liver	11.07%
Sodium nitrite solution (2%)	0.37%



## **NON-FOOD USES**

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### **BIODEGRADABLE GLUTEN PLASTICS**

Wheat gluten	100 g
Plasticiser (glycerol, ethylene glycol or diethylene glycol)	0-10 g
Additives - Urea	0-36 g
Silicone oil emulsion	0-10 g
Sulfur	0-5 g

### **WALL-PAPER COATING COATING Recipe A.**

**Epoxidised gluten in alkaline solution 17.5 g**

White china clay	100 g
Sodium hexametaphosphate	0.25 g
Ammonia	0.75 g
Butadiene-styrene latex	17.5 g
Pine oil	0.23%
O-phenylphenol	0.025 g
Water	90 g

### **COATING Recipe B.**

Epoxidised gluten in alkaline solution	100 g
White china clay	100 g
Sodium hexametaphosphate	0.02 g
Hexamethylene tetramine	1.4 g
Water	169 g

### **PAPER COATINGS**

Coating kaolin	60.0 g
Calcium carbonate	40.0 g
Synthetic dispersion binder	8.0 g
Modified wheat protein	5.0 g
Dispersion agent (polysalt)	0.4 g
Wet-reinforcer	0.5 g

### **GLUE**

Urea dry resin	54.0%
Hardener (60% flour)	5.4%
Water	30.6%
Filler (modified wheat protein)	10.0%

### **PRESSURE-SENSITIVE ADHESIVE TAPE**

Ethylene oxide-treated wheat gluten hydrolysate	100 g
Hydroxyethyl methacrylate	100 g
Tertiary butyl peroxide	2 g

### **CIGARETTE FILTERS**

Wheat gluten	25%
Wheat flour	25%
Water	50%