GR

ORIGINAL SUBMISSION

000021

SRA International, Inc.

21 December 2001

RECEIVED
DEC 2 6 2001
OFFICE OF
FOOD ADDITIVE SAFETY

Dr. Linda Kahl
Office of Food Additive Safety (HFS-206)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Re: GRAS Notification for Transglutaminase

Dear Dr. Kahl:

The purpose of this letter is to inform the Agency of a recent GRAS determination for transglutaminase in which scientific procedures were employed.

BACKGROUND

As you know, the use of the enzyme transglutaminase as a food additive in various human foods has been evaluated a number of times by the U.S. Food and Drug Administration (FDA). These evaluations resulted in the issuance of a series of 'no-objection letters including: Rulis to Post (15 January 1998), and Rulis to Bernard (22 June 1998 [GRN No. 4], 29 December 1999 [GRN. No. 29], and 30 January 2001 [GRN No. 55]).

CURRENT SUBMISSION

This submission is intended to inform the Agency of the results of a recent GRAS determination for transglutaminase. Again, the determination employed scientific procedures and addressed three topic areas:

1. **Expansion of Uses**

   This notification is intended to alert the U.S. Food and Drug Administration (FDA) to a recent independent GRAS determination that expanded the use of this material to all foods, and its use at Good Manufacturing Practice (GMP) levels.

2. **Change in the Method of Determining Use in Foods**

   In the past, use levels of TG in foods have been based upon the amounts of TG in food (e.g., ppm TG/g of food). Experience obtained in working with this enzyme, has demonstrated that expressing use levels based upon *the protein content of the food* (e.g., mg TG/g of protein) is a more accurate and more useful method. This notification is intended to alert the FDA to this change.

1920 L Street, N.W., Suite 420, Washington, D.C. 20036
Telephone: (202) 728-1400 Telefax: (202) 331-3393

3. Addition of an Alternate Formulation

Based upon ongoing research, it has been determined that the addition of small amounts of glutamine peptide at two specific steps in the original TG manufacturing process, significantly increases the ability of TG to resist oxidation when exposed to air. The purpose of this notification is to alert the FDA to this alternative formulation of an oxygen-stable TG (STGP).

Three (3) copies of this cover letter and the GRAS notification to FDA are attached. All data reviewed by the Expert Panel in conjunction with this GRAS determination is available for FDA to evaluate at the SRA offices listed below.

If you have any questions regarding the process employed in this determination, the members of the expert panel or the underlying scientific data employed in the decision, we will be pleased to assist you.

Sincerely,

(b)(6)

Bruce K. Bernard, Ph.D.
President, SRA International
Authorized Representative of
Ajinomoto USA

BKB/km

ATT: GRAS Notification (3)

CC: Ajinomoto USA

SRA International, Inc.

Transglutaminase

GRAS Notification

Submitted to the

U.S.

Food and Drug Administration

December 21, 2001

1920 L Street, N.W., Suite 420, Washington, D.C. 20036
Telephone: (202) 728-1400 Telefax: (202) 331-3393

I. OBJECTIVE OF THIS GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION

A. Expansion of Uses

Microbially-produced transglutaminase (TG) has been the subject of a number of past independent GRAS determinations and FDA notifications. This notification is intended to alert the U.S. Food and Drug Administration (FDA) to a recent independent GRAS determination that expanded the use of this material to all foods, and its use at Good Manufacturing Practice (GMP) levels.

B. Change in the Method of Determining Use in Foods

In the past, use levels of TG in foods have been based upon TG in food (e.g., ppm TG/g of food). Experience obtained in working with this enzyme, has demonstrated that expressing use levels based upon the protein content of the food (e.g., mg TG/g of protein) is a more accurate and more useful method. This notification is intended to alert the FDA to this change.

C. Addition of an Alternate Formulation

Based upon ongoing research, it has been determined that the addition of small amounts of glutamine peptide at two specific steps in the original TG manufacturing process, significantly increases the ability of TG to resist oxidation when exposed to air. The purpose of this notification is to alert the FDA to this alternative formulation of an oxygen-stable TG (STGP).

II. IDENTITY

A. Chemical Name

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), transglutaminase has the following designation:

1. Formal Name

Glutaminyl-peptide γ-glutaminyltransferase

2. **Systematic Name**

Glutaminyl-peptide: amine γ-glutaminyl-transferase, EC 2.3.2.13

3. **Synonyms**

There are various synonyms used for transglutaminase, whose names are based upon the organ(s) or tissue(s) of origin (e.g., Factor XIII in blood coagulating system).

B. CAS Number

80146-85-6

C. Empirical Formula

Not applicable

D. Structural Formula

The transglutaminase enzymes are normally identified by their activity as transferases. Enzymes can also be identified by their amino acid sequence. The amino acid sequence of the microbial transglutaminase (TG) has been determined by mass spectrometry and standard Edman degradation method (Kanaji et al., 1993). The nucleotide sequence is determined after polymerase chain reaction (PCR) amplification (Washizu et al., 1994). The amino acid sequence deduced from the nucleotide sequence shows complete homology with one determined from the enzyme itself.

E. Molecular Weight

The molecular weight estimated from the 331 amino acid sequence is 37,863 and is in agreement with the value obtained by the sodium dodecylsulfate polyacrylamide gel electrophoretic (SDS-PAGE) experiment (Ando et al., 1989).

F. Enzymatic Properties

See Prior GRAS Notice #4, Section I.F

G. Quantitative Composition

Quality Control Data for Representative Lots Manufactured with and without glutamine peptide (Oxygen Stable TG Product [STGP])

Component		Original Process (TG) Mean†	Alternate Process		
			Mean	Lot # AAK-1	Lot # AAK-2
Moisture	(%)	3.6	5.1	5.2	4.9
Protein *	(%)	11.5	16.1	15.9	16.3
Protein **	(%)	--	7.4	7.3	7.4
Lipid	(%)	0.2	0	0	0
Ash	(%)	1.4	2.3	2.3	2.3
Carbohydrate	(%)	88.5	80.7	80.7	80.7
Minerals					
Pb (ppm)				ND	ND
As (ppm)			0.5	0.5	0.5

* Value equals Total N x 6.25
** Bio-Rad protein assay kit
ND Not Detected
† Mean of 5 lots

III. METHOD OF MANUFACTURING

Ajinomoto USA has determined that the addition of glutamine peptide at two points in the separation and purification steps of the manufacturing process imparts greater stability (i.e. resistance to oxidation upon exposure to air) to the final enzyme product. This product is designated as **Oxygen-Stable TG Product (STGP)**. The alternative formulation, STGP, and the original formulation, TGP, will be marketed in the US.

The source of the enzyme is exactly the same for both TGP and STGP. Detailed information on the enzyme source can be found in Prior GRAS Notice #4, Section I.H.

A schematic of the processes employed to manufacture microbial TGP is presented in Figure 1. Points at which glutamine peptide is added to the process to make STGP are identified. The comparative formulations for these products are presented in Figure 2.

Glutamine peptide is a wheat protein hydrolysate manufactured using food grade enzymes developed for supplementation in dry and moist foods. Current regulatory approvals include:

A. U.S. Regulations

Peptones (protein hydrolysates) are GRAS according to the FDA (21 CFR § 184.1553).

B. European Union

Glutamine peptide was reviewed by the Ministry of Agriculture, Fisheries, and Food (UK) and the following statement was issued: "Glutamine peptide would not fall within the scope of the impending EC Regulation of Novel Foods and Processes" (MAFF/UK, 1996).

IV. POTENTIAL HUMAN TOXICANTS

A. Testing for Biologically Active Substances (BASs) Produced by the Genus Streptoverticillium

1. Literature Search

See prior FDA GRAS Notice #4, Section IV.C.12.a

2. Categorization of Biologically Active Substances (BASs)

See prior FDA GRAS Notice #4, Section IV.C.12.b

3. Rationale for Selection of Hazardous Macromolecular BASs

See prior FDA GRAS Notice #4, Section IV.C.12.c

4. **Selection of the enzyme preparation**

See prior FDA GRAS Notice #4, Section IV.C.12.d

5. **Trypsin Inhibitory Activity**

See prior FDA GRAS Notice #4, Section IV.C.12.e

6. **β-Lactamase**

See prior FDA GRAS Notice #4, Section IV.C.12.f

7. **Endotoxin**

See prior FDA GRAS Notice #4, Section IV.C.12.g

8. **Phenomycin**

See prior FDA GRAS Notice #4, Section IV.C.12.h

9. **Teleocidins**

See prior FDA GRAS Notice #4, Section IV.C.12.i

a. **Extraction and Mass spectrometric analysis**
See prior FDA GRAS Notice #4, Section IV.C.12.i(1)

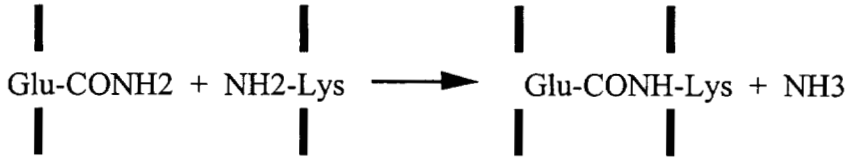
b. **Epstein-Barr Virus Early Antigen induction assay (EBV-EA)**
See prior FDA GRAS Notice #4, Section IV.C.12.i(2)

10. **Specifications for Food-Grade Material**

See Prior Gras Notice #4, Section I.H

IV. SELF-LIMITING USE

Transglutaminase is the name assigned to enzymes that catalyze an acyl-transfer of the γ–carboxyamide group of protein- and peptide-bound glutaminyl residue to various primary amines. When lysine residues in proteins are the substrate in the primary amine, protein crosslinking through ε-(γ-Glu)Lys bonds are catalytically formed between the γ-carboxyamide side chain of the glutamine residues and ε-amino side chain of the lysine residues.



Transglutaminase is added to food to bring about the cross-linking of glutamine and lysine residues within and between proteins in the food. As the number of cross-linked glutamine-lysine residues increase, changes occur in the size and structure of the food proteins present resulting in a meaningful and measurable modification in some physical properties of the food e.g. breaking strength, texture, moisture retention, etc. These modified physical properties translate to changes in sensory attributes like appearance, bite, 'mouth-feel', juiciness, etc. Use levels of the enzyme in excess of those needed to produce an enhancement in the sensory quality of the food may cause untoward effects on those attributes, e.g. excessive chewiness, diminished breaking strength, etc. Hence, like enzyme in many biological systems, there appears to be an optimal use level of transglutaminase in foods at which it functions to enhance the sensory attributes of the food, but above which it has a negative impact on quality. Interestingly, the optimal use level (i.e. concentration of added transglutaminase) is surprisingly consistent in different food systems in which it is known to function. Examples of that phenomenon are presented below.

A. Seafood Product (Kamaboko [fish cake])

Kamaboko is a surimi-based fish cake in which the addition of transglutaminase may be used to 'firm up' the product to enhance its 'mouth-feel'. As can be seen in Figure 3, as the concentration of added transglutaminase increases, there is a corresponding increase in breaking strength and resistance to deformation, up to a particular concentration. However, addition of transglutaminase above that concentration provides no additional enhancement of breaking strength while causing a reduction in the extent to which the product may be deformed before 'breaking'.

Based upon these data and the result of taste panel evaluations, it has been determined that there exists an optimum concentration of transglutaminase in this food product at which firmness is increased in the absence of increased brittleness.

B. Meat Products

1. Sausage

Sausage is prepared by processing pieces of meat through a series of steps that include chopping, mincing, the addition of transglutaminase, cutting, stuffing (into the casing), heating and cooling. The shelf life of some sausage products may be dramatically extended by canning and retorting or by lowering its pH (e.g., by the addition of acid). Both of these processes tend to soften the sausage and dramatically alter its texture and mouth feel. As with the Kamaboko product described above, the purpose of the transglutaminase is to increase texture as indicated by both breaking stress and breaking strain. Both of these parameters increase with the addition of enzyme, but both quickly reach a plateau (at approximately 3 U/g protein). At higher TG concentrations, the former (breaking stress)

remains at the plateau while the latter begins to slowly decline (see Figure 4). Five units of enzyme/g protein produce a decrease in the acceptability of the product due to toughness and brittleness (as determined by taste panel evaluation).

Hence, these data indicate that there is an optimum concentration of transglutaminase that can be used in sausage products to improve texture without making the product excessively tough or brittle.

2. Pork Loin Ham

This product is manufactured by subjecting trimmed pork loin pieces to a progression of steps involving pickling, tumbling, stuffing (into a casing), heating and cooling. The purpose of using transglutaminase is to join together smaller pieces of pork loin into a larger piece (like a ham). In addition to the desired properties of increased breaking stress and breaking strain, the retention of moisture (producing juiciness) is also an important variable in the acceptability of this product. With the addition of transglutaminase at the 0.1 U/g protein level, all three parameters are altered in the desired direction (i.e. there is a significant increase in both breaking stress and strain and an increase in water retention). The addition of 1.0 U enzyme /g protein further increases both breaking stress

and strain, however, less water is retained than even that of the untreated product (see Figure 5). Furthermore, sensory analyses indicate a reduction in product acceptance at this enzyme use level due to an increased product brittleness and a rubber-like texture.

Thus, these data again indicate that there is an optimum use level of transglutaminase in this ham-like product.

3. Restructure Steak

Pork steaks can be made by restructuring pork trimmings using transglutaminase and sodium caseinate. The pork trimmings are mixed with the enzyme and sodium caseinate by sprinkling a powdered mixture of the two on to the meat pieces (Powder Sprinkle Method) or by dissolving them in water and mixing the meat with the enzyme/sodium caseinate solution (Water Solution Method). The coated meat pieces are stuffed into a casing, allowed to set and frozen (before slicing). The optimal use level of the enzyme/sodium caseinate mixture will bind the meat pieces so they remain intact during slicing and cooking without causing the meat to become excessively tough. Using either the Powder-Sprinkle or Water Solution Methods of enzyme/sodium caseinate addition, there is a concentration of the mixture at which optimal binding occurs without causing an adverse effect on the tenderness of the meat. Use of amounts of the enzyme/sodium caseinate mixture above that level produces no additional effect of binding but adds to the toughness of the meat (see Figure 6). Hence, there is an optimal concentration of the enzyme, (2-3 U/gram protein) when it is used for binding.

C. Dairy Products (Yogurt)

Yogurt may be produced using transglutaminase by addition of the enzyme directly to milk at 25°C for approximately 2 hours. The milk is then heated (to 90°C) to inactivate the enzyme and cooled before adding standard starter cultures. The enzyme treated milk containing starter culture is poured into cups and allowed to "set" (at 44°C) for a short time before being cooled in preparation for distribution and sale.

Use of small amounts of transglutaminase helps form the yogurt gel by trapping water in the network of cross-linked milk proteins. Under usual yogurt production methods (without use of transglutaminase), the mesh of milk proteins formed by the acidity of the yogurt continues to contract causing separation of some liquid from the gel (a process termed syneresis). Use of a small amount of the enzyme in yogurt production allows development of a gel with optimal textures (breaking strength) while preventing the unsightly syneresis (see Figure

7). Use of additional quantities of the enzyme contributes no added benefit to either product texture or the prevention of syneresis. Hence, as in other applications there is an optimum use level of the enzyme, an amount similar to that seen to be optimal in other food applications. Subtle changes in the yogurt production process can allow even lower use levels of the enzyme.

D. Soybean product (Tofu)

Tofu is manufactured by treating cooled soy milk with the enzyme and subsequently coagulating, setting, heating and cutting the curd. Consumers prefer a tofu product with a texture that has a moderate breaking strength and exhibits some degree of deformation. However, they also want the product to be soft and smooth. The addition of enzyme up to 4.0 U/g protein significantly increases both product strength and deformation resistance (see Figure 8). However, loss of smoothness and softness occurs as the concentration increases, and curd strength and resistance to deformation are also adversely affected.

Hence, like other food systems, there appears to be an optimal concentration of transglutaminase that may be used in tofu products.

E. Bakery Products (Bread)

Bread is produced by combining a wheat flour mix with transglutaminase and then subjecting it to a series of processes including repeated kneading and resting, fermenting, molding, a second fermentation and baking. The enzyme has been shown to help increase loaf volume (a desired characteristic) but only when applied in low concentrations (i.e. 0.1 U/g protein, see Figure 9). At higher concentrations of enzyme use, there is actually a decrease in loaf volume.

Hence, like other food systems there appears to be an optimum concentration of transglutaminase (although an order of magnitude lower than other applications) useful for increasing bread loaf volume. Levels or enzyme higher than that optimal amount actually depress loaf volume.

In conclusion, the concentrations of transglutaminase required to achieve the desired results, in a wide variety of foods, are remarkably similar. These range from a low of 0.1 U/g protein in bread to a high of 2-3 U/g protein for restructured steak and all of these levels are at or below the levels presented in previous GRAS notifications (see Table 1). In all cases, when the concentration required to achieve the desired technical effect is exceeded, there is a decline in that or another important characteristic of the food. These data demonstrate that the use of transglutaminase is clearly self-limiting in a wide variety of foods.

