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Original Submission

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Dr Linda S. Kahl
Regulatory Policy Branch, HFS-206
Office of Premarket Approval
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
200 C St., SW
Washington DC 20204

30th June 1999

Dear Dr Kahl

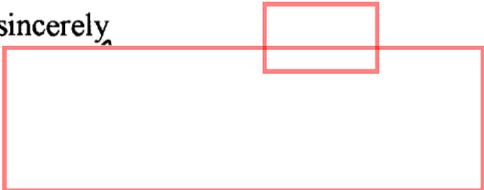
Re: GRAS Notification for Isolated Wheat Protein

Further to our correspondence by e-mail during April and May of this year, I now enclose our "Notice of a Claim for GRAS Exemption Based on a GRAS Determination". Thank you for your valuable advice in the preparation of these documents. Enclosed are the "one-pager" constituting our GRAS Exemption Claim including the information which you indicated as necessary in this, and a second major document which describes in detail the outcomes of the scientific procedure undertaken to determine GRAS eligibility.

These documents have been sent to you in triplicate by mail as stipulated in the guidelines; a further copy has been sent by electronic mail.

From the guidelines, I expect to receive notification of receipt of these documents within 30 days of their receipt by your department. I then expect to be advised within 90 days if you or your scientific advisers consider that the Claim is not substantiated. I understand that I may not receive a subsequent notification if the Claim is considered satisfactory. If these are incorrect interpretations of the guidelines, please inform me of their correct interpretation and what subsequent steps will ensue.

Yours sincerely,


Dr. R. John Pearce
Research & Development Manager

Cc Mr. J.T. Honan, Chairman, Manildra Group.

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Notice of a Claim for GRAS Exemption Based on a GRAS Determination

Notification by: The Manildra Group

Address: The Crescent
Auburn
Sydney, NSW 2144
AUSTRALIA

Chairman: Mr. J. T. Honan

Name of substance for which GRAS eligibility is sought : Isolated Wheat Protein

Use for substance for which GRAS eligibility sought: Functional ingredient applications in manufactured foods, including: emulsification, foam stabilisation, water holding, thickening and gelling, microencapsulation, film formation, adhesion and stretchability

Types of foods substance will be applied to: powdered shortenings in baked goods
milk-like beverages
beverage whiteners
cheese analogues
manufactured meat and fish products
whole muscle cooked meats
soups, sauces and marinades
mousses and meringues
edible films and coatings

Determination method for GRAS eligibility: through scientific procedures;
all information sourced in support of eligibility will be made available to FDA upon request.

**Notice of a Claim for GRAS Exemption based on a GRAS
Determination for Functional Ingredient Applications of
Isolated Wheat Protein**

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Notification

Notification by: **The Manildra Group**

Address: **The Crescent
Auburn
Sydney, NSW 2144
AUSTRALIA**

Chairman: **Mr. J. T. Honan**

1. Basis for GRAS Determination

This Notice of a claim for GRAS Exemption based on a GRAS Determination for Isolated Wheat Protein is made following the outcomes of a scientific process which, firstly, provided an analytical assessment of information concerning the present consumption of wheat, its currently consumed derivatives and equivalent products. Secondly, direct scientific data relating to the physical and chemical nature of Isolated Wheat Protein was considered and its application as a functional ingredient in a range of human foods.

2. Isolated Wheat Protein - process, product and applications in outline

Water-insoluble wheat protein, gluten, is isolated and treated under acidic conditions resulting in solubilisation of the protein due to conversion of the natural amino acids, glutamine and asparagine, to their non-amidated derivatives, glutamic acid and aspartic acid that are also naturally occurring amino acids.

The partially or wholly deamidated protein product is recovered after several steps of purification as Isolated Wheat Protein in the form of a powder.

The Isolated Wheat Protein may be used directly as a functional food ingredient in certain food formulations to confer physical attributes to the final food form. Alternatively, it may be re-solubilised prior to use using one or more of a range of substances that facilitate its functional performance prior to use. Commercial products may have incorporated the latter step prior to drying.

3. Identity of Isolated Wheat Protein

Isolated protein products are being manufactured which can be unequivocally characterised and identified as being derived from wheat.

While certain trade names may be attached to such Isolated Wheat Protein for the purpose of marketing, a number of common names are appearing in the scientific and technical literature which are synonyms for similar products. The following list is representative, but not necessarily comprehensive, of names currently in use:

Common name	Acronym
Solubilised wheat protein	SWP
Wheat solubilised protein	WSP
Isolated Wheat Protein	IWP
Wheat Protein Isolate	WPI
Wheat protein concentrate	WPC
Acid-modified wheat protein	
Deamidated wheat protein	
Solubilised gluten	
Deamidated gluten	
Acid-modified gluten	

While the common names are, in general, quite precisely descriptive of the products, some of the acronyms in common use are ambiguous with respect to other protein products currently produced elsewhere and marketed from other raw material sources,(eg WPC is also used for whey protein concentrate, and WPI is used for whey protein isolate). It is recommended that acronyms as shown should not be used in isolation.