VI. BASIS FOR GRAS DETERMINATION EMPLOYING SCIENTIFIC PROCEDURES

A. Safety Information

1. *Streptoverticillium Mobaranese*

a. Classification

See prior FDA GRAS Notice #4, Section IV.A.1

b. General Microbiology of the Organism

See prior FDA GRAS Notice #4, Section IV.A.2

c. Genetic stability, Cultural purity and Strain Integrity

See prior FDA GRAS Notice #4, Section IV.A.3

d. Non-Pathogenicity of the Organism

1) **Search of the Literature for Pathogenicity**
See prior FDA GRAS Notice #4, Section IV.A.4.a

2) **Pathogenicity Test of the Organism**
See prior FDA GRAS Notice #4, Section IV.A.4.b

2. TG Enzyme Broth (following organism removal)

a. Lack of Antibiotic Production by the Organism

See prior FDA GRAS Notice #4, Section IV.B.1

b. Antimicrobial Activity

See prior FDA GRAS Notice #4, Section IV.B.2

c. **β-Lactamase**

See prior FDA GRAS Notice #4, Section IV.B.3

d. **Phenomycin**

See prior FDA GRAS Notice #4, Section IV.B.4

3. Transglutaminase: The entire preparation

a. **Chromosomal Aberration**

See prior FDA GRAS Notice #4, Section IV.C.1 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

b. **Reverse Mutation**

See prior FDA GRAS Notice #4, Section IV.C.2 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

c. **Mouse Micronucleus**

See prior FDA GRAS Notice #4, Section IV.C.3 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

d. **Dermal Sensitization**

See prior FDA GRAS Notice #4, Section IV.C.5 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

e. **Dermal Maximization**

See prior FDA GRAS Notice #4, Section IV.C.6 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

f. Allergenicity (Amino Acid Sequence)

See prior FDA GRAS Notice #4, Section IV.C.7

g. Acute Toxicity

See prior FDA GRAS Notice #4, Section IV.C.8 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

h. One-Month Toxicity

Since submission of previous GRAS Notifications (#0004, 00029 and 00055) relating to the use of transglutaminase in different foods, a 30-day assessment of potential toxicity in dogs has been completed. The design and results of that study are summarized below.

Diets containing 0, 0.1 inactive transglutaminase product, 0.1, 1.0 and 5.0% active transglutaminase product were fed to groups of beagle dogs for 28 days. The mean intake of enzyme during the administration period was 48.8 mg/kg/day for males and 47.6 mg/kg/day for females in the 0.1% inactive concentration group, 48.5 mg/kg/day for males and 50.1 mg/kg/day for females in the 0.1% active concentration group, 496.5 mg/kg/day for males and 501.7 mg/kg/day for females in the 1.0% active concentration group, and 2410.6 mg/kg/day for males and 2380.6 mg/kg/day for females in the 5.0% active concentration group. Each group consisted of 4 animals of each sex. All animals were subjected to autopsy (immediate sacrifice) at the end of the 28-day test period.

The dogs were observed for clinical signs of toxicity twice daily during the period of test article administration. Vomiting was observed once in 2 male animals from Group 5 (5.0% active enzyme) and soft stools were observed twice in one male from Group 4 (1.0 active enzyme). The effects were not considered related to administration of the test material because there was no dose-response effect.

Third eyelid swelling was noted in the right eye of one female animal from Group 2 (0.1% inactive enzyme) and two females in Group 5. This swelling of the nicitating membrane was present in the animals prior to initiation of the study and was not test material-related.

Body weights were recorded pretest, approximately weekly and prior to necropsy. No significant differences in changes in body weight were observed between the control and treated groups over the 30-day duration of the study. Compared to controls, a significant decrease in body weight was noted in males from Group 5 during Study Days 1-8, and significant increases were observed in males from Groups 4 and 5 during Study Days 22-29. The mild decrease in body weight changes is likely due to the slight decrease (nonsignificant) in feed consumption during the same period. These effects were not considered test-material related.

Feed consumption was measured daily. There were no significant effects of inactive or active enzyme ingestion on daily absolute feed consumption in males when compared to controls. However during Study Days 1-6, there was a trend towards a decreased feed consumption for males in Group 5. On study Day 1, females in Groups 3 (0.1% active enzyme), 4 and 5 exhibited significant increases in feed consumption. A similar increase was observed for females in Group 5 on Study Day 23. This is likely due to the quantity of feed offered. The effect is not considered toxicologically significant because the overall feed consumption was not different from that of the controls.

All animals were subjected to ophthalmologic examinations by a board-certified ophthalmologist prior to study initiation and prior to necropsy. No abnormalities were observed.

A semi-quantitative urinalysis was conducted pretest and prior to necropsy on all animals. Feed and water were withheld during the overnight collection period. Pretest urinalysis indicated the majority of male dogs had blood in their urine, while blood was less frequently noted in the urine of females. Due to the inherent false positives associated with the analysis procedure, these findings were not considered biologically significant. Other pretest findings were uniform throughout all groups. Analysis of the urine at the termination of the study revealed no test material-related effects.

Hematology parameters were observed pretest and prior to necropsy. There were no significant differences between the control and treated groups in any of the parameters evaluated.

Clinical chemistry was performed pretest and prior to necropsy. Pretest analyses showed a marginally elevated creatinine level for

males in Group 5 and females in Group 3, globulin levels in males in Group 3 and phosphorus level in females in Group 4. These findings were not considered biologically significant. Analytical values at the termination of the study showed marginal increases in sodium levels for males in Groups 4 and 5. The effect was not considered test-material related because there was no dose-response effect. There were no significant differences between the control and treated groups.

Electrocardiograms were performed pretest and near the end of the study. All dogs were within normal ranges. There were no test material-related effects.

Results of serum protein electrophoresis conducted pretest, were remarkable for males in Groups 3, 4, and 5. When compared to control values, gamma-2-globulin was decreased approximately 33%. The effects were not considered biologically significant. Results of serum protein electrophoresis at the termination of the study showed no test material-related effects.

A complete necropsy was performed on all dogs. Gross pathology observations were limited to female dogs. Observed lesions included a pituitary gland cyst, discoloration and paleness of the lungs, an enlarged uterus, and an enlarged right eyelid. These finds were considered spurious and not test material-related.

With exception of the prostate gland, there were no significant differences in organ weights (relative and absolute). The absolute weights of the prostate gland were significantly lower in all treatment groups than in the control dogs. However, when the prostate weights were expressed as a percentage of brain weight, the inactive and high-dose active enzyme group values were similar to control values. In addition, there was no dose-response relationship, and there were no microscopic correlates to suggest a test material effect. The reduction in the prostate weights was not considered test material-related.

Histopathology was performed on all tissues from all animals and all gross lesions. No treatment-related microscopic changes were observed in any group. Microscopic changes that were observed in various organs and tissues were considered to have been incidental and unrelated to treatment.

The most common microscopic finding was mild to marked acute congestion of the spleen. This change occurred in all males and

female dogs of all groups and is likely related to the to barbiturate anesthesia used for the euthanasia procedure. Another microscopic change that occurred in most dogs, including the control dogs, consisted of multifocal mineralization in the renal papilla. Also, multifocal accumulations of pigmented macrophages were seen in the mandibular lymph nodes of male and/or female dogs of all groups, including the controls.

Microscopic examination of the lung revealed several different microscopic changes which consisted of chronic peribronchitis, foreign body granulomas, foci of alveolar macrophages, and multifocal chronic or focal proliferative inflammation. These pulmonary changes occurred at single or similar frequencies among the various treatment groups and also were considered to be incidental and unrelated to treatment.

The authors conclude that: "There were no transglutaminase-related effects established for any of the toxicological parameters (clinical observations, body weights, feed consumption, opthamological examinations, electrocardiograms, hematology, clinical chemistry, protein electrophoresis, urinalysis, necropsy, organ weights and histopathology). For the duration of the study, all levels of dietary exposure to transglutaminase (inactive and active) were well tolerated and no treatment-related adverse effects were observed." Redfield Laboratories, Study #099-001, Final Report, Jan. 3, 2001

i. 13-Week Toxicity

See prior FDA GRAS Notice #4, Section IV.C.9 and toxicity summaries prepared using the JECFA method, which were provided to Dr. Linda Kahl October 30, 1997.

j. Determination of the Presence of Aflatoxins

See prior FDA GRAS Notice #4, Section IV.C.10.

k. Antibiotics Evaluations

See prior FDA GRAS Notice #4. Section IV.C.11.

l. Antimicrobial Activity

See prior FDA GRAS Notice #4, Section IV.C.12.

4. Estimated Consumption in Intended Uses

a. Chronology: Previous Approvals

The use of TG has been declared to be Generally Recognized as Safe Under Conditions of Intended Use (GRAS) in a number of food categories by a series of SRA-sponsored Expert Panels. The chronology of these approvals is provided below.

1) On February 11, 1997, an independent panel of experts declared the use of TG (at specified levels) to be GRAS in **processed meats**. A notification summary of this designation was submitted to the US Department of Agriculture (USDA) on June 9, 1997. The USDA requested a review of this information by the US Food and Drug Administration (FDA).

2) On February 2, 1998 an approval was received from USDA. In addition, on May 15, 1998 the USDA issued a "no-objection letter."

3) On February 11, 1997, the use of TG (at specified levels) was declared GRAS in **processed seafood.** A GRAS Notification (GRN) was submitted to FDA for this use on March 3, 1998. That Agency reviewed the submission and on June 22, 1998 issued a "no-objection letter."

4) On May 19, 1998, the use of TG (at specified levels) was declared GRAS in **dairy products and meat substitutes.** A GRAS notification for this use was submitted to the FDA on July 26, 1999. On December 29, 1999 the Agency issued a "no-objection letter."

5) On September 13, 1999, the use of TG (at specified levels) was declared GRAS in **pasta, pastry, cereal, pizza dough and grain mixtures**. A GRAS notification was submitted to the FDA for this use on August 7, 2000. On January 30, 2000 the Agency issued a "no-objection letter."

b. Use Levels: Previous Approvals

The previously approved food categories and usage levels are listed below.

Food Category	Usage Level of TG In **Food** (ppm)
Processed Seafood – regular and low-fat	65
Processed Meat – regular and low fat	65
Processed Cheeses	250
All Natural Hard Cheeses (Domestic)	100
Cream Cheese	70
Refrigerated Yogurt	30
Frozen Desserts	20
Vegetable Protein Dishes/ Vegeburgers/Meat substitutes	25
Pasta	25
Bread	15
Pastries (pies, cakes, etc.)	20
Ready to eat cereals	45
Pizza dough	20
Grain mixtures (burritos, tortillas, etc.)	25

B. Calculation of Exposure Based Upon Food Consumed

1. Previous Exposure Calculations: Based Upon Food Consumed

a) Estimated Consumption Employing MRCA Data: Methodology

MRCA (Chicago, IL) was retained to provide frequency distributions of intake of TG from all of the previously considered food categories. The data provided by MRCA is based on the Menu Census Studies of July 1993 to June 1995 and the USDA Survey of 1987 to 1988 for Average Grams/Eating Occasion, assuming incorporation of TG, at the maximum concentration levels as stated in Section A.4b, above.

b) Estimated Consumption Employing MRCA Data: Results

The table below lists the previously approved food categories and the associated consumption levels.

Food category	Mean consumption Eaters only (89% of the U.S. population)	90th Percentile Eaters only consumption	Mean consumption US population	90th Percentile US population
Processed seafood and meats, regular and low fat	4.1 mg/p/day	7.0 mg/p/day	0.88 mg/p/day	3.8 mg/p/day
Dairy products	5.5 mg/p/day	10 mg/p/day	2.5 mg/p/day	8.2 mg/p/day
Meat substitutes	2.1 mg/p/day	3.0 mg/p/day	0.0052 mg/p/day	--
Pasta, bread, pastry, cereals (ready to eat), pizza dough, grain mixtures	1.8 mg/p/day	5.6 mg/p/day	1.4 mg/p/day	5.3 mg/p/day
Total estimated consumption of all food categories	4.9 mg/p/day	13.0 mg/p/day	4.2 mg/p/day	12 mg/p/day

2. Estimated Consumption Employing Protein Content of Food:

a) Rationale

All of the past GRAS considerations have estimated exposure based upon the amount (ppm of food on a weight basis) of TG in the quantity of **food** consumed. We have always known, however, that the amount of TG required in particular types of foods (TG concentration) to achieve the desired technical effect is a function of the **amount of protein** in the particular food. As previously discussed in this GRAS petition, TG acts to form ε-(γ-glutamyl) lysine linkages by catalyzing an acyl-transfer of the γ-carboxyamide group of protein- and peptide-bound glutaminyl residue to various primary amines. When lysine residues in proteins are the substrate in the primary amine, protein crosslinking through ε-(γ-glu)lys bonds are catalytically formed between the γ-carboxyamide side chain of the glutamine residues and ε-amino side chain of the lysine residues (see Appendix A).

Thus, the greater the percentage of protein in a particular food, the more TG is required when expressed as mg TG/g **food.** The previous GRAS approvals, although not based upon protein content, clearly were closely related to it (see Table 2).

Knowing the above (i.e. TG concentration is a function of protein content of the food), why in the past did we calculate consumption based upon food intake instead of protein intake? There are several reasons. First, there was not enough experience available to *a priori* permit the prediction of the concentration required in a particular food based solely upon the protein content. Thus, each food was individually tested to determine the proper level of TG required. The efficiency of TG is an inverted U-shaped function, with both too little and too much TG providing a sub-maximal technical effect (see Appendix A). Second, the food groups that were considered for 'intended use' contain relatively similar percentages of protein (i.e. generally in the 10% -30% range (see Table 2). Thirdly, in spite of the fact that these calculations grossly over estimated consumption (and thus exposure), even this over estimation produced relatively small exposure estimates.

What has changed that requires the rethinking of this type of analysis? First, the applicant desires to have the use of TG approved for use in all remaining food groups. These food groups, while accounting for a significant portion of the energy derived from consumed food (≈50%) have a *very small protein content* (see Table 3) and, hence, collectively represent only 15% of all protein being consumed. In addition, future uses of transglutaminase will include creative new formulated foods where the functional benefits of the enzyme will be delivered via protein-based ingredients (e.g., soy, whey, etc.) added to the product formulations. Use levels of the enzyme in these applications will be dependent upon the type and amount of protein used in the formulation. Also, for some of these remaining food groups, TG may never be employed. Indeed, the very nature of the function of transglutaminase, cross-linking proteins, programmatically precludes its uses in food like raw fruits or vegetables. This will eliminate the necessity for GRAS consideration for each of the remaining and all new food formulations.

b) Background Data

As discussed above, the food groups for which TG is now considered GRAS have a protein content generally of 10% - 30% (see Table 2). *En toto* these groups have consistently constituted approximately 82% - 88% of the protein consumed by the U.S. population (see Tables 4A and 4B). This includes meat, poultry, fish, dairy foods, most grain-based products and some of the soy, legume and nut protein used in meat analogs.

The remaining food groups, while they constitute approximately 50% of the total food energy consumed (see Table 3), contributed only 12-18% of the protein ingested. When viewed individually, these food groups generally provide, on average, $\leq$ 1% of the total protein consumed (see Tables 5A and 5B).

The overall protein intake for Americans has been well known for some time based upon a number of government sponsored surveys. These surveys indicate that the relative and absolute intake of protein has remained fairly constant over the last 88 years (see Tables 6 and 7). The results of a number of surveys (USDA, NHANES, etc.) evaluating protein consumption, as a function of sex and age has also been consistent (see Tables 8, 9, and 10). These data indicate that the mean protein intake is approximately 75 g/p/day while the 90th percentile for this parameter is approximately 140 g/p/day (see Table 11).

c) Projecting Use Levels in Low Protein Foods

The concentrations of TG that will be required in these low protein foods to achieve the technical effects are not well defined. However, the concentrations required in the food categories that were the subject of previous notifications provide a good basis for generating such an estimate. Based upon the percentage of protein and the concentrations of TG required in the food groups, it can be observed that the range of TG concentration to protein (mg TG/g protein) varies from 0.16 to 0.92 (see Table 2).

Given the paucity of knowledge regarding required use levels of the TG in additional (low protein) food groups, the applicant, in this notification, addresses levels based on the protein content of the food, similar to those previously approved. The TG levels in three previously approved categories (i.e. processed cheese, bread and dough) were considered inappropriate for inclusion in this calculation due to the issue identified in Table 2.

Using the amount of TG (mg) per gram of protein in all previously approved food categories (see Table 12) and USDA data on protein content of these major food groups (see Table 6), a weighted mean of the amount (mg) of TG per gram of protein was determined. This value (0.38 mg TG/gram of protein) was assumed to be the appropriate estimated use level of TG in the food categories contained in this notification, and was subsequently employed to determine the additional exposure to the enzyme from these new uses.

d) Projecting Exposure Estimates in All Foods

1) Use of MRCA Consumption Data

As noted earlier, the protein ingested from TG-containing foods which were the subject of previous GRAS notifications is about 82% - 88% of the total protein intake (see Table 6) of Americans. Thus, the protein content of all food groups in the present notification represents only 12% - 18% of total protein intake. If the mean concentration of TG (mg/gram of protein) used in these numerous foods is equal to that of the foods in earlier notification (0.38 g of protein), one can use earlier (MRCA generated) TG exposure estimates to project potential future exposures.

Using the aforementioned assumptions, exposure estimates and the conservative 18% addition to protein intake, the use of TG in all protein containing foods will increase the mean and 90th percentile of exposure by approximately 18%. Under these conditions the average intake of the enzyme for the entire population will be 6.5 mg/person/day (up from 5.5 mg/person/day in approved categories), while that of the 90th percentile of intake will be 14.2 mg/person/day (an increase from 12 mg/person/day). Similar estimates for 'Eaters Only' change from 6.2 mg/person/day for the average consumer to 7.3 mg/person/day, with the 90th percentile of intake increasing from 13 mg/person/day to 15.3 mg/person/day.

2) Use of Protein Consumption Data

A second and distinct method for estimating consumption of transglutaminase, as included in all food groups, is possible based upon the known distribution of protein consumption.