4. Composition of Isolated Wheat Protein

4.1 Chemical procedures

The identity of Isolated Wheat Protein as a derivative from wheat may be unequivocally determined by detailed biochemical analysis of the product especially with respect to the following attributes:

- Protein content
- Amino acid composition
- Extent of deamidation
- Molecular size distribution

Methodology appropriate for each of the above is described below in Section 5.5.2

4.2 Variations in Isolated Wheat Protein products for different food applications

Isolated Wheat Protein will be produced and marketed as powder products. Two distinct variations will be supplied for particular applications as follows:

- Insoluble isoelectric form
 - Sub-variations will be produced using different acidulants for isoelectric precipitation of Isolated Wheat Protein, singly or in combination, including hydrochloric acid, sulphuric acid, phosphoric acid, citric acid, malic acid, acetic acid, tartaric acid, or other food approved acidulants.
- Totally or partially soluble forms
 - Sub-variations will be produced that have been re-solubilised in the pH range 5.5 to 8.5 prior to drying using bases including sodium hydroxide, potassium hydroxide, trisodium phosphate, disodium hydrogen phosphate, ammonium hydroxide, calcium hydroxide or other food approved bases.

5. The Scientific Process forming the basis of the GRAS Determination for Functional Ingredient Applications of Isolated Wheat Protein

Data has been gathered indirectly from literature and common experience, and directly by physical and chemical analysis. This information has been considered with respect to any potential hazard to human health that may result from contact with or consumption of Isolated Wheat Protein.

The data analysis has been directed towards the raw material, processing conditions, product composition and applications and these are considered below under the following headings:

Natural origin of wheat and consumption
Gluten, a GRAS ingredient
Manufacturing process
Similar products
Chemical specification for Isolated Wheat Protein
Intended technical use and benefit

5.1 Natural origin of wheat and consumption

Wheat is a wild grass (*Gramineae* family) native to the arid countries of western Asia, Its use as a food goes back to the Stone-Age era. Altogether about 600 genera of grasses have evolved , among them the various forms of the genus *Triticum* of which the following are the main groups:

<i>aestivum (vulgara)</i>	- Common wheat
<i>durum</i>	- Durum wheat
<i>compactum</i>	- Club wheat
<i>turgidum</i>	- Poulard wheat
<i>dicoccum</i>	- Emmer wheat
<i>spelta</i>	- Spelt wheat
<i>polonicum</i>	- Polish wheat

For commercial purposes, wheat is generally classified as hard or soft, red or white, spring or winter.

Wheat and related grasses such as barley and rye have always been important for food in Europe, the eastern Mediterranean region and western part of Asia.

Wheat has become the leading cereal crop as bread, especially leavened varieties have become an important part of the daily diet. Wheat is the only grain suitable for leavened bread. This is due to the presence of a unique elastic protein complex called “gluten” that provides a matrix for evolved gases to form the characteristic open cellular texture of leavened bread.

Large quantities of wheat are produced in Europe, Asia, North and South America and Australia. Altogether about 600 million tonnes of wheat are produced and consumed per annum around the world.

The wheat grain consists of an outer layer (epidermis) inside of which is contained a starchy endosperm which is the material from which white flour is made. It comprises starch granules embedded in a matrix of proteins. The proteins consist of albumins, globulins, gliadins and glutenins. The combination of gliadins and glutenins is referred to as the “gluten complex” or “gluten” and is regarded as storage protein.

5.2 Gluten, a GRAS food ingredient

Gluten, representing about 80% of the total endosperm protein of the wheat grain is virtually insoluble in water. It is readily isolated commercially by washing wheat flour with water to remove starch granules and other solubles and dispersibles. After drying it is marketed as vital wheat gluten and is widely used in the baking industry especially for strengthening dough in bread making. Devitalised gluten is also marketed for other food applications.

Gluten may be separated nominally into the gliadins and glutenins by virtue of the solubility of gliadins in 70% ethanol in water. All gluten proteins are referred to as prolamines because of their high contents of the amino acids, proline and glutamine. This distinction between gliadins and glutenins is considered due to glutenins being polymeric and gliadins being monomeric.

Gluten is characterised by the presence of

- a high percentage of amide nitrogen contributed mainly from glutamine amino acid residues, The gliadins are particularly rich in glutamine, it being the principal amino acid and representing about 35% of amino acids present.
- A high percentage of proline, being about 20% of amino acids present
- Much intra- and inter-molecular disulphide bonding due to cysteine residues
- Significant amounts of amino acids with hydrophobic side chains particularly valine, leucine, isoleucine, and phenylalanine,

Hydrogen bonding of amide side chains of glutamine and asparagine is a major factor in the development of the cohesive gluten complex when flour is mixed with water.

The glutenin proteins are diverse in their characteristics with two major groupings being identified

- (a) unassociated fractions of molecular weight 15,000 to 150,000 daltons
- (b) associated fractions of molecular weight 150,000 to 3,000,000 daltons

The gliadins are also heterogeneous with at least 50 different proteins present. They are characterised by

- (a) a single polypeptide chain
- (b) molecular weights 30,000 to 50,000
- (c) solubility in 70% ethanol/water

5.3 Manufacturing process

5.3.1 Processing chemicals

All chemicals used for processing gluten to Isolated Wheat Protein meet the recognised standards of performance and quality for their application as specified in *Food Chemicals Codex* 4th Ed. (1996). Food chemicals are handled according to standards of “good manufacturing practice”. The following chemicals may be used in the manufacture of Isolated Wheat Protein:

Sulphuric acid, or other strong acid such as hydrochloric acid or phosphoric acid .

Sodium hydroxide, or other food grade bases such as potassium hydroxide, trisodium phosphate, disodium hydrogen phosphate, ammonium hydroxide, calcium hydroxide or other food approved bases.

Other food grade acids such as citric acid, malic acid, acetic acid or tartaric acid.

5.3.2 Procedure

Gluten is recovered from wheat flour by initially forming an aqueous dough which is subsequently thinned and ultimately extensively washed to remove starch granules and other soluble or dispersible materials. The gluten may be dried or used directly according to need or convenience.