According to a number of USDA surveys (i.e. Economic Research Service [1970 – 1991, 1988 – 1991], Household Food Consumption Survey [1994 – 1996]), the intake of protein in the various U.S. subpopulations ranges from 25 – to 111 g/p/day. The estimate of overall mean protein intake is 75 g/p/day. Estimates of the 90th percentile of intake based upon published surveys (USDA Household Food Consumption Survey [1994 – 1996], HHS-NHANES III) range from 84 – 154.5 g/p/d, with an overall estimate of 140 g/p/day. If transglutaminase were added to all food containing protein (100% of the food consumed), an obvious worst case and clearly unachievable situation, total consumption would be 28.5 g/p/day with a 90th percentile consumption of 53.2 g/p/day.

Estimates of exposure levels of transglutaminase, like most such intake assessments, tend to substantially over estimate the amount of TG ingested for a host of reasons, like that noted above. These overestimates are especially seen when use level is based on the protein content of a food. For example, there are a large number of protein containing foods consumed in which the enzyme would likely never be used, e.g. nuts consumed as snacks, whole cuts of fish or meat, etc. Hence, taking the unattainable worst case scenario, the addition of transglutaminase to all protein-containing food would result in a consumption of approximately 50 g/p/day, a level that is clearly consistent (and in many cases lower) than consumption of other enzymes currently permitted in US foods.

3) Application of Transglutaminase at Good Manufacturing Practice (GMP) Levels of Use

Previous requests for determination of the GRAS status of TG have involved food applications in which use levels of the enzyme in commercial practice had been somewhat established by prior extensive laboratory testing, pilot scale implementation and full production experience in other

countries. To date, use levels of the enzyme (e.g. 65-ppm in meat, etc.) in previous notifications have been shown to adequately achieve the functional purposes of its intended use. Research in some of the areas of intended use have uncovered methods for reducing substantially the level of TG required for the desired functionality (e.g. the notified use level in processed cheese was 250 ppm, processing methods have been devised which will permit use level of less than 10 ppm).

Much of the research to identify processing modifications that would allow lower use levels of the TG is being driven by cost. It is currently estimated that the use of TG to restructure meat (at use levels of less than 65 ppm) adds $0.20 - $0.25 per pound to the cost of the meat. Like all enzymes used in food processing, the added product cost by the use of TG will continue to drive food processors to identify alternative processing systems and conditions that will further reduce use levels of the enzyme.

Little is known about the required use levels of TG in a number of the products that are the subject of this notification because experience with the enzyme in these applications has been limited. As noted earlier, because the functionality of the enzyme relates to the protein present in such foods, it is possible to estimate potential use levels. However, as applications of the technology extend to more complex, multi-ingredient formulated food systems, the relationship between protein concentration (in the food) and TG use levels will vary. In like manner, other variables that can impact enzyme activity in a food (e.g. pH, water activity, etc.) may similarly alter the TG to protein relationship.

Hence, the present notification involves the use of TG at those amounts required by Good Manufacturing Practice (GMP). Currently approved use levels of TG (meat, poultry, seafood, etc.) meet or exceed those which might be considered GMP, although as creative new applications of the enzyme are found in even these food categories, use levels may need to be modified. Approval of commercial enzymes at GMP use levels is well established and documented. All of the more than 20 enzymes affirmed by the U.S. Food and Drug Administration as GRAS listed in Part 184 of the Code of Federal Regulations have no use

limits other than current Good Manufacturing Practice (GMP) (see Appendix B). During 2001, the FDA has responded with 'no objection' to some 13 GRAS notifications involving enzymes proposed for use at GMP levels.

Unlike many ingredients added to foods, the levels of enzymes that can be used tend to be self-regulating. That is, concentrations of enzyme in foods that are inadequate to achieve the intended purpose result in quality attributes that make the product either undesirable or inedible. Conversely, addition of excessive levels of an enzyme to a food system can similarly have a substantial and negative impact on product quality. As previously discussed in this notification, a number and variety of food types were evaluated and demonstrate the existence of a narrow range of optimal use levels and the negative impact of enzyme concentrations in excess of those optimal levels.

In addition to the above noted negative impact of excess TG concentrations on its functionality and product quality, like most microbial-derived enzymes, the high cost of transglutaminase similarly serves to limit its over use. As noted earlier, the current cost to the food processor of using TG in a restructured meat product application is in the range of $0.20 - $0.25 per pound of meat. For example, for a poultry processor wishing to restructure chicken leg or thigh meat, this translates into a more than doubling of product cost by using TG. Hence, ingredient cost becomes a major driving force to insure the use of minimal TG levels that produce the optimum functionality. Although technological improvements may over time increase the efficiency of TG production and potentially reduce its cost, for the foreseeable future, cost is anticipated to be a use-limiting factor.

Estimates of use levels (concentrations) to be employed in various food groups covered in previous notifications have proved to be more than adequate (i.e. in some cases, overestimates) in actual use.

These food uses and related use levels resulted in the following estimates of consumption:

Group	Mean (mg/p/d)	90th Percentile (mg/p/day)
Eaters and Non-Eaters	5.5	12.0
Eaters Only (89% of the U.S. population)	62.	13.0

Estimates of the **increase** in consumption posed by the use of transglutaminase in the remaining food groups, the subject of this notification (i.e. an additional 18% consumed protein), are:

Group	Mean (mg/p/d)	90th Percentile (mg/p/day)
Eaters and Non-Eaters	1.0	2.2
Eaters Only	1.1	2.3

Thus, it is estimated that consumption of transglutaminase if employed in all food groups would be:

Group	Mean (mg/p/d)	90th Percentile (mg/p/day)
Eaters and Non-Eaters	6.5	14.2
Eaters Only	7.3	15.3

Based upon a past history of use in 82% of the protein consumed in the U.S., the projections based upon consumption of 100% protein are likely to be very accurate. However, if these estimates should prove to be incorrect and consumption in these new groups has been underestimated (unlikely) by even 25% (highly unlikely), the resulting consumption is at the low end of the

consumption range compared with most other enzymes currently permitted in the U.S. food supply by FDA:

Group	Mean (mg/p/d)	90th Percentile (mg/p/day)
Eaters and Non-Eaters	6.75	14.75
Eaters Only	7.6	15.9

3. **Estimated Consumption of Possible Contaminants**

See prior FDA GRAS Notice #4, Sections IV.A.4. and IV.B.

4. **Cumulative Effect of TG in Diet**

TG has been found to be widely distributed in nature. A variety of TGs have been discovered in many tissues and organs of animals, fish, shellfish, plants and microorganisms. There are five types of TG found in the tissues and organs of a host of living organisms.

For instance, Factor XIII in warm-blooded animals, including humans, is a TG that aid in blood clotting. Extensive studies have revealed that beef, pork, chicken and rabbit muscles contain fair amounts of tissue-type TG. In addition to blood TG (Factor XIII) and tissue type-TG, there are three other types of TG in living matter. They include epidermal, keratinocyte and prostate TG. TG activity was also found in the floral buds of broccoli, leaves of silver beet, spinach and soybean, sprout apices of sunflower and alfalfa. TG activity was also reported in *Bacillus, Streptoverticilliums* and *Physarums.*

A number of foods, many of which have been consumed by humans for centuries, have been evaluated for TG specific activity and content (see Table 13). Based upon these data, it is apparent that natural consumption of TG is likely to exceed consumption of TG employed as a food additive.

C. Inconsistent Reports

There are no reports that contain conclusions inconsistent with the information presented in this notification.

D. Conclusion

In accordance with the FDA's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS), this notification is hereby submitted.

Transglutaminase has been the subject of a number of GRAS notifications previously submitted to the FDA and a petition submitted to the USDA. The FDA issued 'no-objection letters' to all notifications and the USDA issued an approval.

The purpose of this notification is to inform the Agency that a Panel of Experts has declared as GRAS, a number of changes in the use of TG in foods (see Appendix C). These changes include:

1. Expansion of Uses

The GRAS status of TG has been expanded to encompass its use in all foods at the minimum levels necessary to achieve the desired effect in accordance with current good manufacturing practices.

2. Change in the Method of Determining Use in Foods

The Panel concluded that expressing use levels based upon the protein content of the food (e.g., mg TG/g of protein) is more accurate and more useful.

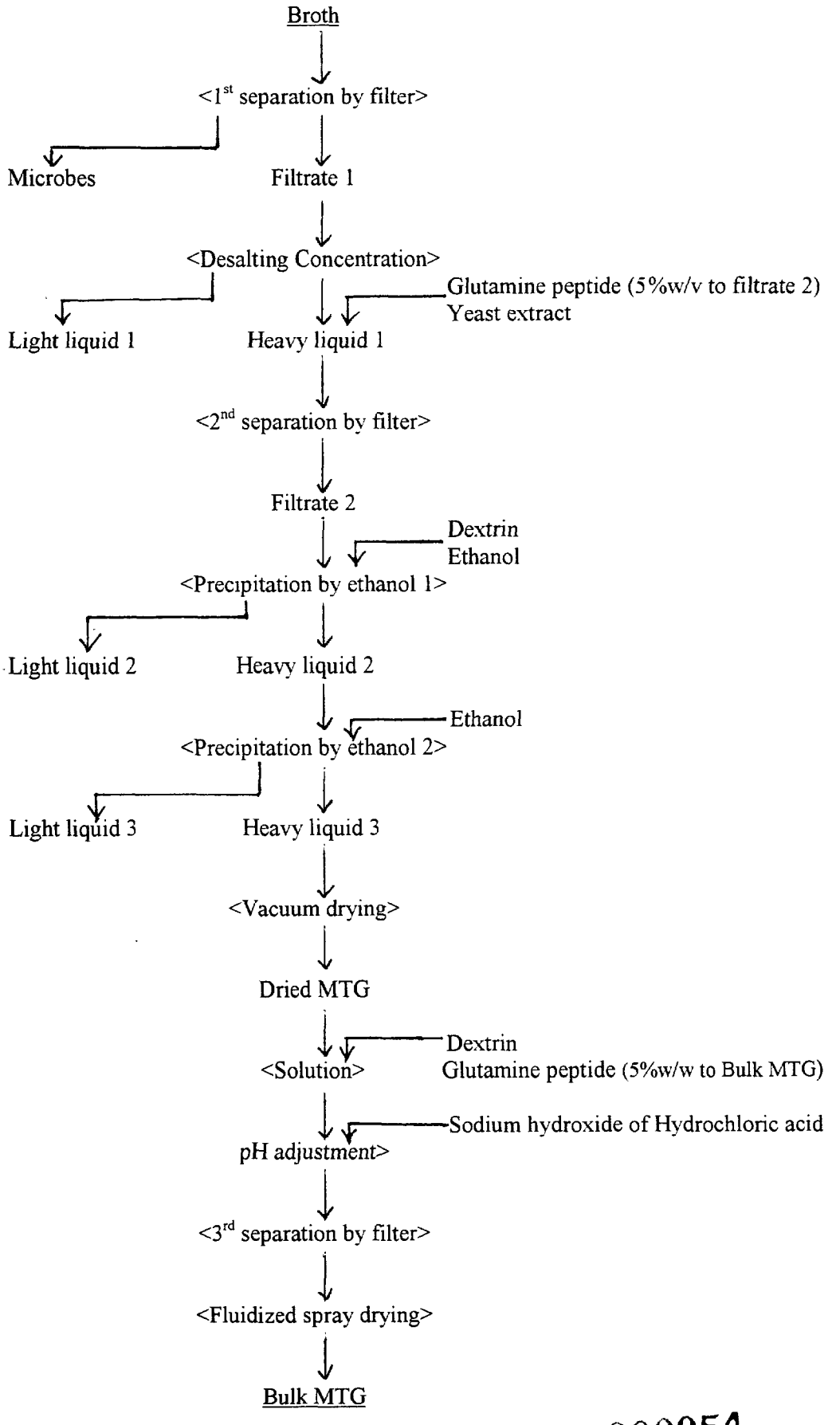
3. Addition of an Alternate Formulation

The Panel concluded that the addition of small amounts of glutamine peptide at two specific steps in the original TG manufacturing process was GRAS.