Using dispersive machinery, the gluten is finely divided and treated with dilute sulphuric acid (or other acid). Temperatures applied may be in the range ambient to 90C depending on the predetermined timescale of conversion and extent of deamidation required. When the gluten has been solubilised, the protein may be recovered by isoelectric precipitation

at or about pH4.5 using sodium hydroxide solution (or other base). The isoelectric precipitate may be dried directly or washed with water and then dried.

Alternatively, the isoelectric isolate may be redispersed at alkaline pH by addition of more sodium hydroxide (or other base). The dispersed product may be dried directly.

5.4 Similar products

In Section 4.2 a number of variations in composition of Isolated Wheat Protein were identified dependent on the type of chemicals, extent of processing and final state of the products. In the following discussion the compositional similarity is considered with respect to determination of all such variations as GRAS.

5.4.1 Products arising from different chemicals applied in processing

Hydrolysis of glutamine and asparagine residues in gluten is achieved in suspension in dilute strong acid with application of heat. Acidic conditions and temperature are sufficiently moderate to result in minimal hydrolysis of peptide bonds and consequent polypeptide fragmentation, so the amino acid content of gluten is maintained and oxidised products are not generated. Sulphuric, hydrochloric and phosphoric acids are similarly effective at equivalent acid concentration, however, commercial manufacturing and economic conditions favour the use of sulphuric acid. Products resulting from each of these acids contain a predominance of sulphate, chloride or phosphate respectively in their residual ash content but otherwise are chemically similar after the same processing conditions. To facilitate application of Isolated Wheat Protein residual ash is reduced to less than 5% so it does not significantly affect the GRAS nature of the product.

5.4.2 Products arising from variation in processing conditions

Apart from acid type, acid concentration, processing temperature and duration of heating are the major variables which may be used to achieve desired specific variation in functional properties of Isolated Wheat Protein products. The consequence of increasing any of these parameters is an increase in the proportion of the amide groups hydrolysed to their corresponding acid. As glutamic acid and glutamine, and aspartic acid and asparagine are considered to be substantially equivalent in nutritional terms, assuming overall nitrogen adequacy in the diet, the extent of deamidation is not considered to affect the GRAS status of the Isolated Wheat Protein.

5.4.3 Products arising from variation in final physico-chemical state

In Section 4.2, soluble and insoluble forms of the product were identified resulting from variation in the final steps of processing. Having precipitated the deamidated protein at its isoelectric pH, the material may be dried directly or after washing with water.

Alternatively, other dilute food-grade acids, as listed above, may be used as a washing medium to achieve substitution of non-protein components in the final insoluble form of the Isolated Wheat Protein product.

To produce a soluble product, the isoelectric form of the Isolated Wheat Protein is dispersed in either acid or base. A variety of such materials may be used, as listed above, to achieve suitability for specific food formulation and particular functionality as a food ingredient. All the acids and bases used are food-approved materials of food grade as listed in Food Chemicals Codex 4th Ed. (1996). Consequently, the finished Isolated Wheat Protein contains an amount of the acid or base sufficient to achieve the required dispersability.

It is considered that incorporation of such materials in Isolated Wheat Protein products does not adversely affect the nutritional value of the protein, or consumer health and so does not affect the GRAS status of Isolated Wheat Protein.

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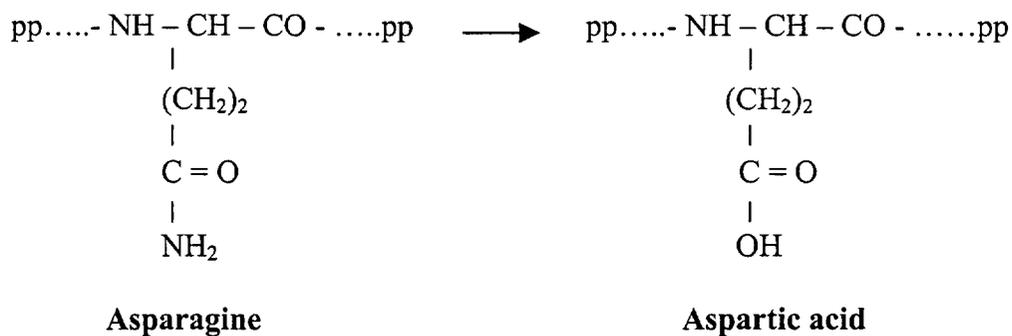
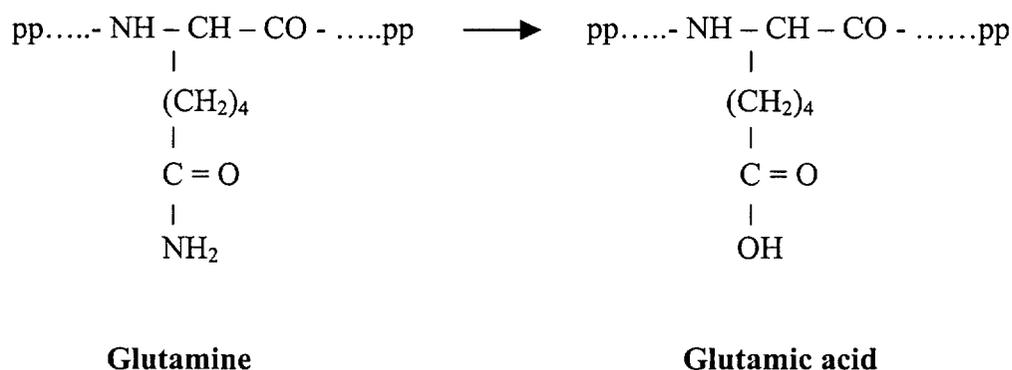
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It is considered that incorporation of such materials in Isolated Wheat Protein products does not adversely affect the nutritional value of the protein, or consumer health and so does not affect the GRAS status of Isolated Wheat Protein.