Figures 1 – 9

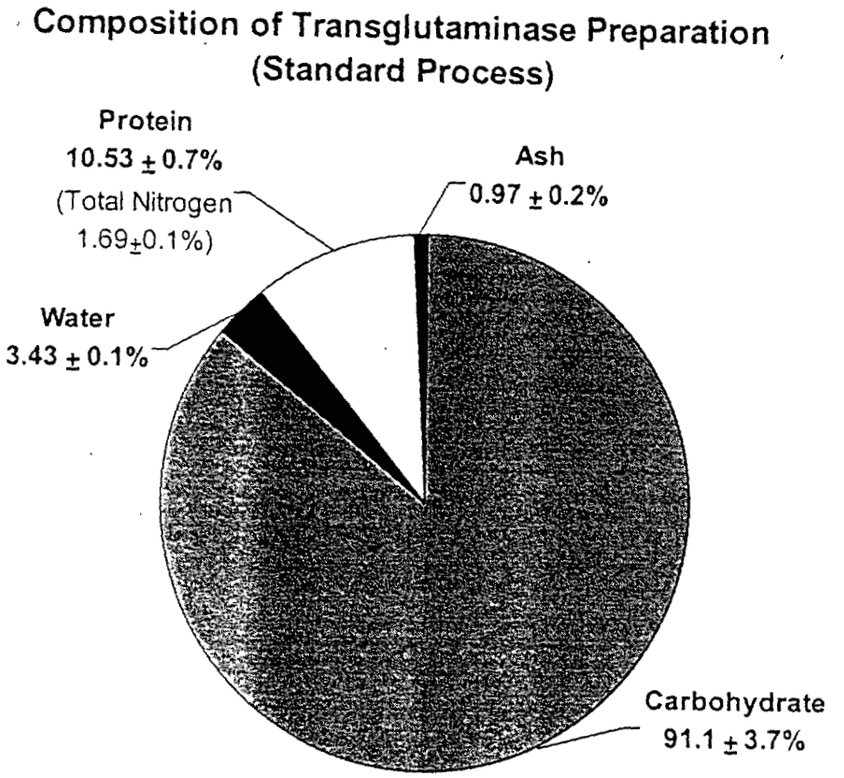
Figure 1

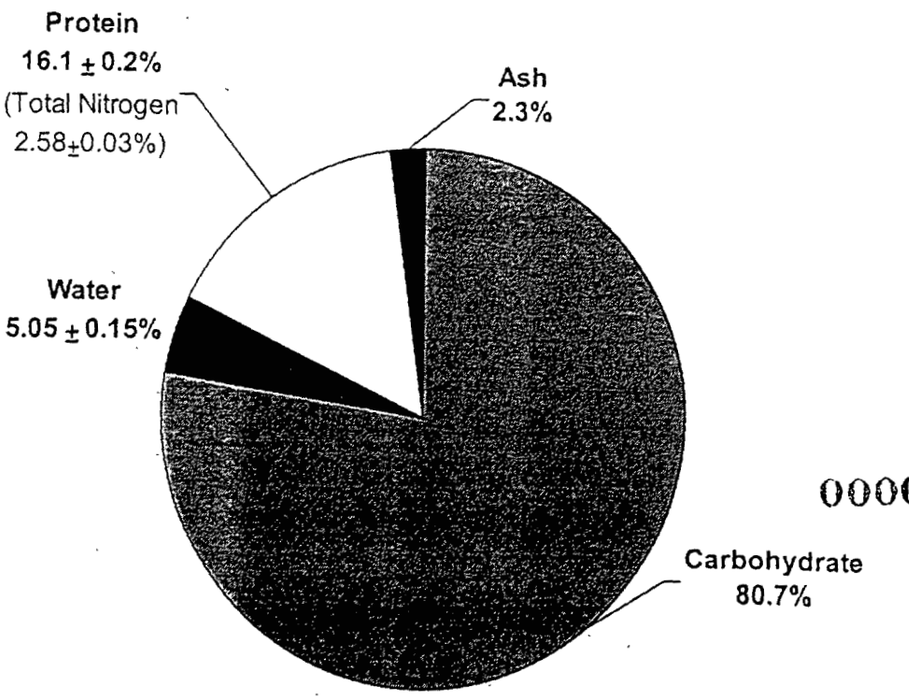
Production Process Flowchart for New TG



000054

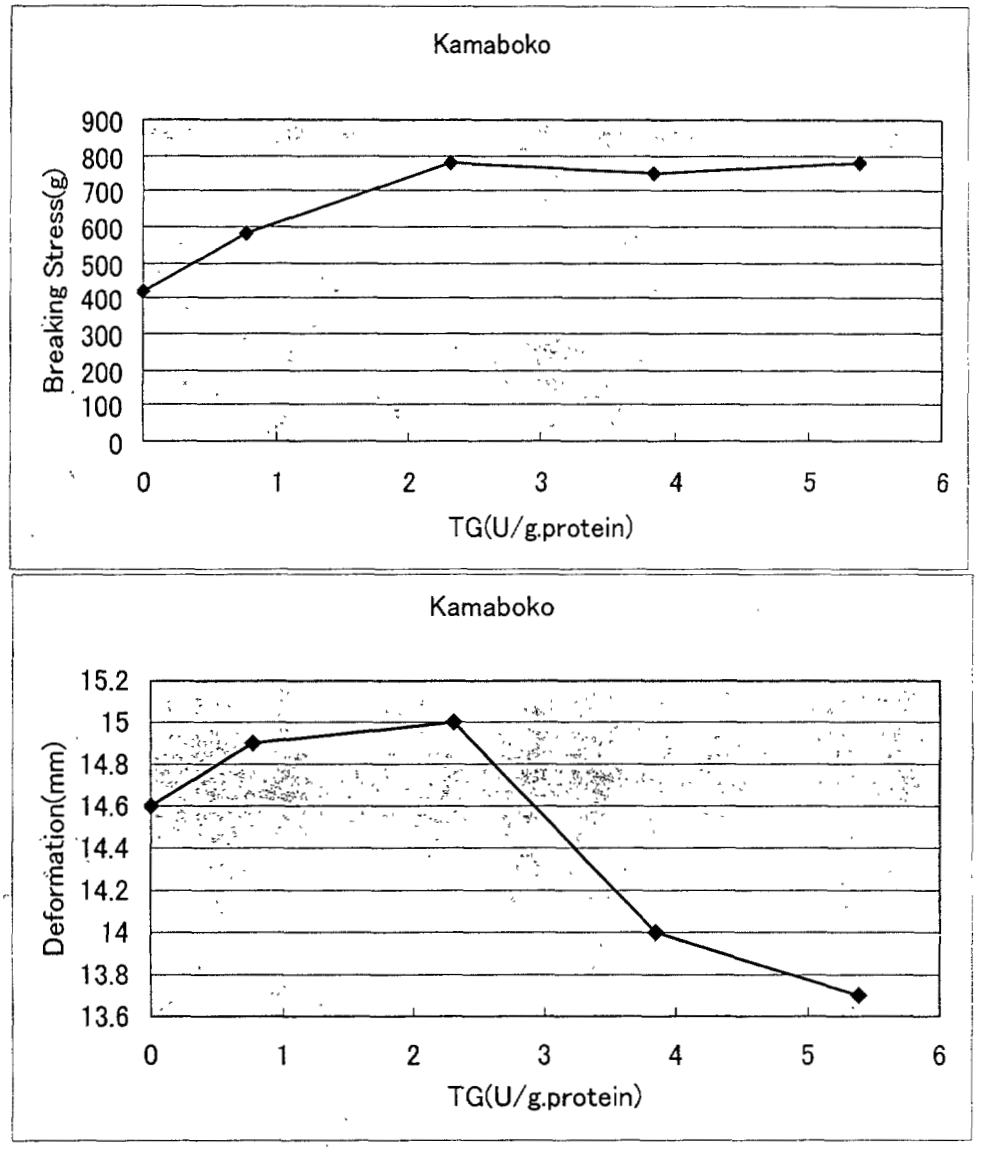
Figure 2





000055

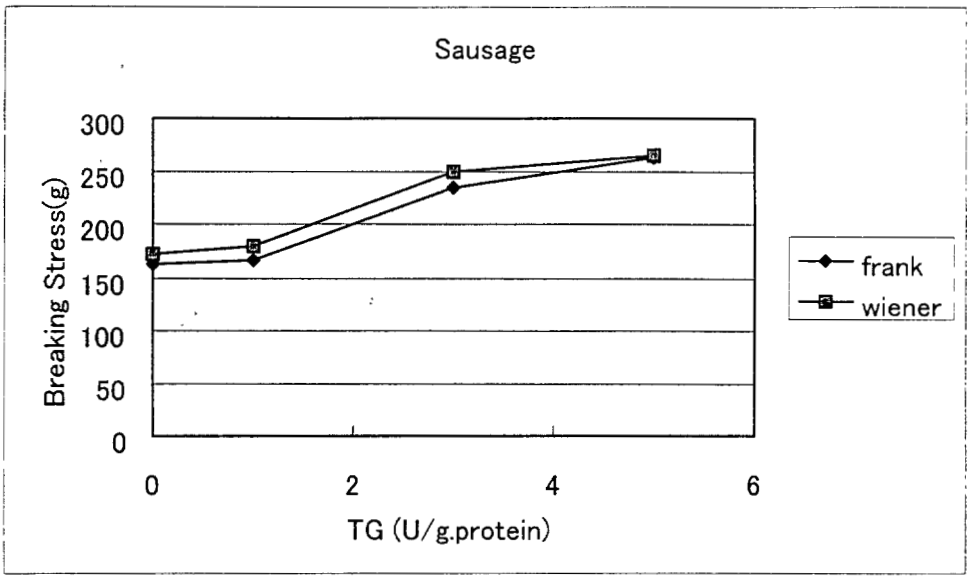
Figure 3

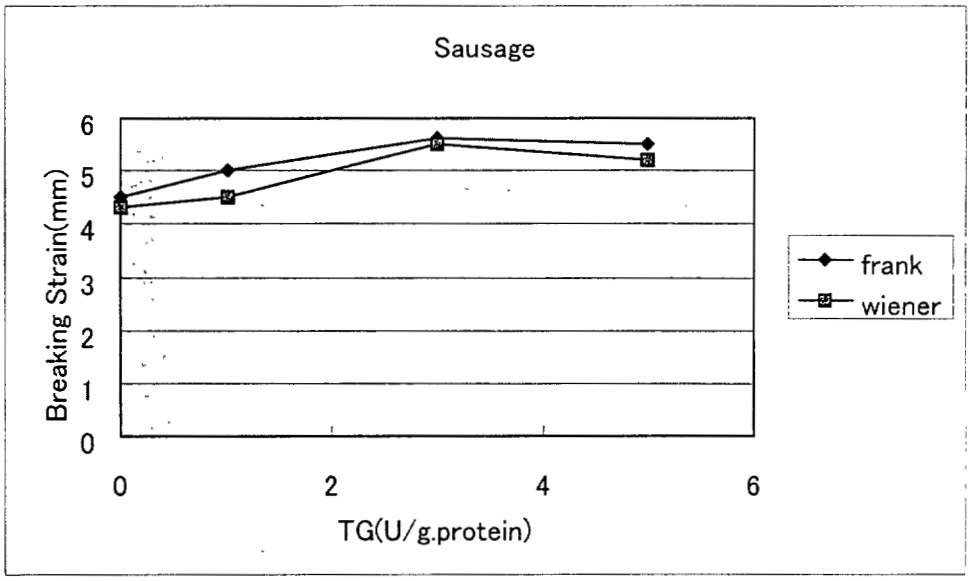


Breaking stress increases with TG amount and becomes plateau more than 2 U/g protein. Breaking stress decreases more than 2 U/g protein after increase. Desirable texture of *kamaboko* is given between 0.7 to 0.12 U/g protein of TG addition.

000056

Figure 4

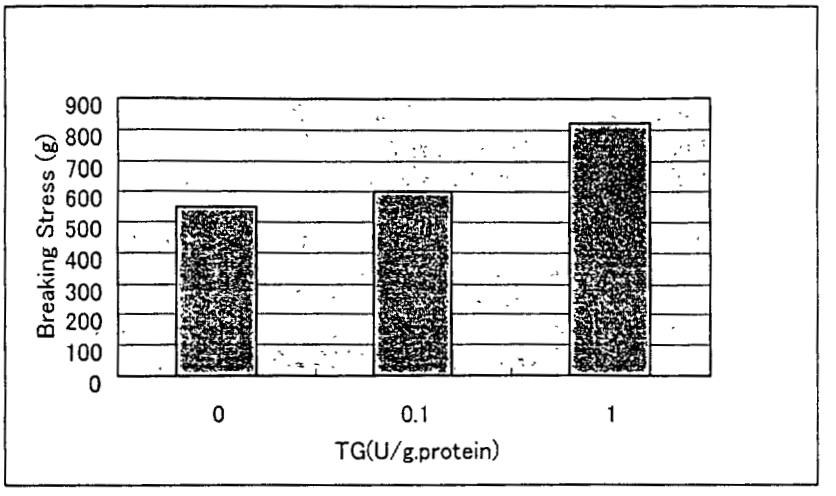


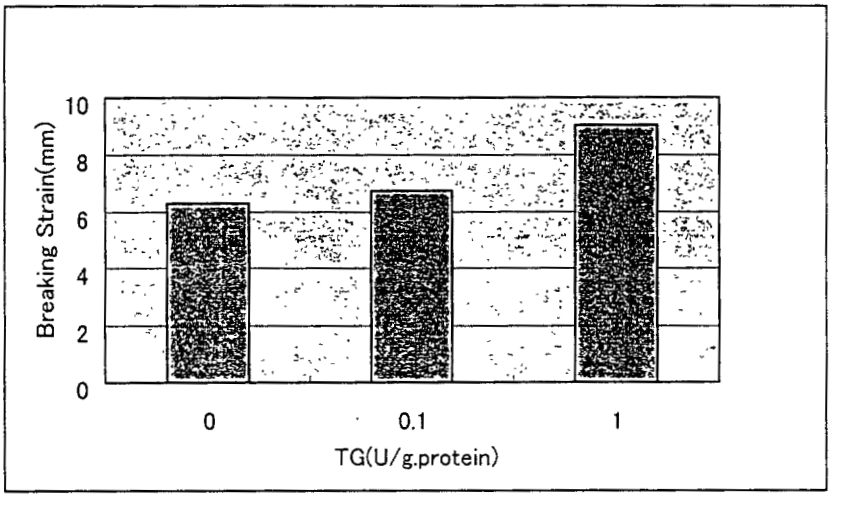


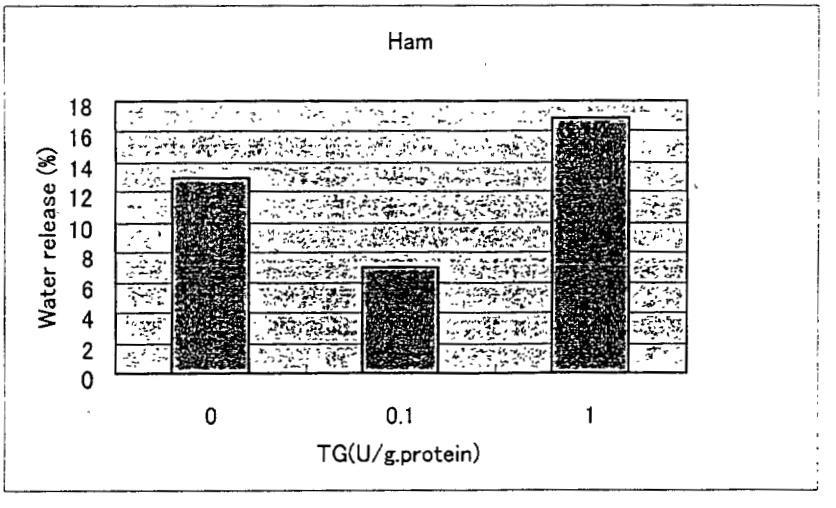
Breaking stress increases with TG amount while breaking strain decreases gradually at TG level more than 3 U/gram protein after it reaches maximum. Although breaking stress does not drop at 5 U/gram protein, too tough and brittle texture is observed by sensory analysis (data are not shown).

The best texture can be obtained between 3 and 4.5 u/gram protein. Too much enzyme, such as more than 5 U/gram protein, causes undesirable texture.

Figure 5





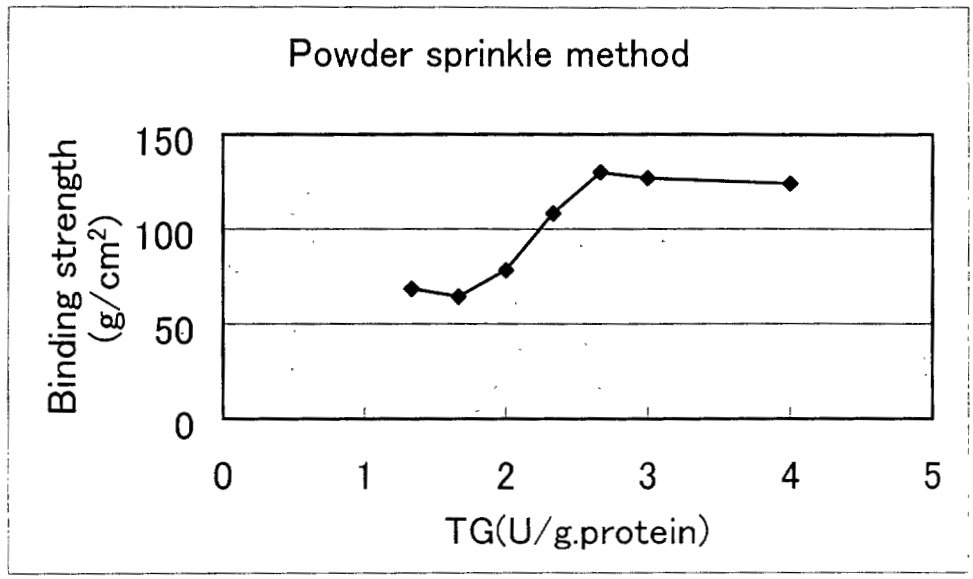


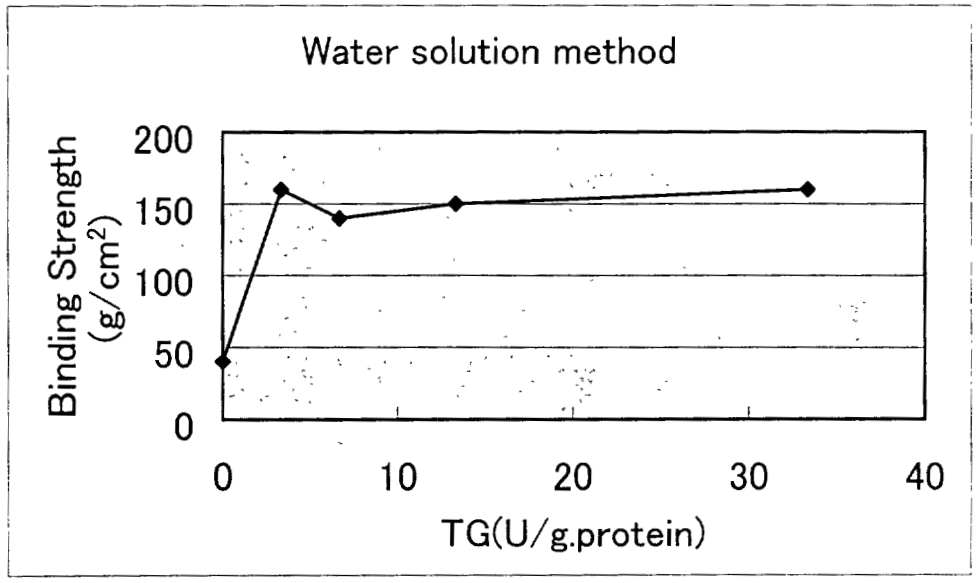
Both breaking stress and strain increase with TG amount. Water release* can be suppressed at 0.1 U/gram protein while it increases at 1 U/gram protein. Undesirable texture, such as too brittle and rubber-like texture, is observed at 1 U/gram protein by sensory analysis (data are not shown). Best texture can be obtained between 0.5 and 1.0 U/gram protein.

$$*\text{water release (\%)} = \frac{\text{product weight Z(g)} - \text{product weight compresses (g)}}{\text{product weight (g)}} \times 100$$

000058

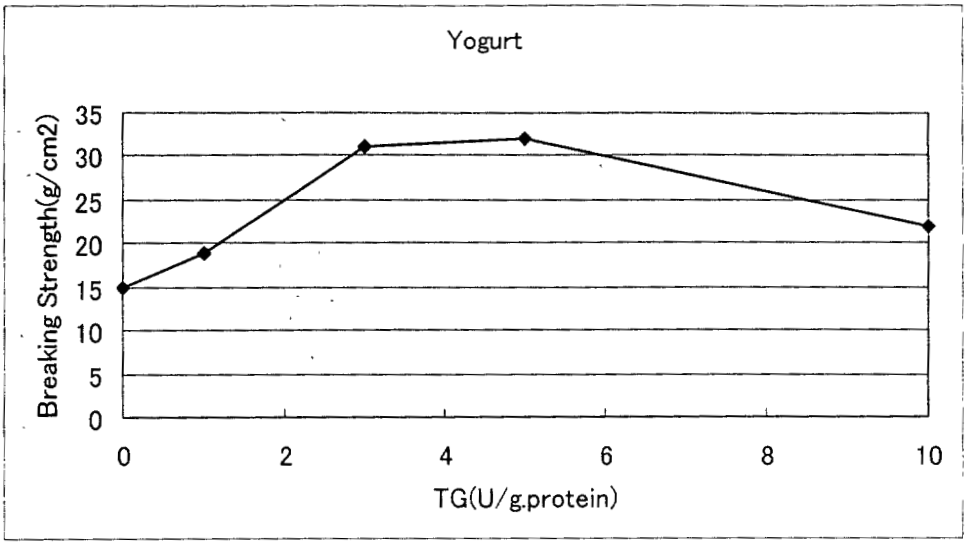
Figure 6

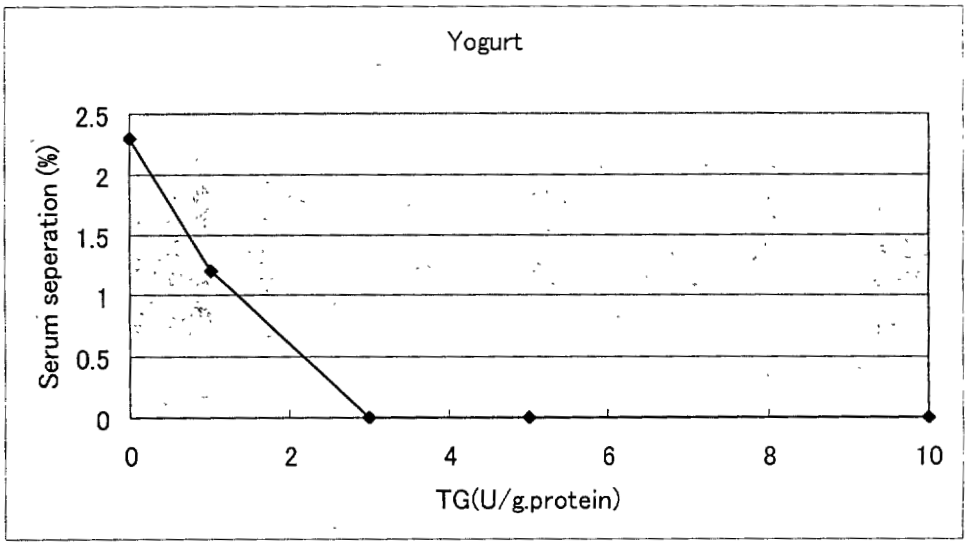




Feasible binding strength is more than 80 g/cm^2. Appropriate TG range is between 2.0 and 2.5 U/gram protein in Powder Sprinkle Method. In case of Water Solution Method, appropriate TG range is between 2.0 and 3.0 U/gram protein. It becomes plateau more than 3U and 5 U/gram protein in Powder Sprinkle and Water Solution Method.