5.5 Chemical Specification for Isolated Wheat Protein

5.5.1 Description of Isolated Wheat Protein

By the above manufacturing process, all or a proportion of the glutamine and asparagine residues of the gluten polypeptide chain are converted to glutamic and aspartic acids respectively in a process of acid-catalysed deamidation/ hydrolysis as shown in the diagram below.



pp.... = polypeptide chain

Both glutamic acid and glutamine, and both aspartic acid and asparagine are widely occurring amino acids in the proteins of all animal and plant materials. The proportions of the amide form and the acid form are equally diverse amongst proteins according to the function of the protein and the role of the amino acid within the protein. The amide

forms of the amino acids are quite hydrophobic and tend to be found in the interior of globular proteins whereas, because of the very hydrophilic nature of the acid forms, they tend to be found on the surface of globular proteins facilitating their interaction with water and encouraging solubility.

The high content of glutamine and asparagine in gluten enables the wheat seed to store more nitrogen for subsequent germination and growth; in the seed the low moisture environment is therefore conducive to hydrophobicity. In the presence of small amounts of water, the high amide content of gluten contributes greatly to the elasticity of dough. However, the high amide content prevents solubility of gluten in water or other aqueous media. Conversion of the glutamine to glutamic acid and asparagine to aspartic acid results in the presentation of hydrophilic residues to an aqueous environment instead of the native hydrophobic surface and consequently the deamidated gluten is greatly more soluble in water and aqueous media than native gluten.

Amino acid analysis of proteins has relied for many years on the hydrolysis of peptide bonds by concentrated hydrochloric acid (usually about 6M) at elevated temperature, typically about 105C. However under these conditions the aliphatic amides of glutamine and asparagine are also converted to their respective acid forms. Consequently, in a typical amino acid analysis, glutamic and aspartic acids are not discriminated from their corresponding amides.

Metabolically there is ready transfer of nitrogen between the two forms and none of these four amino acids is regarded as essential. Both aspartic acid and glutamic acid and their corresponding amide forms are synthesised as required in mammalian cells. Thus from the point of supply of amino acids to the digested protein pool, the manufacturing process of deamidation is of little consequence, however, from the point of utilisation as a functional food ingredient, deamidation of gluten can produce a dramatic and useful effect.

5.5.2 Identification tests

1. Protein content
2. Amino acid analysis
3. Molecular size analysis
4. Extent of deamidation.

5.5.2.1 Protein content

To be regarded as a protein isolate by the food industry, a wheat solubilised protein product should contain 90% or more of protein.

Routinely in the food industry, protein content of a raw material, ingredient or product has been determined from the nitrogen content after oxidative decomposition by the Kjeldahl or Dumas procedures and multiplying by a conversion factor to obtain the protein content. A typical and average value for the conversion factor of 6.25 has been utilised widely by the food industry especially in the absence of a more specifically determined value.

Each amino acid contributes a unique amount of nitrogen to the protein, with the exception of isomers such as leucine and isoleucine. Hence, dependent on the relative proportions of each amino acid, the composite proportion of nitrogen differs for each protein. Amino acids having nitrogen in their side chain moieties contribute a greater proportion of nitrogen; this group includes lysine, arginine, histidine, asparagine and glutamine. Many proteins have approximately similar proportions of amino acids which accounts for the similar relevant conversion factor of 6.25.

Certain well defined protein materials are estimated using alternate conversion factors when these are known more accurately. An example of this is gluten for which a value of 5.7 has been routinely used because glutamine and asparagine represent such a high proportion of its amino acid composition and it has an unusually high nitrogen content. As glutamine and asparagine are progressively converted to glutamic and aspartic acids during the gluten deamidation process, so too must the value of the conversion factor applied be increased to account for the decreasing content of amide nitrogen. Total deamidation results in a required conversion factor of about 7; complete aqueous dispersion of gluten requires about one third of the amide residues to be hydrolysed. This approximates then to a conversion factor of about 6.25 and is considered to be an appropriate value for many commercial Isolated Wheat Protein products. For detailed research purposes, and commercial products representing significantly lesser or greater extents of deamidation, the precise extent of deamidation of each product of interest should be determined and hence the use of an exact conversion factor.

5.5.2.2 Amino acid analysis

Amino acid analysis is a routine automated procedure for measuring the amount of each amino acid in a protein providing rapid reliable characterisation of proteins. Whilst many proteins have approximately similar proportions of particular amino acids, each protein has a unique amino acid composition and sequence. Certain proteins have very different amino acid compositions which cause them to be separately and specifically identified. Gluten is one such protein having a very distinctive amino acid composition rich in proline (~20%) and glutamine (~35%).

As described above in Section 5.5.1, by conventional amino acid analysis, the amino acid composition of gluten and its deamidated derivatives will be the same as shown in Appendix 1. Consequently, conventional amino acid analysis will provide identification of a product as having a gluten source but, alone, it will not differentiate between gluten and a deamidated gluten product.

5.5.2.3 Molecular size analysis

It was noted above that conditions appropriate for hydrolysis of peptide bonds and so for amino acid analysis also bring about hydrolysis of the amide bonds of glutamine and asparagine. However, the reverse is not the case. The hydrolysis of aliphatic amides is achieved at relatively low acid concentrations viz. 0.2 to 2.0 M acid at temperatures as low as 40C; these conditions are not effective for the hydrolysis of peptide bonds being a special internal amide configuration stabilised by electron delocalisation. Consequently, the polypeptide chain length of gluten component molecules is not affected by the deamidation conditions.

Using solubilisation conditions suitable for both gluten and deamidated gluten and analysis by Size Exclusion HPLC, the molecular size distribution of protein components does not change as a result of deamidation as shown in Appendix 2. Guanidinium hydrochloride at a concentration of 6M in the presence of 2% 2-mercaptoethanol to reduce disulphide crosslinks in dilute aqueous buffer is suitable for solubilising and chromatographic analysis of gluten and deamidated gluten. In this system all non-covalent associations between molecules are disrupted and the molecular sizes of constitutive gluten or deamidated gluten molecules are revealed.

Molecular size analysis alone will not permit discrimination between gluten and deamidated Isolated Wheat Protein, however, together with amino acid analysis, it provides a comparative characterisation procedure to identify differences between products similar in amino acid composition. Molecular size analysis allows discrimination between Isolated Wheat Protein and wheat protein hydrolysates in which the polypeptide chains of gluten have been deliberately cleaved by hydrolysis of peptide bonds either enzymatically or under more vigorous chemical conditions.

5.5.2.4 Extent of deamidation

The deamidation of glutamine and asparagine residues in gluten has been described in terms of the chemical changes occurring in the protein. The corollary to the hydrolysis of the amide bonds is the evolution of ammonia which remains in the acidic hydrolysing medium as ammonium ion. Consequently, during hydrolysis estimation of the amount of ammonia produced relative to amount of gluten treated provides an assessment of the extent of deamidation achieved.

In practical processing terms, to achieve an accurate, on-going determination of the extent of deamidation during hydrolysis of a particular gluten source it is necessary to determine the total amide content of the source gluten and compare this with the amount of amide remaining in the deamidated product derived from the amount of ammonia evolved during the deamidation process.

Estimation of the extent of deamidation in a finished isolated wheat protein product necessitates the complete deamidation of a sample of the product, under standard test conditions, with collection of the ammonia evolved. This result is then compared to an average value of total amide content for whole gluten or to the specific parent gluten if available.

A published method for determination of amide nitrogen content of a protein recommends treatment of the protein in hydrochloric acid (1M) at 100C for 2 hours. This procedure may be adequate but does not give an indication that all amide nitrogen has been evolved; a modified procedure in which measurements are made between 1 and 4 hours of treatment enables a plateau value to be obtained representing the total amide content. Nitrogen evolved, as ammonium ion, may be estimated accurately by several different procedures such as steam distillation in alkali and titration, colorimetric determination of ammonia by, for example, the phenol-sulphuric acid method or direct combustion analysis of deproteinated liquor using the Dumas method.

5.5.3 Physico-chemical characteristics of Isolated Wheat Protein

a. Appearance and colour

- pale buff to light tan coloured, free-flowing powders

b. Solubility

- isoelectrically precipitated isolates < 5% soluble protein in water dispersion

- resolubilised form >95% soluble protein in water dispersion

c. Moisture content

3 – 5 %

d. Ash content

1 – 5%

e. pH

insoluble form – aqueous dispersion at pH 4.2 – 4.8

soluble form – aqueous solution at pH 5.5 to 8.0

5.6 *Intended technical use and benefit*

5.6.1 Technical effects for which Isolated Wheat Protein may be used

- Surface active agent
- Water holding ingredient
- Microencapsulation
- Extension or replacement of casein

Both emulsions and foams may be effectively stabilised through the use of Isolated Wheat Protein. Certain emulsions may be dried to yield microencapsulated fats and oils for use as powdered shortenings. Isolate Wheat Protein demonstrates water holding properties advantageous to foods such as manufactured meat and fish products which have a tendency to lose water and succulence during cooking. Melting and stretching attributes of Isolated Wheat Protein closely resemble those of casein which, together with the similarities in emulsifying and water holding properties, enable a variety of non-dairy analogue products to be made.

5.6.2 Types of food likely to be manufactured with Isolated Wheat Protein

- Coffee whiteners and creamers, powdered and liquid
- Baked goods containing powdered shortenings including cakes and pastries
- Beverages such as milk alternatives
- Manufactured meat products including chopped and emulsified meat products
- Cheese analogues for pizza and cheeseburgers etc.
- Dairy analogue desserts, toppings, yoghurts etc.

5.6.3 Ingredient content in food applications

In the above list the total solids content of the various foods may range from as little as 10% in a milk-like beverage through to more than 90% in some baked goods. In many of the food types where the Isolated Wheat Protein is functioning as an emulsifier, the Isolated Wheat Protein to fat ratio may be reasonably constant whereas the overall fat content may vary widely from as little as 0.5% to 50%. As an effective emulsifier, Isolated Wheat Protein is expected to be used at the 2 – 10 % rate with respect to fat.