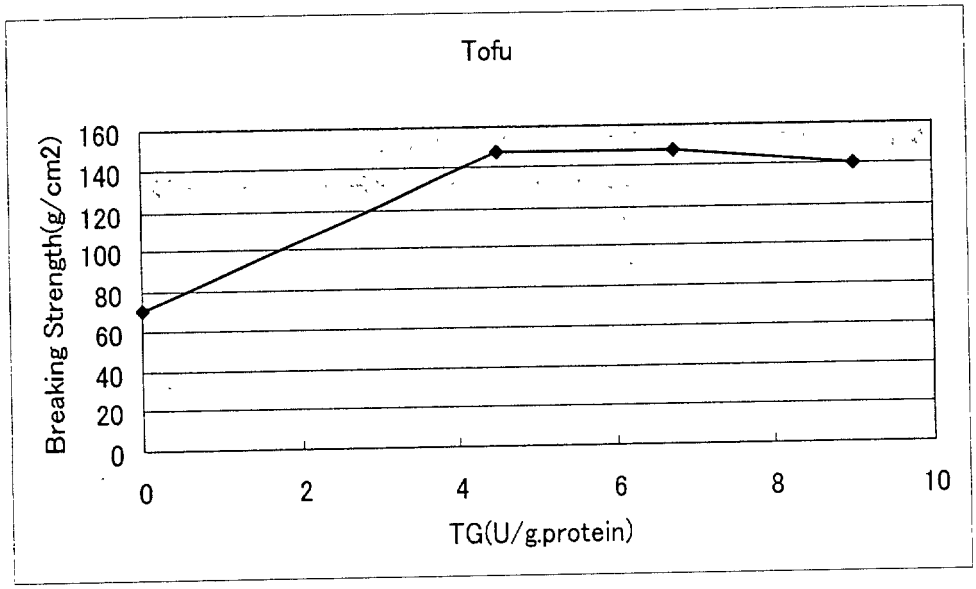
Figure 7

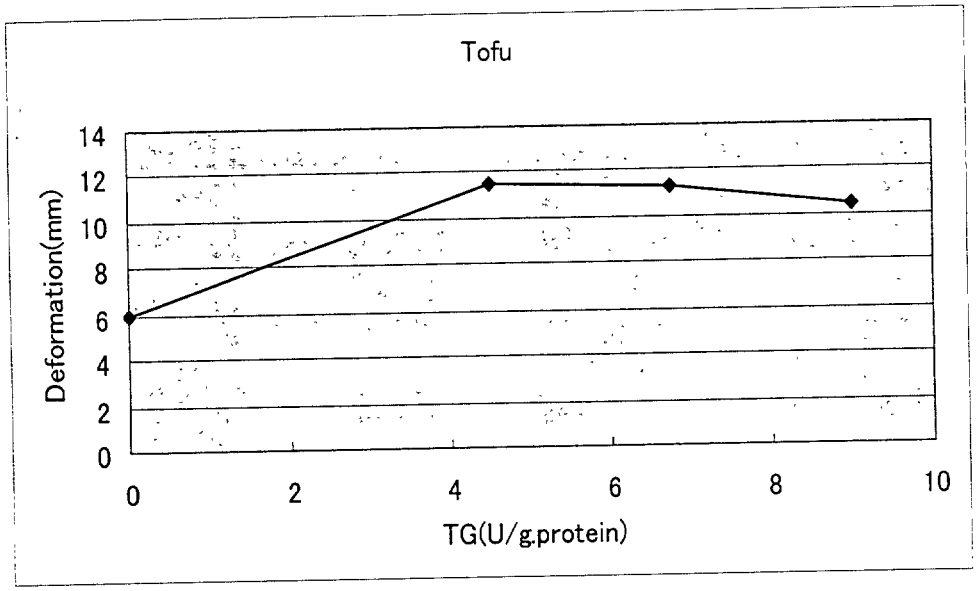




Breaking strength increases almost linearly with TG amount and reaches plateau at around 3 U/gram protein. Serum separation is suppressed completely at more than 3 U/gram protein. The yogurt curd becomes brittle and develops a rough texture when TG is added by more than 3 U/gram protein. TG addition of 2 to 3 U/gram protein is the appropriate range for obtaining smooth and good texture. This range is also good enough to suppress serum separation. In this procedure, material milk is heated to inactivate TG activity before fermentation. When TG is added with starter culture during fermentation, greater effects can be obtained. In this case, 0.5 to 0.75 U/gram protein is large enough.

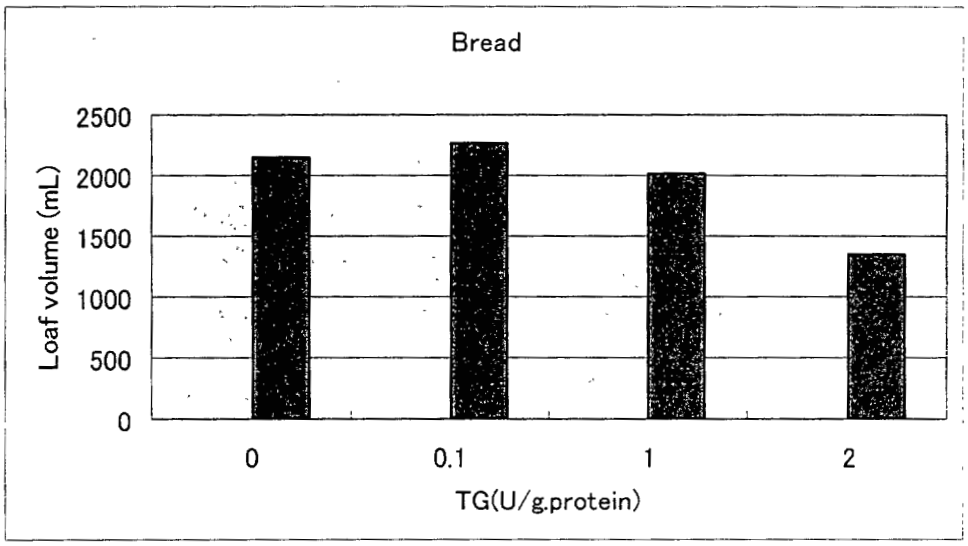
Figure 8





Both breaking strength and deformation reaches almost plateau at more than 4 U/gram protein. Tofu requires a soft and smooth texture. Appropriate TG range is between 0.5 and 1.0 U/gram protein.

Figure 9



Loaf volume decreases with TG amount after it reaches maximum at 0.1 U/gram protein. The dough becomes hard and rough with TG addition. Too hard and poor 'mouth feeling' in the final product is observed by sensory analysis when TG is added more than 0.1 U/gram protein (data are not shown). Appropriate TG amount is around 0.1 U/gram protein.

Tables 1- 13

Table 1: **Summary of Appropriate TG Range in Various Food Items**

item	protein content (%)	TGase (U/gram protein)	TGase (ppm)*
Kamaboko	12.0	0.7-1.2	9.2-13.8
Sausage	13.0	1.5-2.3	20.0-30.0
Ham	14.0	0.7-1.5	10.0-20.0
Yogurt	3.5	2.0-3.0	7.0-10.5
Tofu	6.0	0.5-1.0	3.0-6.0
Bread	8.5	0.01-0.1	0.085-0.85
Restructured Steak powder sprinkle method	15.0	2.0-2.5	30.0-37.5
Restructured Steak water solution method	15.0	2.0-3.0	30.0-45.0

*Amount in final product.

000064

Table 2

Protein Content of Selected Foods or Food Groups and Amount (milligrams) of Transglutaminase Currently Approved for Use in Those Foods*

Product	Protein Content (grams/100 g)	Approved TG Level in **Food** (ppm)	Approved TG Level in **Protein** (mg/kg)
Meats			
Beef	13.0 – 19.0	65	0.41
Pork	13.5 – 16.0		0.44
Chicken	23.0 - 31.0		0.25
Turkey	30.0		0.22
Seafood		65	
Fish	30.0		0.31
Scallops	20.0		0.33
Shrimp	20.0 – 24 0		0.30
Dairy Products			
Natural cheese		100	
Cheddar	25.0		0.40
Brick	22.2		0.45
Swiss	27.5		0.36
Processed cheese	23.2	250*	
Cottage cheese	13.5 – 16.0		
Cream cheese	7.6	70	0.92
Frozen desserts	4.5	20	0.44
Yogurt	3.0 – 3.5	30	0.92
Grain Products			
Macaroni (pasta)	4.0 – 5.0	25	0.56
Bread	8.7 – 9.0	15*	
Rolls	8.4 – 9.9	15	0.16
Pastry	5.4 – 7.4	20	0.31
Grain mixture (tortilla)	5.7 – 8.7	25	0.37
Ready-To-Eat cereal	3.8 – 20.5 (8.0)	45	0.56
Dough	9.0	20*	

Average (weighted for percent of total ingested protein) amount of transglutaminase (in milligrams) per gram of protein in food uses approved to date – 0.38mg/gram protein

000065

- Inappropriate comparisons –
  A. Processed cheese – need 250 ppm to crosslink whey proteins in cheese – crosslinking can be done prior to addition of whey using low level of TG
  B. Bread and dough – by using protease enzymes with TG, level of TG needed is substantially lower than that which might be expected (or approved)

Table 3

Nutrients Contributed from Major Food Groups, per capita per day, 1970 and 1994
Source: USDA Center for Nutrition Policy and Promotion

Food Group	Protein				Food Energy	
	1970		1994		% of Total Intake	
	grams	% total	grams	% total	1970	1994
Meat, poultry and fish	38	40.3	43	39.3	19.8	14.3
Dairy products	20	21.2	21	19.3	10.6	9.3
Grain products	18	19.1	26	23.7	19.6	25.1
Subtotal	76	80.6	90	82.3	50.0	48.7
Legumes, soy, and nuts	5	5.4	7	6.0	2.9	2.9
Eggs	5	5.7	4	3.8	2.0	1.3
Vegetables	5	5.8	5	5.3	5.2	4.7
White potatoes	2	2.5	3	2.3	2.8	2.6
Dark green, deep yellow	-	0.4	-	0.4	0.4	0.4
Other vegetables	3	3.0	3	2.6	2.1	1.8
Fruits	1	1.2	1	1.3	2.9	3.4
Citrus fruits	1	0.5	1	0.6	0.9	1.0
Noncitrus fruits	1	0.7	1	0.8	2.1	2.4
Fats and oils	-	0.1	-	0.1	17.8	19.5
Sugars and sweeteners	-	-	-	-	18.1	18.3
Miscellaneous	1	1.2	1	1.2	0.9	1.2

000066

Table 4A

Nutrients Contributed from Major Food Groups, per capita per day, 1970 and 1994
Source: USDA Center for Nutrition Policy and Promotion

Food Group	Protein			
	1970		1994	
	grams	% total	grams	% total
Meat, poultry and fish	38	40.3	43	39.3
Dairy products	20	21.2	21	19.3
Grain products	18	19.1	26	23.7
Subtotal	76	80.6	90	82.3
Legumes, soy, and nuts	5	5.4	7	6.0
Eggs	5	5.7	4	3.8
Vegetables	5	5.8	5	5.3
White potatoes	2	2.5	3	2.3
Dark green, deep yellow	-	0.4	-	0.4
Other vegetables	3	3.0	3	2.6
Fruits	1	1.2	1	1.3
Citrus fruits	1	0.5	1	0.6
Noncitrus fruits	1	0.7	1	0.8
Fats and oils	-	0.1	-	0.1
Sugars and sweeteners	-	-	-	-
Miscellaneous	1	1.2	1	1.2

000067

Table 4B

Protein (%) Contributed to Diet from Existing GRAS Approvals, in Selected Years

Year	Major Food Groups					
	Meat, Fish Poultry	Dairy Products	Grain Products	Subtotal	Legumes, Soy & Nuts	Total
1909-14	31.2	14.2	36.6	82.0	4.5	86.5
1925-29	30.8	17.0	32.4	80.2	4.9	85.1
1935-39	30.5	19.3	29.2	79.0	5.8	84.8
1945-49	33.3	22.3	23.9	79.5	5.2	84.7
1955-49	37.3	22.5	20.3	80.1	5.3	85.4
1965-69	42.7	20.2	18.9	81.8	5.0	86.8
1970	44.0	19.8	18.1	81.9	5.0	86.9
1971	44.4	19.8	17.9	82.1	4.9	87.0
1972	44.4	19.9	17.8	82.1	4.8	86.9
1973	42.4	20.3	18.6	81.3	5.8	87.1
1974	44.2	19.6	18.3	82.1	5.1	87.2
1975	43.1	19.5	19.0	81.6	5.6	86.7
1976	44.0	19.4	18.9	82.3	5.1	87.4
1977	44.2	19.5	18.6	82.3	5.2	87.5
1978	44.0	19.9	18.6	82.5	4.9	87.4
1979	43.3	19.7	19.0	82.0	5.3	87.3
1980	43.9	19.6	19.1	82.6	4.7	87.3
1981	44.0	19.4	19.1	82.5	5.0	87.5
1982	42.9	19.7	19.4	82.0	5.5	87.5
1983	43.4	19.6	19.0	82.0	5.4	87.4
1984	43.5	19.9	19.0	82.4	5.0	87.4
1985	43.1	19.9	19.3	82.3	5.5	87.8
1986	42.6	20.0	19.6	82.2	5.4	87.6
1987	42.7	20.1	20.1	82.9	4.9	87.8
1988	43.3	19.5	20.1	82.9	5.2	88.1

Data extracted from 'Nutrient Content of the U.S. Food Supply, 1909 – 1988*' N.R. Raper, C. Zizza, and J. Rourke
*Based upon USDA Food Available for Consumption Data

000068

Table 5A

Protein Contributed from Major Food Groups to the U.S. Food Supply, Selected Years

Year	Meat, Poultry, Fish				Dairy Products					Eggs	Legumes, Nuts, and Soy	Grain Products
	Meat	Poultry	Fish	Total	Fluid Milk		Cheese	Other	Total			
					Whole	Lowfat						
	Percent											
1909-14	25.1	3.4	2.8	31.2	9.0	2.6	1.4	1.2	14.2	5.1	4.5	36.6
1925-29	24.2	3.4	3.2	30.8	10.4	2.2	1.7	2.6	17.0	5.9	4.9	32.4
1935-39	23.9	3.5	3.2	30.5	11.1	2.1	2.3	3.9	19.3	5.6	5.8	29.2
1945-49	25.9	4.6	2.8	33.3	13.0	1.5	2.7	5.1	22.3	6.6	5.2	23.9
1955-59	28.6	5.5	3.3	37.3	12.2	1.1	3.6	5.5	22.5	6.7	5.3	20.3
1965-69	31.5	7.5	3.7	42.7	9.9	1.7	4.3	4.3	20.2	5.6	5.0	18.9
1970	32.1	8.0	3.9	44.0	9.0	2.2	4.6	4.0	19.8	5.4	5.0	18.1
1971	32.5	8.0	3.8	44.4	8.8	2.4	4.8	3.9	19.8	5.4	4.9	17.9
1972	31.9	8.4	4.1	44.4	8.5	2.6	5.2	3.6	19.9	5.3	4.8	17.8
1973	29.8	8.3	4.3	42.4	8.3	2.9	5.3	3.9	20.3	5.1	5.8	18.6
1974	31.7	8.3	4.1	44.2	7.8	2.9	5.5	3.4	19.6	5.0	5.1	18.3
1975	30.7	8.2	4.1	43.1	7.6	3.2	5.5	3.3	19.5	4.9	5.6	19.0
1976	31.4	8.5	4.2	44.0	7.1	3.3	5.7	3.4	19.4	4.6	5.1	18.9
1977	31.3	8.8	4.1	44.2	6.8	3.5	5.9	3.3	19.5	4.6	5.2	18.6
1978	30.4	9.3	4.4	44.0	6.6	3.6	6.2	3.5	19.9	4.8	4.9	18.6
1979	29.1	10.0	4.2	43.3	6.3	3.7	6.2	3.5	19.7	4.8	5.3	19.0
1980	29.6	10.1	4.2	43.9	6.0	3.9	6.3	3.4	19.6	4.8	4.7	19.1
1981	29.4	10.4	4.2	44.0	5.8	4.0	6.5	3.1	19.4	4.7	5.0	19.1
1982	28.1	10.7	4.1	42.9	5.5	4.0	7.0	3.1	19.7	4.7	5.5	19.4
1983	28.5	10.7	4.3	43.4	5.3	4.0	7.1	3.2	19.6	4.5	5.4	19.0
1984	28.2	10.9	4.4	43.5	5.1	4.2	7.3	3.3	19.9	4.5	5.0	19.0
1985	27.5	11.2	4.4	43.1	4.9	4.3	7.5	3.2	19.9	4.3	5.5	19.3
1986	26.6	11.5	4.5	42.6	4.6	4.5	7.6	3.4	20.0	4.2	5.4	19.6
1987	25.6	12.4	4.6	42.7	4.4	4.6	7.8	3.4	20.1	4.2	4.9	20.1
1988	25.7	12.7	4.9	43.3	4.1	4.6	7.6	3.2	19.5	4.0	5.2	20.1

**** Means value is less than 0.05 but more than 0.

000069

Table 5B

Fruits			Vegetables				Fats and Oils						Sugars and Sweeteners	Miscellaneous
Citrus	Non-citrus	Total	White Potatoes	Dark Green, Deep Yellow	Other	Total	Butter	Margarine	Shortening	Lard and Beef Tallow	Salad, Cooking and Other Edible Oils	Total		
Percent														
0.1	0.9	1.0	3.7	0.5	2.5	6.8	0.2	0.0	0.0	0.0	0.0	0.2	****	0.4
.2	1.1	1.3	3.2	.7	2.9	6.7	.2	.0	.0	.0	.0	.2	****	.8
.4	1.1	1.4	3.0	.8	3.2	7.0	.2	.0	.0	.0	.0	.2	****	1.0
.5	.9	1.4	2.4	.6	3.1	6.2	.1	.0	.0	.0	.0	.1	****	.9
.5	.8	1.3	2.4	.5	2.8	5.6	.1	.0	.0	.0	.0	.1	****	1.0
.4	.7	1.1	2.3	.4	2.5	5.2	.1	.0	.0	.0	.0	.1	****	1.1
.5	.6	1.1	2.3	.4	2.6	5.4	.1	.0	.0	.0	.0	.1	****	1.1
.5	.6	1.2	2.3	.4	2.7	5.3	.1	.0	.0	.0	.0	.1	****	1.1
.5	.6	1.1	2.3	.4	2.6	5.3	.1	.0	.0	.0	.0	.1	****	1.2
.5	.6	1.2	2.3	.4	2.7	5.4	.1	.0	.0	.0	.0	.1	****	1.2
.6	.6	1.2	2.2	.4	2.7	5.4	****	.0	.0	.0	.0	****	****	1.2
.6	.7	1.3	2.4	.4	2.7	5.5	.1	.0	.0	.0	.0	.1	****	1.1
.6	.6	1.2	2.3	.4	2.7	5.4	****	.1	.0	.0	.0	.2	****	1.1
.6	.6	1.2	2.3	.4	2.7	5.4	****	.1	.0	.0	.0	.2	****	1.0
.5	.6	1.2	2.3	.4	2.6	5.3	****	.1	.0	.0	.0	.2	****	1.0
.5	.7	1.2	2.3	.4	2.7	5.4	****	.1	.0	.0	.0	.2	****	1.1
.6	.7	1.3	2.3	.4	2.7	5.4	****	.1	.0	.0	.0	.2	****	1.1
.6	.7	1.3	2.2	.5	2.7	5.3	****	.1	.0	.0	.0	.2	****	1.1
.6	.7	1.3	2.3	.5	2.7	5.4	****	.1	.0	.0	.0	.2	****	1.1
.6	.7	1.3	2.3	.4	2.6	5.3	.1	.1	.0	.0	.0	.2	****	1.2
.5	.8	1.3	2.3	.5	2.7	5.4	.1	.1	.0	.0	.0	.2	****	1.2
.5	.7	1.3	2.2	.5	2.6	5.3	.1	.1	.0	.0	.0	.2	****	1.3
.6	.7	1.3	2.3	.5	2.6	5.4	****	.1	.0	.0	.0	.2	****	1.2
.6	.8	1.3	2.2	.5	2.5	5.3	****	.1	.0	.0	.0	.2	****	1.2
.6	.7	1.3	2.3	.5	2.5	5.3	****	.1	.0	.0	.0	.2	****	1.2

0000070

Table 6

Protein (%) Contributed to Diet from Existing GRAS Approvals, in Selected Years

Year	Major Food Groups					
	Meat, Fish Poultry	Dairy Products	Grain Products	Subtotal	Legumes, Soy & Nuts	Total
1909-14	31.2	14.2	36.6	82.0	4.5	86.5
1925-29	30.8	17.0	32.4	80.2	4.9	85.1
1935-39	30.5	19.3	29.2	79.0	5.8	84.8
1945-49	33.3	22.3	23.9	79.5	5.2	84.7
1955-49	37.3	22.5	20.3	80.1	5.3	85.4
1965-69	42.7	20.2	18.9	81.8	5.0	86.8
1970	44.0	19.8	18.1	81.9	5.0	86.9
1971	44.4	19.8	17.9	82.1	4.9	87.0
1972	44.4	19.9	17.8	82.1	4.8	86.9
1973	42.4	20.3	18.6	81.3	5.8	87.1
1974	44.2	19.6	18.3	82.1	5.1	87.2
1975	43.1	19.5	19.0	81.6	5.6	86.7
1976	44.0	19.4	18.9	82.3	5.1	87.4
1977	44.2	19.5	18.6	82.3	5.2	87.5
1978	44.0	19.9	18.6	82.5	4.9	87.4
1979	43.3	19.7	19.0	82.0	5.3	87.3
1980	43.9	19.6	19.1	82.6	4.7	87.3
1981	44.0	19.4	19.1	82.5	5.0	87.5
1982	42.9	19.7	19.4	82.0	5.5	87.5
1983	43.4	19.6	19.0	82.0	5.4	87.4
1984	43.5	19.9	19.0	82.4	5.0	87.4
1985	43.1	19.9	19.3	82.3	5.5	87.8
1986	42.6	20.0	19.6	82.2	5.4	87.6
1987	42.7	20.1	20.1	82.9	4.9	87.8
1988	43.3	19.5	20.1	82.9	5.2	88.1

Data extracted from 'Nutrient Content of the U.S. Food Supply, 1909 – 1988*' N.R. Raper, C. Zizza, and J. Rourke
*Based upon USDA Food Available for Consumption Data

000071

Table 7

U.S. food supply: Nutrients and other food components, per capita per day, 1970-94 1/

Year	Food energy	Carbo-hydrate	Protein	Fat				Choles-terol	Vitamins				
				Total fat	Satu-rated Fat	Monoun-saturated fat	Polyun-saturated fat		Vitamin A	Caro-tenes	Vitamin E	Vitamin C	Thia-min
	Kilo-calories	Grams						Milligrams	Micrograms Retinol equivalents		Milligrams Alpha-TE	Milligrams	
1970	3,300	386	95	154	54	63	26	470	1,500	510	13.7	107	2.0
1971	3,300	387	96	154	55	63	26	470	1,510	520	13.5	108	2.0
1972	3,300	386	95	155	54	63	27	460	1,530	550	13.9	108	2.0
1973	3,200	390	94	150	52	61	27	440	1,520	580	14.4	106	2.0
1974	3,200	383	94	151	52	62	27	440	1,560	600	14.2	108	2.1
1975	3,200	385	93	146	50	59	27	430	1,550	620	14.4	112	2.2
1976	3,300	399	97	152	51	60	29	430	1,580	620	14.7	113	2.3
1977	3,300	398	96	149	51	59	28	430	1,530	580	14.2	112	2.3
1978	3,200	392	95	150	51	59	29	430	1,510	580	14.5	108	2.2
1979	3,300	400	96	151	51	60	30	430	1,530	610	14.6	109	2.3
1980	3,300	406	96	153	52	60	30	430	1,520	600	14.6	112	2.3
1981	3,300	394	96	153	51	61	30	430	1,510	600	14.7	109	2.3
1982	3,300	396	96	152	51	60	30	420	1,510	620	15.0	110	2.3
1983	3,300	400	97	157	53	62	31	430	1,500	600	15.4	115	2.3
1984	3,400	404	98	155	53	62	29	430	1,530	640	14.9	112	2.3
1985	3,500	420	101	163	55	65	32	430	1,520	630	16.2	114	2.4
1986	3,500	425	102	162	54	65	32	420	1,500	610	16.3	118	2.4
1987	3,500	436	103	160	53	64	32	420	1,530	640	16.4	115	2.5
1988	3,600	443	105	161	53	64	33	420	1,470	610	16.9	116	2.5
1989	3,500	445	104	156	51	63	32	410	1,500	640	16.5	115	2.6
1990	3,600	458	105	156	51	63	32	400	1,530	670	16.8	111	2.6
1991	3,600	464	107	155	50	63	32	400	1,500	640	17.0	115	2.6
1992	3,700	473	108	158	52	64	32	410	1,540	670	17.1	117	2.7
1993	3,700	482	108	161	52	66	32	410	1,530	670	17.6	122	2.7
1994	3,800	491	110	159	52	65	31	410	1,520	660	16.9	124	2.7

1/ Data are based on ERS estimates of per capita quantities of food available for consumption from "Food Consumption, Prices, and Expenditures, 1970-94," (SB-928, ERS, USDA, April 1996), on imputed consumption data for foods no longer reported by ERS, and on estimates from USDA's Center for Nutrition Policy and Promotion (CNPP) of quantities of produce from home gardens. Historical data for this table are available from CNPP's Shirley Gerrior, (202) 606-4839, or Lisa Bente, (202) 208-2447.

Source: USDA/Center for Nutrition Policy and Promotion (CNPP).

000072

Table 8

Protein intake in grams by age, sex, and race/ethnicity: United States, 1988–91

Sex and age	Total population[1]			
	Sample size	Mean	Standard error of the mean	Median
Both sexes				
All ages[2]	14,801	78	0.6	69
2–11 months[2]	871	26	0.7	22
1–2 years[2]	1,231	48	0.8	45
3–5 years	1,547	57	0.8	53
6–11 years	1,745	67	0.9	64
12–15 years	711	76	2.0	68
16–19 years	765	89	2.5	77
20–29 years	1,682	89	1.7	77
30–39 years	1,526	88	1.6	80
40–49 years	1,228	81	1.6	75
50–59 years	929	78	1.8	72
60–69 years	1,106	73	1.5	67
70–79 years	851	65	1.4	60
80 years and over	609	58	1.6	54
Male				
All ages[2]	7,322	92	0.9	82
2–11 months[2]	439	27	0.9	23
1–2 years[2]	601	50	1.1	47
3–5 years	744	59	1.2	54
6–11 years	868	71	1.3	67
12–15 years	338	89	3.0	82
16–19 years	368	111	3.8	100
20–29 years	844	110	2.7	96
30–39 years	735	106	2.4	97
40–49 years	626	96	2.3	90
50–59 years	473	93	2.8	88
60–69 years	546	84	2.4	78
70–79 years	444	74	2.2	70
80 years and over	296	69	2.5	64
Female				
All ages[2]	7,479	64	0.6	60
2–11 months[2]	432	25	0.9	20
1–2 years[2]	630	45	0.9	43
3–5 years	803	54	1.0	50
6–11 years	877	63	1.1	60
12–15 years	373	62	2.0	58
16–19 years	397	67	2.2	62
20–29 years	838	69	1.5	66
30–39 years	791	70	1.6	66
40–49 years	602	67	1.5	64
50–59 years	456	64	1.7	59
60–69 years	560	64	1.6	60
70–79 years	407	58	1.6	55
80 years and over	313	52	1.7	49

[1]Includes data for race/ethnicity groups not shown separately.
[2]Excludes nursing infants and children.

000073

Table 9

Nutrient Intakes: Mean amount consumed per individual, by sex and age, 1 day, 1994-96

Sex and age (years)	Percentage of population	Food energy	Protein	Total fat	Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids	Cholesterol
	Percent	*Kilocalories*	*Grams*					*Milligrams*
Males and females:								
Under 1	1.1	838	21.6	36.4	15.4	11.1	7.8	46
1-2	3.1	1,312	49.2	47.9	19.5	17.5	7.3	189
3-5	4.7	1,577	55.4	57.4	21.6	21.9	9.6	183
5 and under	8.9	1,393	49.1	51.5	20.1	19.0	8.6	168
Males:								
6-11	4.6	2,026	70.3	74.3	27.3	28.7	12.7	228
12-19	5.8	2,766	97.5	102.8	36.5	40.2	18.4	323
20-29	7.3	2,821	104.1	103.3	35.4	40.1	19.6	348
30-39	8.3	2,665	102.7	102 8	35.3	39.4	20.1	352
40-49	7.0	2,435	95.3	91.8	30.6	35.6	18.3	331
50-59	4.6	2,270	90.3	87.4	28.5	33.9	18.1	332
60-69	3.4	2,072	83.5	78.8	25.9	30.1	16.5	307
70 and over	3.4	1,834	72.9	68.6	22 8	26.4	13.9	270
20 and over	33.9	2,455	94.9	92.7	31.3	35.8	18.4	331
Females:								
6-11	4.4	1,807	62.4	66.1	24.2	25.5	11.5	200
12-19	5.6	1,910	65.3	69.3	24.5	26.6	12 9	216
20-29	7.0	1,841	65.9	65.9	22.3	25.2	13.5	219
30-39	8.8	1,710	65.3	63.2	21.3	24.1	12.8	217
40-49	6.9	1,682	63.5	63.5	21.0	24.0	13.6	222
50-59	5.2	1,600	64.1	59.4	19.1	22.6	13.1	209
60-69	4.1	1,489	60.4	55.2	17.9	20.8	12.1	218
70 and over	4.9	1,384	56 6	49.2	15.9	18.7	10.6	188
20 and over	36.8	1,646	63.2	60.5	20.0	23.0	12.8	213
All individuals	100.0	2,002	75.1	74.4	25.6	28.6	14.6	256

Excludes breast-fed children.

Continued

000074

Table 10

Protein Intake of Individuals in the United States, 1994-96 – USDA Nationwide Survey*
*NFS Report No. 96.2 (USDA)

Protein Intake of Americans (as % of RDA) by Percentile of Age and Sex Groups

Age and Sex (Years)	Protein RDA (grams/day)	% of Sample Population	Protein Intake at Selected Percentile 25th (% RDA)	50th (% RDA)	90th (% RDA)	(g/day)
Male & Female						
Under 1		1.1	107.1	135.1	258.2	
1 – 2	16*	3.1	227.5	288.2	445.8	71.3
3 – 5	24*	4.7	193.0	245.8	380.4	91.3
5 & under		8.9	187.1	248.2	400.0	
Males						
6 – 11	28*	4.6	177.4	228.9	350.1	98.0
12 – 19	45/59*	5.8	132.1	172.0	275.4	143.2
20 – 29	58/63*	7.3	113.6	154.7	253.0	154.5
30 – 39	63*	8.2	115.6	153.2	238.9	150.5
40 – 49	63	6.9	111.6	139.0	224.0	141.1
50 – 59	63	4.6	105.7	135.9	216.4	136.3
60 – 69	63	3.4	101.1	126.0	190.7	120.1
70 & over	63	3.3	87.7	110.4	165.7	104.4
20 & over		33.9	107.1	140.1	226.8	142.9
Females						
6 – 11	28*	4.4	154.1	196.6	302.6	84.7
12 - 19	44/46*	5.6	102.0	135.3	215.0	96.8
20 – 29	46/50*	7.0	96.0	122.0	196.1	94.1
30 – 39	50	8.7	94.9	121.4	194.8	97.4
40 – 49	50	6.9	94.8	121.3	182.6	91.3
50 – 59	50	5.2	92.5	122.6	175.5	87.8
60 – 69	50	4.2	91.5	116.3	172.2	86.1
70 & over	50	4.8	82.9	107.6	167.9	84.0
20 & over		36.8	92.7	119.4	184.1	92.0
All Individuals		100.0	106.2	141.5	257.7	

* Age/sex categories of survey don't exactly match age categories of RDA's

000075

Table 11

Protein Intake of Americans from Various USDA and HHS Surveys

Mean Intake

USDA – Economic Research Service 1970 – 1994 (Based on production)
Mean Intake: 93 – 110 grams/day

USDA - 1988 – 1991
Mean Intake:

Male and female, all ages	78 grams/day	(range 26 – 89)
Male only, all ages	92 grams/day	(range 27 – 111)
Female only, all ages	64 grams/day	(range 25 – 70)

USDA – Continuing Survey 1989 – 1991
Mean Intake:

All individuals	71.7 grams/day
Males age 20 – 29	99.5 grams/day

USDA – Household Food Consumption Survey 1994 – 1996
Mean Intake:

Male and female, all ages	75.1 grams/day	
Males only, over 20 years of age	94.9 grams/day	(range 70.3 – 103.3)
Females, over 20 years of age	63.2 grams/day	(range 56.6 – 65.9)

ESTIMATE OF OVERALL MEAN PROTEIN INTAKE 75 grams/day

90th Percentile of Intake

HHS – NHANES III 1988 - 1994
Value quoted 133 grams

USDA – Household Food Consumption Survey 1994 – 1996

Females over 20 years	92.0 grams	(range 84 – 97.4 grams)
Males over 20 years	142.9 grams	(range 98 – 154.4 grams)
Males age 20 – 29	154.5 grams	

ESTIMATE OF 90TH PERCENTILE OF PROTEIN INTAKE 140 grams/day

000076

Table 12

Previously Approved and Proposed Maximum Use Levels of Transglutaminase in All Foods (by Category)

Product	Previously Approved TG Uses		Proposed Maximum TG Use	
	Level (ppm)	mg/gram protein	Level (ppm)	mg/gram protein
Meats				
Beef	65	0.41	81	0.51
Pork		0.44		0.55
Chicken		0.25		0.31
Turkey		0.22		
Seafood	65		81	
Fish		0.31		0.39
Scallops		0.33		0.41
Shrimp		0.30		0.38
Dairy Products				
Natural cheese	100		125	
Cheddar		0.40		0.50
Brick		0.45		0.56
Swiss		0.36		0.45
Processed cheese	250*			
Cottage cheese				
Cream cheese	70	0.92	88	1.15
Frozen desserts	20	0.44	25	0.55
Yogurt	30	0.92	38	1.15
Grain Products				
Macaroni (pasta)	25	0.56	31	0.70
Bread	15*		19	
Rolls	15	0.16	19	0.20
Pastry	20	0.31	25	0.39
Grain mixture (tortilla)	25	0.37	31	0.46
Ready-To-Eat cereal	45	0.56	56	0.70
Dough	20*		25	
Requested Other Food Categories		0.38		0.48

Average (weighted for percent of total ingested protein) amount of transglutaminase (in milligrams) per gram of protein in food uses approved to date – 0.38mg/gram protein

*Approved levels significantly exceed actual use levels

000077

Table 13

Category	Sample	Specific Activity (mg^{-1})	TG Content (mg/100g)
Meat	Pork	0.04	2.00
	Beef	0.04	2.49
	Poultry	n.d.	n.d.
Organ Meats	Pork Liver	0.08	4.46
	Beef Liver	0.13	8.00
	Chicken Liver	0.46	12.0
Fish and Shellfish	Crab meat	0.37	84.0
	Spiny lobster	1.63	55.1
	Lobster	0.56	23.54
	Oyster	5.61	232
	Clam	2.12	84.0
	Scallop	0.11	3.13
	Mussel	1.09	9.06
	Sea urchin	0.33	11.7
	Sardine	0.07	9.10
	Pollack	0.15	24.1
	Surimi	0.04	0.32

n.d.
not detected

000078

Appendix A

APPENDIX A

Lee, H.G., Lanier, T.C., Hamann, D.D. and Knopp, J.A. (1997) Transglutaminase Effects on Low Temperature Gelation of Fish Protein Sols, *J Food Sci.*, 20 (1) 20-24.