Some typical amounts in foods are likely as follows:

Type of food	Maximum IWP g / kg	Typical IWP g / kg
Coffee whitener, 20% solids, 4% fat	4	2
Cake , 70% solids, 30%fat	30	15
Biscuits/ cookies, 90% solids, 50% fat	50	25
Beverage 10% solids 2% fat	2	1
Sausage product 40% solids 25% fat	25	13
Cheese analogue 60%solids 35% fat	35	18

5.6.4 Technical effect of Isolated Wheat Protein in food

Type of food	Technical effect of WPI
Coffee whitener, 20% solids, 4% fat	Emulsification / heat stability at low pH
Cake, 70% solids, 30% fat	Emulsification / microencapsulation
Biscuits/ cookies, 90% solids, 50% fat	Emulsification/ microencapsulation
Beverage 10% solids, 2% fat	Emulsification
Sausage product 40% solids, 25% fat	Emulsification and water holding
Cheese analogue 60% solids, 35% fat	Emulsification, casein extension, melting

5.6.5 Fate of Isolated Wheat Protein after consumption

Isolated Wheat Protein incorporated as a functional ingredient in food will experience and be subject to similar degradative digestion processes as gluten. The products will be naturally occurring amino acids and partially digested peptides. The enhanced solubility of isolated wheat protein in neutral or alkaline digestion systems may facilitate digestion

5.6.6 Directions for use and labeling

Isolated Wheat Protein will be marketed with recommendations for specific functional applications in foods, including usage levels, formulation guidelines and processing conditions.

The term “wheat protein” is proposed to describe Isolated Wheat Protein as an ingredient on food labels for manufactured foods.

6. Dietary exposure

It is considered that dietary exposure to Isolated Wheat Protein is unlikely to present a basis for a safety concern. This conclusion has been reached on the collective evidence which indicates substantial equivalence between gluten and the gluten derivatives referred to as Isolated Wheat Protein. Thus, it is concluded that the consumption of Isolated Wheat Protein is likely to be as safe as consuming gluten. The evidence leading to this conclusion has been presented in detail above in Section 5; it is summarised as follows:

- the natural origin of wheat as the source material, and its wide utilisation and long history as a safe foodstuff,
- the GRAS status of gluten, as the primary protein derivative from wheat grain, from which the Isolated Wheat Protein is prepared
- the use of only food-grade, food-approved processing materials
- applying processing conditions which result only in conversion by chemical modification of certain constituent amino acids of the wheat protein to other naturally occurring amino acids, where neither the original nor the derivative amino acids are considered “essential” in dietary intake terms.
- the level of usage of Isolated Wheat Protein in manufactured foods as functional ingredients being unlikely to significantly increase the already substantial intake of gluten and equivalent materials in target markets.