Kuraishi, C. Sakamoto, J. Yamazaki, K. Susa, Y. Kuhara, C. and Soeda, T. (1997) Production of Restructured Meat using Microbial Transglutaminase without Salt or Cooking, *J. Food Sci.*, 62(3) 488-490 and 515.T

Negative Effect of High Amount of TG on Processing Foods

000080

Pages 000081 - 000085 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this
request.

Pages 000086 - 000089 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

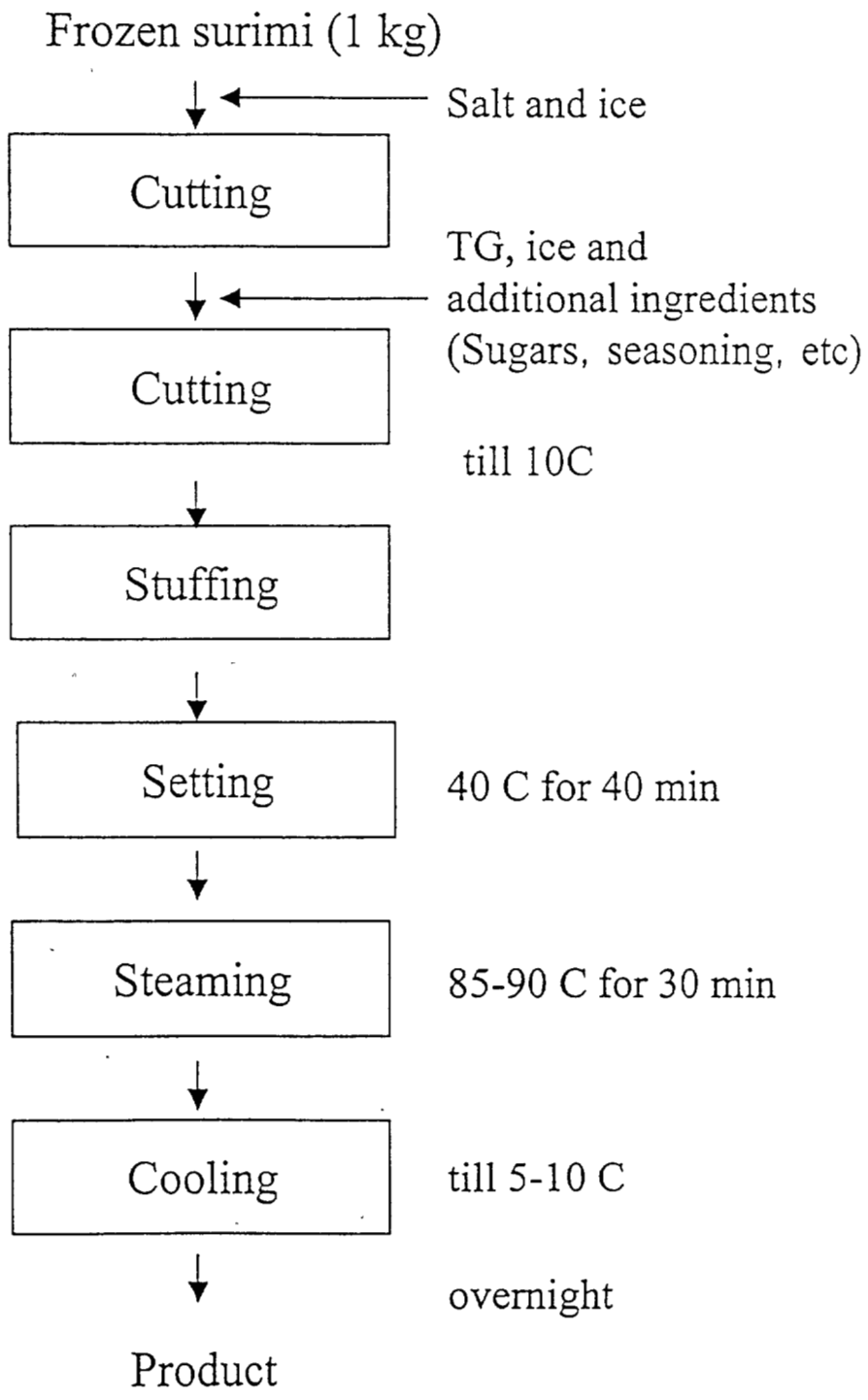
Negative effects of high amount of TG on Processing Foods

1. Sea food product
2. Meat products
3. Dairy
4. Soybean
5. Bakery
6. Restructured steak
7. Summary

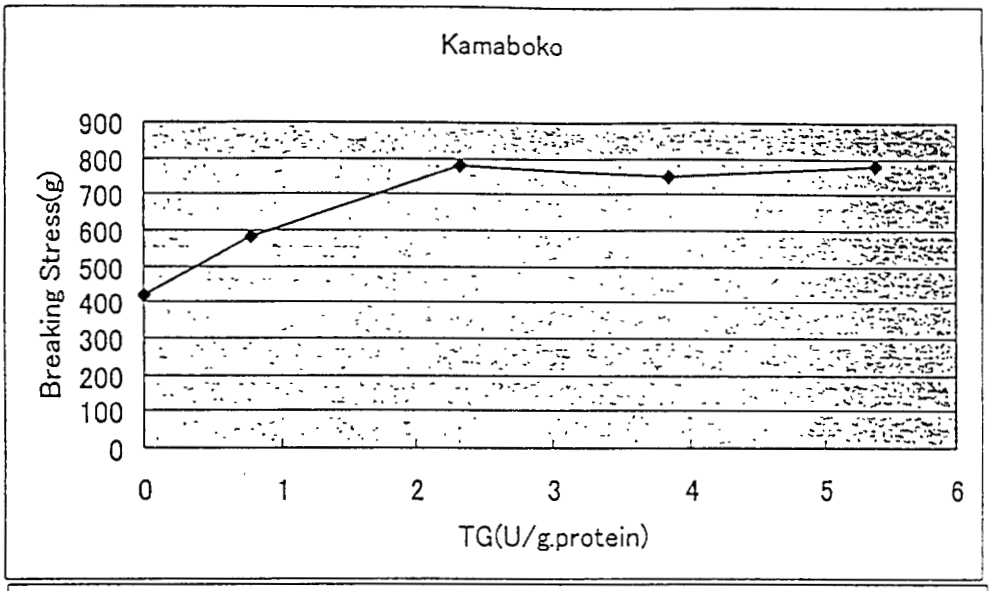
000090

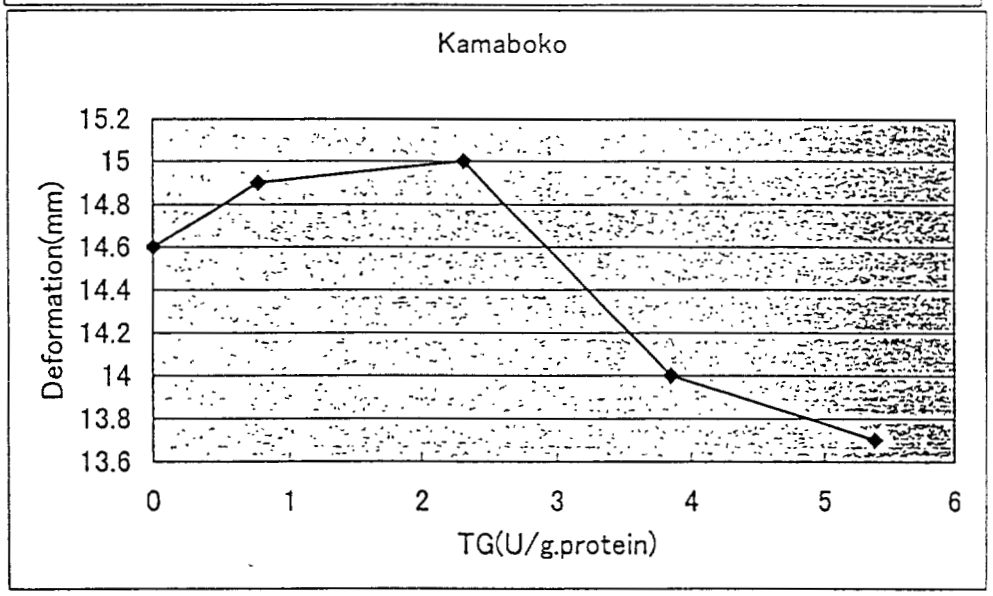
1. Sea food product (*Kamaboko,* fish cake)

Kamaboko manufacturing procedure



000091





Breaking stress increases with TG amount and becomes plateau more than 2 U/g protein. Breaking stress decreases more than 2 U/g protein after increase. Desirable texture of *kamaboko* is given between 0.7 to 0.12 U/g protein of TG addition.

000092

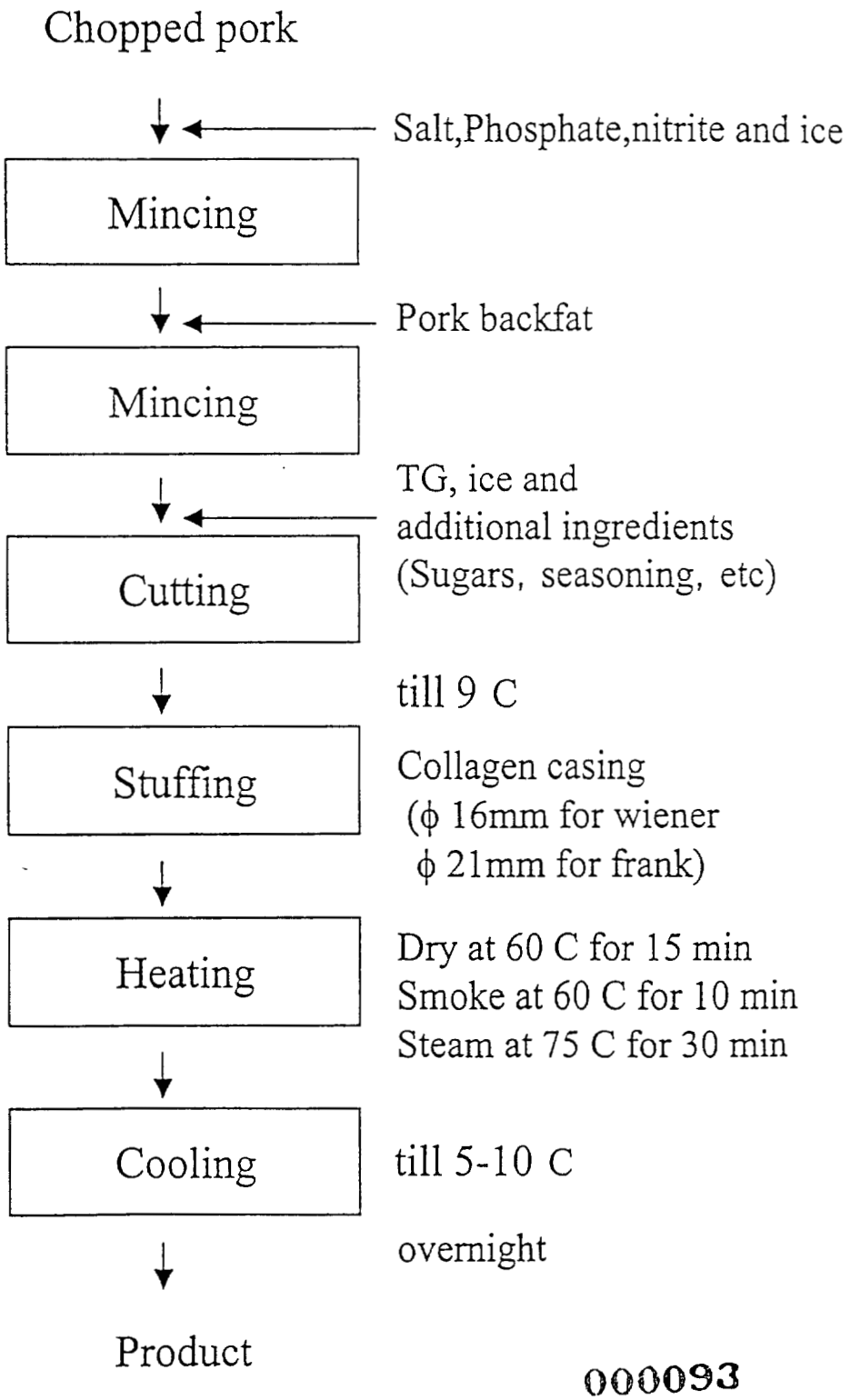
2. Meat products

2-1. Sausage

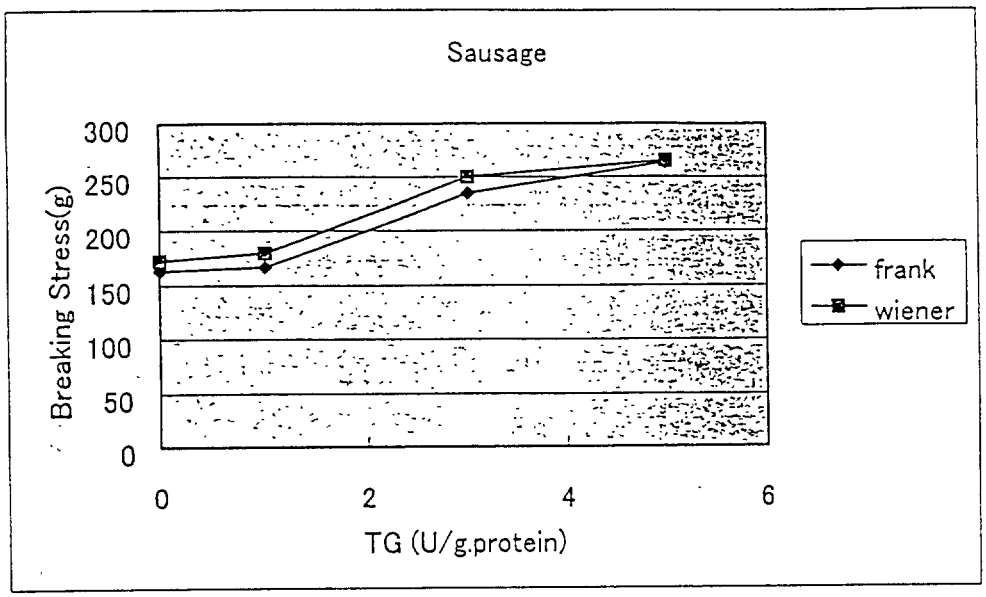
Sausage manufacturing procedure

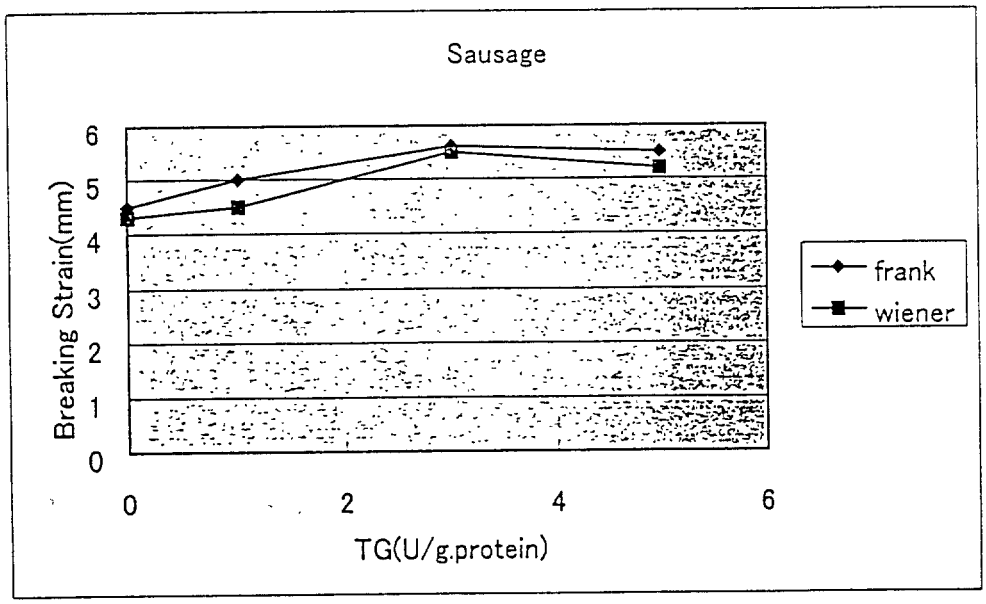
Table Ingredients for sausage manufacturing

Ingredients	Weight(%)
Pork trimmings	45.00
Pork backfat	23.10
Sodium caseinate	1.00
Soy protein	1.50
ice	25.60
Salt	1.70
Sodium Nitrite	0.02
Sodium Ascorate	0.08
Tripolyphosphate	0.30
Sugar	0.20
MSG	0.20
Spice mix	0.20
	100.00



000093





Breaking stress increases with TG amount while breaking strain decreases gradually at TG level more than 3U/gram protein after it reaches maximum. Although breaking stress does not drop at 5U/gram protein, too tough and brittle texture is observed by sensory analysis (data is not shown).