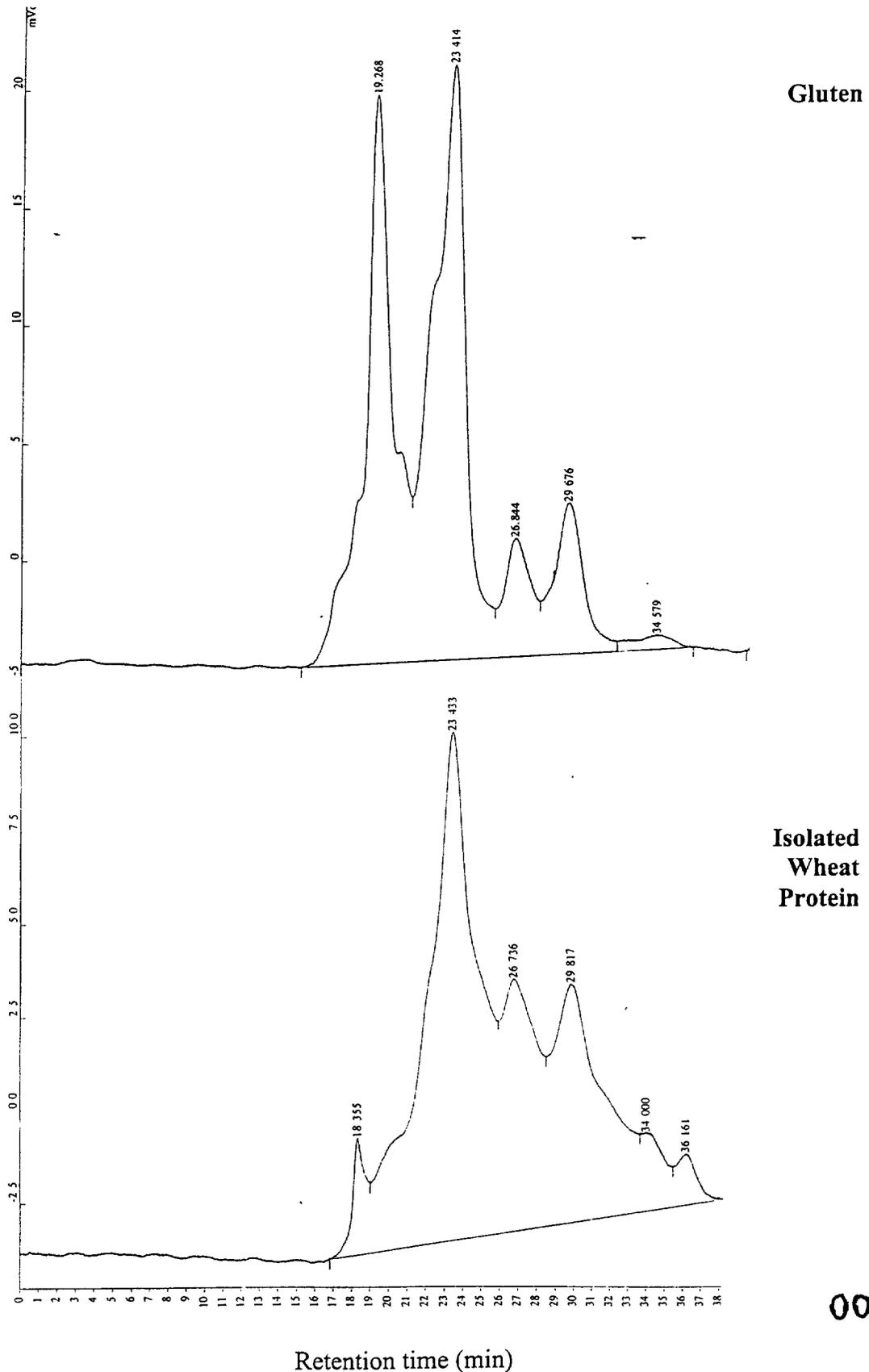
APPENDIX 1

Comparison of Amino Acid Analyses for Gluten and Isolated Wheat Protein

Amino Acid	Gluten	Isolated Wheat Protein
Asx	3.6	3.2
Ser	6.8	5.9
Glx	30.2	33.7
Gly	7.2	5.1
His	1.9	1.8
Arg	2.8	2.7
Thr	3.2	2.9
Ala	4.1	4.0
Pro	15.1	14.2
Cys	2.7	1.3
Tyr	2.4	2.7
Val	4.8	4.5
Met	1.4	1.4
Lys	1.8	1.4
Ile	3.8	3.9
Leu	6.6	7.1
Phe	3.7	4.2

APPENDIX 2

Comparison of the Molecular Size Distribution of gluten and Isolated Wheat Protein by Size-exclusion High Performance Liquid Chromatography in 6M guanidine



000023

APPENDIX 2 continued

Comparison of the Molecular Size Distribution of gluten and Isolated Wheat Protein by Size-exclusion High Performance Liquid Chromatography in 6M guanidine

Method

Gluten and Wheat Protein Isolate samples were dissolved in dilute phosphate buffer, 0.02M pH 6.8 containing 6M guanidine hydrochloride. 2-Mercaptoethanol was added at 5%v/v to ensure complete reduction of all disulphide crosslinks.

Using a Varian HPLC in isocratic mode operating at 0.55mL / minute, samples were analysed using two size-exclusion separation columns in series, TSK 3000 and TSK3000XL. The columns were equilibrated and samples eluted with the same phosphate buffer. The eluent was monitored at 280nm.

The columns were calibrated using well characterised purified proteins from milk as in the following table from which data the molecular size distribution for the gluten and Isolated Wheat Protein samples were determined

Protein	Molecular weight (kDa)	Retention time (min)
α -lactalbumin	14,200	29.27
β -lactoglobulin	18,300	27.17
Bovine serum albumin	67,000	21.04

Results

The molecular components of Gluten and Isolated Wheat Protein eluted essentially at the same retention times. Gluten showed a large peak at 19.27 minutes where this was greatly reduced in Isolated Wheat Protein and appeared to result in some smaller molecular sized material, however, this material was still all in excess of 10kDa indicating minimal molecular fragmentation. Other major peaks at about 23.4, 26.8 and 29.7 minutes were equally represented in both gluten and Isolated Wheat Protein.

Overall a number of different sized molecular components are indicated in both preparations in the range 10 – 80kDa most of which demonstrate corresponding elution profiles.

APPENDIX 2 continued

Comparison of the Molecular Size Distribution of gluten and Isolated Wheat Protein by Size-exclusion High Performance Liquid Chromatography in 6M guanidine

Method

Gluten and Wheat Protein Isolate samples were dissolved in dilute phosphate buffer, 0.02M pH 6.8 containing 6M guanidine hydrochloride. 2-Mercaptoethanol was added at 5%v/v to ensure complete reduction of all disulphide crosslinks.

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Overall a number of different sized molecular components are indicated in both preparations in the range 10 – 80kDa most of which demonstrate corresponding elution profiles.

SUBMISSION END

000026

To: Maribeth LaVecchia@OPA@FDA.CFSAN
From:
Certify: N
Priority: Normal
Subject: RE: GRN 000026-Isolated Wheat Protein
Date: Sun Oct 03 18:20:22 1999
Attached: None

AM



To Maribeth LaVecchia

Thank you for your letter which arrived while I was overseas. I note your concern about the lack of a lead specification in our Notification. I will attend to this matter promptly and communicate with you again in a few days.

John Pearce

-----Original Message-----

From: Maribeth LaVecchia [SMTP:MLavecch@bangate.fda.gov]
Sent: Saturday, 11 September 1999 5:23
To:
Cc: lsk
Subject: GRN 000026-Isolated Wheat Protein

Subject: GRN 000026--Isolated Wheat Protein

Dear Mr. Pearce,

This is in reference to the above referenced notice for isolated wheat protein. In the notice the Manildra group proposed specifications for isolated wheat protein but did not include a specification for lead. FDA is quite concerned about achieving the lowest possible levels of lead in food and would like the Manildra group to set a lead specification. If you are unable to commit to a lead specification at this time, we can flag this issue in our response letter to GRN 000026.

Please notify us if you are in the position to recommend a lead specification for isolated wheat protein. If you have this information available, please forward it to us at this time.

We have not identified any other issues that require a response other than lead specifications. We are currently waiting to hear from USDA before drafting our response letter.

Maribeth LaVecchia
Division of Petition Control (HFS-215)
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

000035

To: Maribeth LaVecchia@OPA@FDA.CFSAN
From:
Certify: N
Priority: Normal
Subject: RE: Isolated wheat Protein--GRN 000026
Date: Mon Oct 04 17:50:19 1999
Attached: None

AM



Dear Ms LaVecchia

Thank you for your response to my note of 3 October 1999 and for the advice concerning a lead specification. Could you advise me as to how the amendment/ insertion should be made to our Notification. Is it adequate to provide a paragraph and insertion point information or should we submit a whole set of revised documents including electronic format? Please advise, thank you.

Kind regards

John Pearce

Dr. R. John Pearce
Research & Development Manager
Manildra Group

-----Original Message-----

From: Maribeth LaVecchia [SMTP:MLavecch@bangate.fda.gov]
Sent: Monday, 4 October 1999 22:25
To:
Cc: lsk
Subject: Isolated wheat Protein--GRN 000026

Dear Mr. Pearce,

This is in response to your e mail of October 3, 1999, notifying FDA that you intend to include a specification for lead for the GRAS notification for isolated wheat protein that you submitted on behalf of the Manildra Group, (GRN 000026). The proposed lead specification should be as low as technically feasible, and for protein ingredients with a dietary exposure such as the expected exposure to isolated wheat protein we would recommend that manufacturers have as a target a level of 0.5-1 ppm.

As you stated in your e mail, we will expect to receive further communication from you.

Maribeth LaVecchia
Division of Petition Control (HFS-215)
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

000037

To: Maribeth LaVecchia@OPA@FDA.CFSAN
From:
Certify: N
Priority: Normal
Subject: RE: GRN 000026--Isolated wheat protein
Date: Thu Nov 25 19:02:04 1999
Attached: grassup1.doc

AM



Dear Ms LaVecchia

Thank you for your advice on how to submit supplementary information to the GRAS Notification for Isolated Wheat Protein in relation to its lead content. Before providing the additional information, it was necessary to screen a number of our products to ascertain that they would indeed meet the suggested specification. This was the cause of the delay; it was necessary to externally contract the analyses.

We are now satisfied that our products all meet the specification, mostly at only one tenth of the specified maximum of 0.5mg/kg (ppm).

I am attaching a copy of a Supplementary Information statement which I ask you to append to the original GRAS Notification.

Kind regards

John Pearce

Dr. R. John Pearce
Research & Development Manager

-----Original Message-----

From: Maribeth LaVecchia [SMTP:MLavecch@bangate.fda.gov]
Sent: Wednesday, 6 October 1999 21:42
To:
Cc: lsk; ssc
Subject: GRN 000026--Isolated wheat protein

Dear Dr. Pearce,

This is in response to your October 4, 1999, e mail requesting information regarding the appropriate way to submit additional information to the GRAS notification for isolated wheat protein that you submitted on behalf of the Manildra Group, (GRN 000026).

The information you are submitting may be provided in a letter with the Manildra Group letterhead. It will be a separate document that we will append to the existing file. We will need 3 copies of the information in hard copy so that all copies of GRN 000026 can be updated. When we receive these copies we can officially initiate our response. If you would like to e mail this information to me prior to our official date of receipt, we can start to process it.

As stated in our previous e mail, the proposed lead specification for lead should be as low as technically feasible, and for protein ingredients with a dietary exposure such as that expected for isolate wheat protein, we would recommend a target level of 0.5-1 ppm.

For your information, the monograph for Whey in the 4th ed., Food Chemical Codex has a lead specification of 0.5 ppm. The monograph details the methodology that is used for this analysis.

If you have any additional questions, please feel free to contact me.

Maribeth LaVecchia
Division of Petition Control (HFS-215)
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

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200 C Street S.W.
Washington, DC 20204

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Happy  Mail!



MANILDRA GROUP

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NAMOI FLOUR MILLS PTYLIMITED - HOOKERS MILK PRODUCTS PTY. LTD. - MANILDRA MILLING CORPORATION U.S.A.

Ms Maribeth LaVecchia
Division of Petition Control (HFS-215)
Office of Premarket Approval
Centre for Food Safety and Applied Nutrition
200 C Street S.W.
Washington, DC 20204

26 November 1999

Dear Ms LaVecchia

Re: GRAS Notification GRN 000026 – Isolated Wheat Protein

Thank you for your advice on how to submit supplementary information to the GRAS Notification for Isolated Wheat Protein in relation to its lead content. Before providing the additional information, it was necessary to screen a number of our products to ascertain that they would indeed meet the suggested specification. This was the cause of the delay; it was necessary to externally contract the analyses.

We are now satisfied that our products all meet the specification, mostly at only one tenth of the specified maximum of 0.5mg/kg(ppm).

I am attaching three copies of a Supplementary Information statement which I ask you to append to the original GRAS Notification.

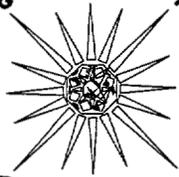
Kind regards

Dr. R. John Pearce
Research & Development Manager

1999 DEC 13 P 3 04

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GEM OF THE WEST



MANILDRA GROUP

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NAMOI FLOUR MILLS PTYLIMITED - HOOKERS MILK PRODUCTS PTY. LTD. - MANILDRA MILLING CORPORATION U.S.A.

Re GRAS Notification GRN 000026 – Isolated Wheat Protein

SUPPLEMENTARY INFORMATION

Note, the following information should be included in GRN 00026 at Section 5.5.3 Physico-chemical characteristics of Isolated Wheat Protein as sub-section 5.5.3.f.:

f. Lead content

- less than 0.5 mg/kg

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Pagination**



Computer Technology Services, Inc.

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MANILDRA GROUP

AM



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Re GRAS Notification GRN 000026 – Isolated Wheat Protein

SUPPLEMENTARY INFORMATION

Note, the following information should be included in GRN 00026 at Section 5.5.3 Physico-chemical characteristics of Isolated Wheat Protein as sub-section 5.5.3.f.:

f. Lead content

- less than 0.5 mg/kg

1999 NOV 29 A 8:53

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