The best texture can be obtained between 3 and 4.5U/gram protein. Too much enzyme, such as more than 5 U/gram protein causes undesirable texture.

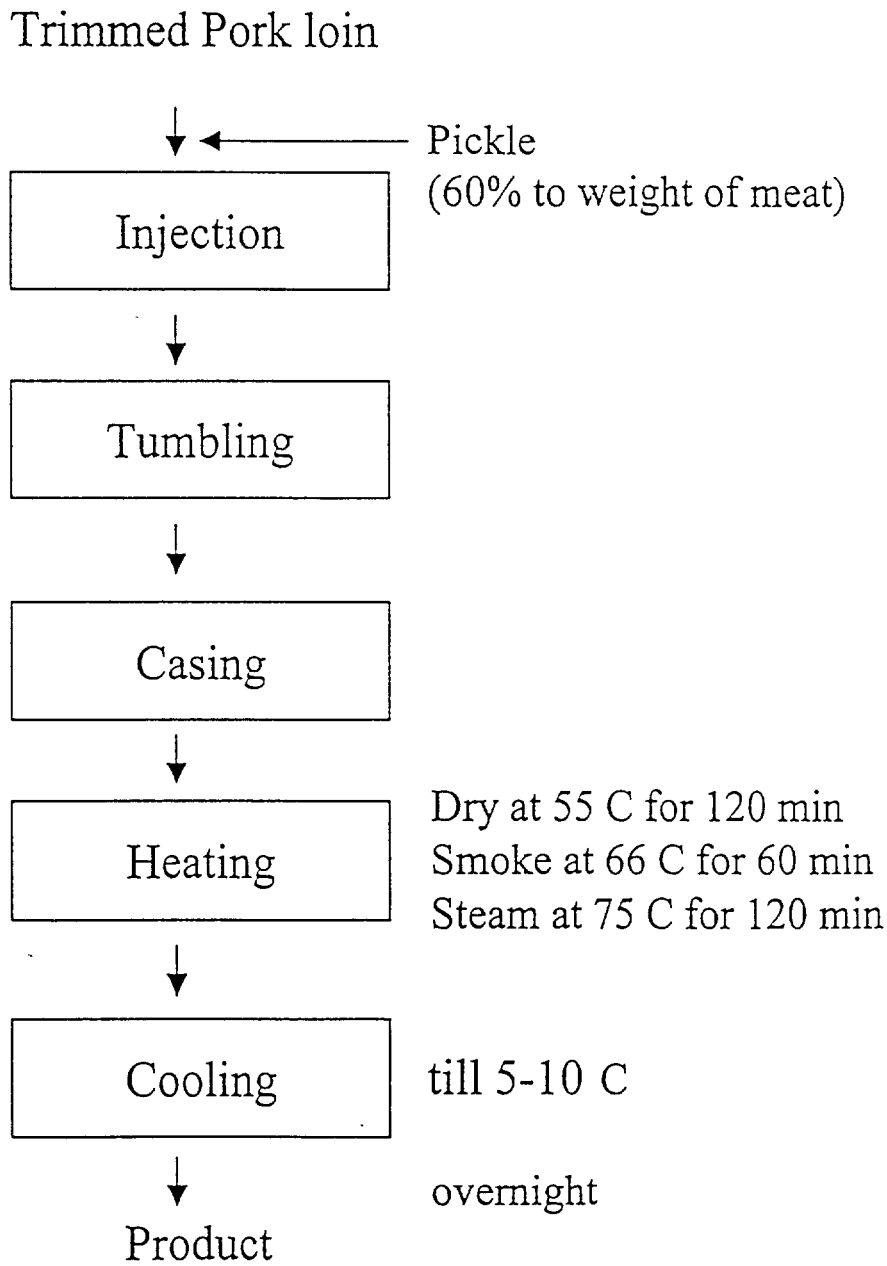
000094

2-2. Pork ham

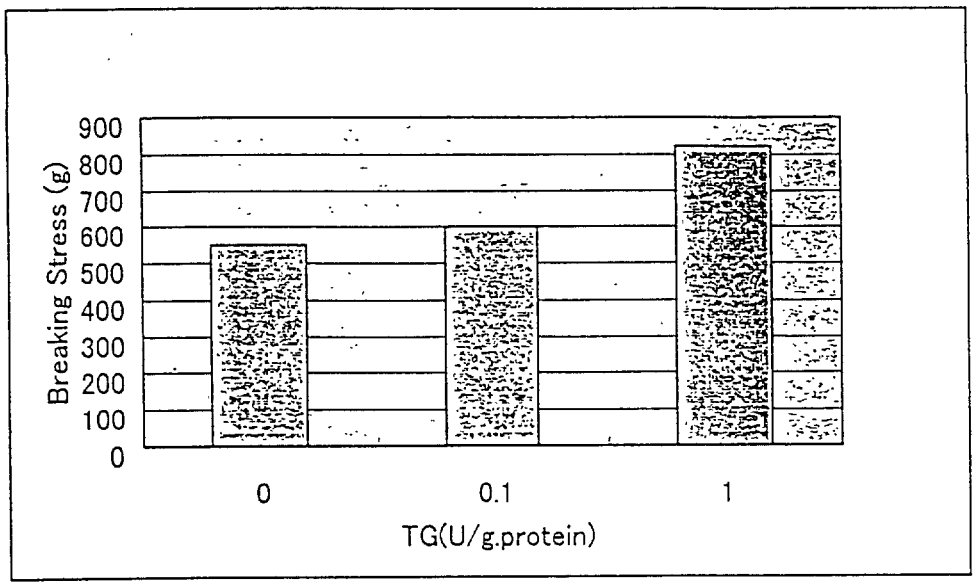
Pork loin ham manufacturing procedure

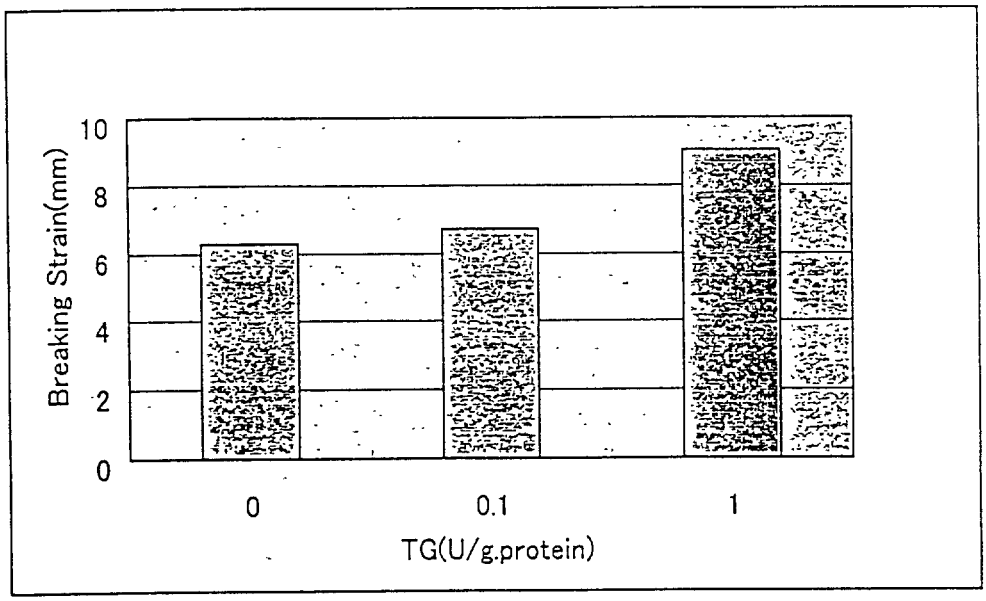
Table Ingredients for Ham

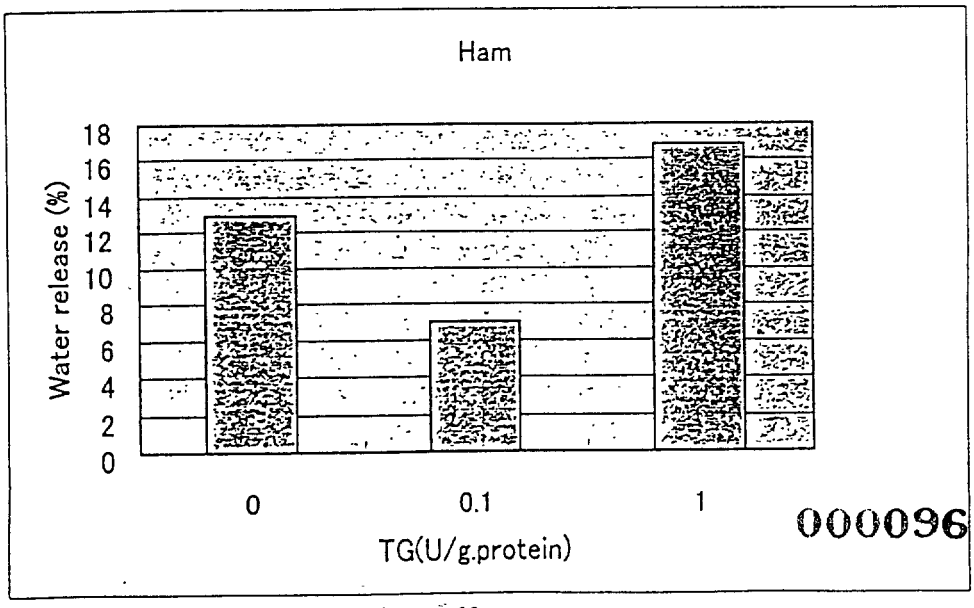
Ingredients	Weight(%) in pickle	Weight(%) in product
Salt	3.6	1.5
Sugar	2.4	1.0
MSG	0.48	0.2
Seasoning	0.96	0.4
Sodium Nitrite	0.048	0.02
Sodium Ascorate	0.192	0.08
Tripolyphosphate	0.72	0.3
Dexitorin	7.2	3.0
Sodium caseinate	2.88	1.2
Soy protein	4.8	2.0
Whey protein	2.4	1.0
Egg white	2.4	1.0
water	71.82	-
	100.00	



000095


Breaking Stress (g)
900
800
700
600
500
400
300
200
100
0
0
0.1
1
TG(U/g.protein)


Breaking Strain(mm)
10
8
6
4
2
0
0
0.1
1
TG(U/g.protein)


Ham
Water release (%)
18
16
14
12
10
8
6
4
2
0
0
0.1
1
TG(U/g.protein)

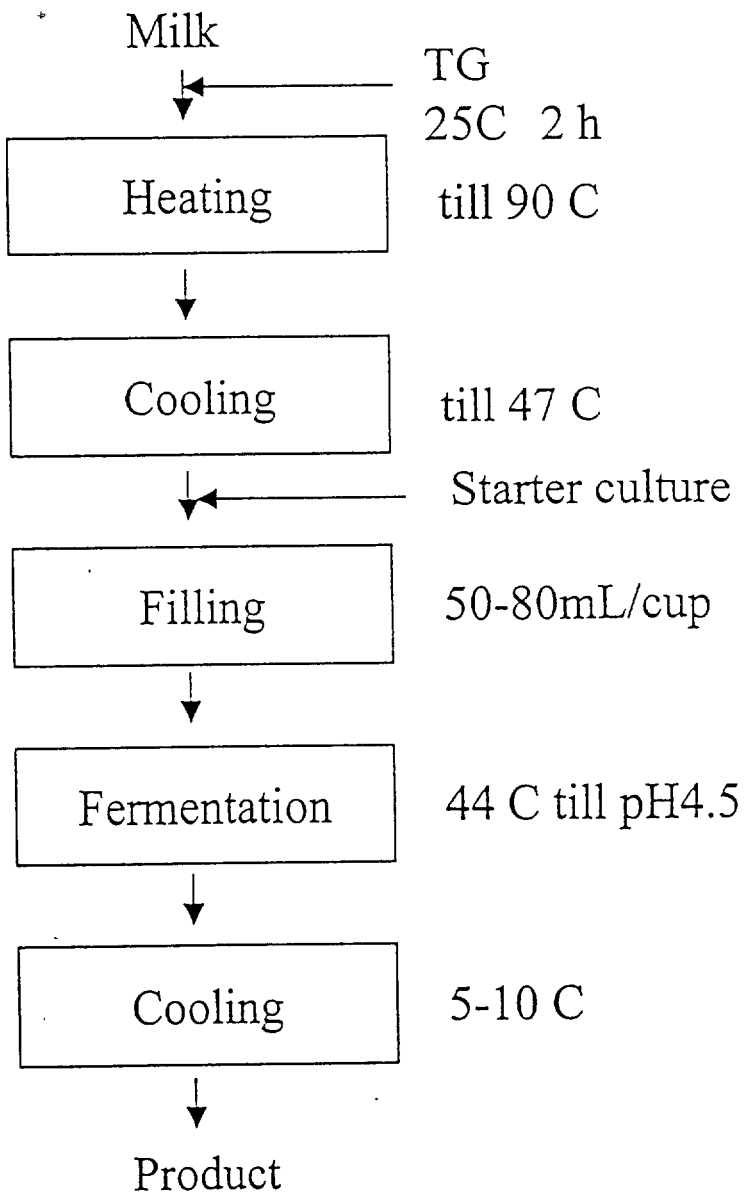
000096

Both breaking stress and strain increase with TG amount. Water release * can be suppressed at 0.1 U/gram protein while it increases at 1U/gram protein. Undesirable texture such as too brittle and rubber like texture, is observed at 1U/gram protein by sensory analysis (data is not shown). Best texture can be obtained between 0.5 and 1.0 U/gram protein .

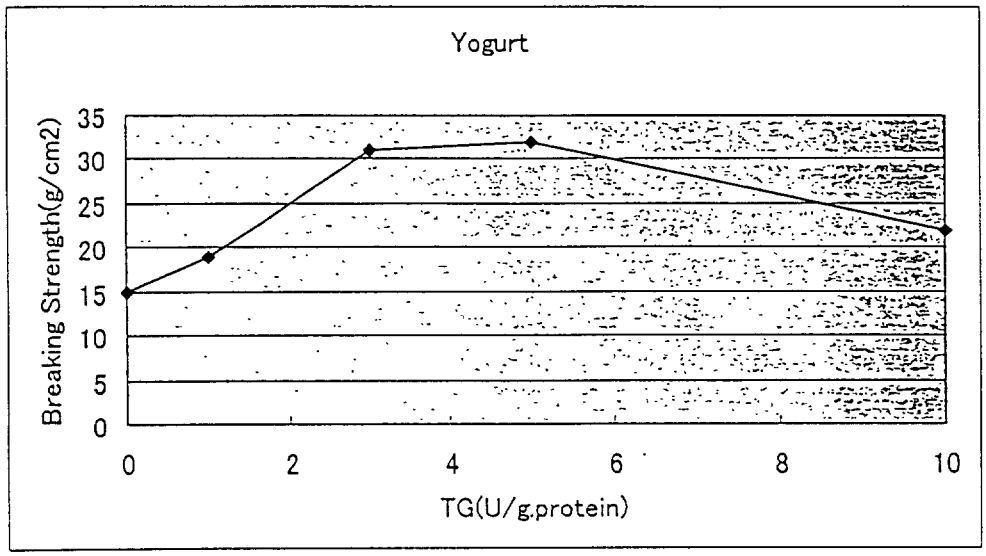
$$* \text{ water release } (\%) = \frac{\text{product weight(g) - product weight compressed (g)}}{\text{product weight(g)}} \times 100$$

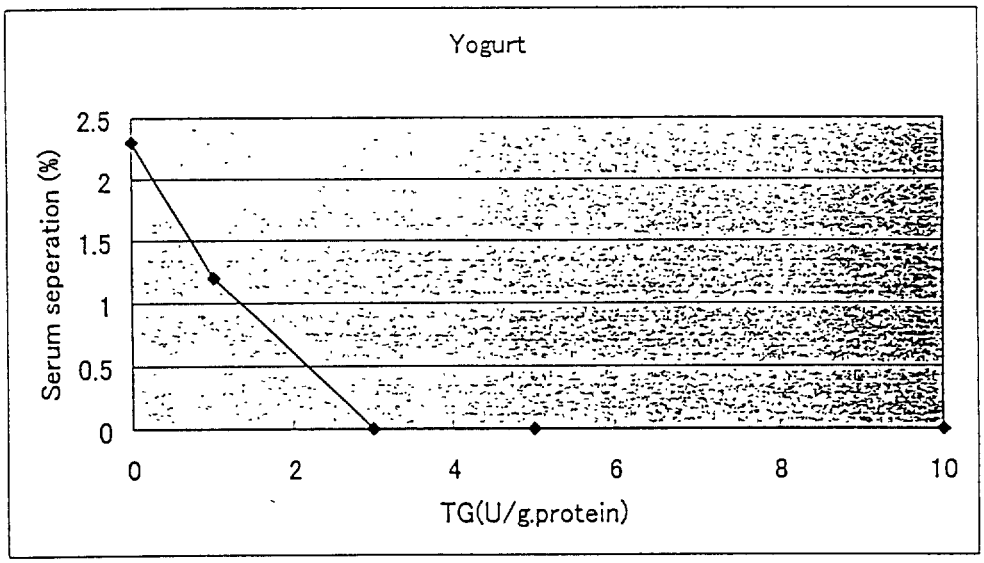
3. Dairy product (yogurt)

Yogurt manufacturing procedure



000098



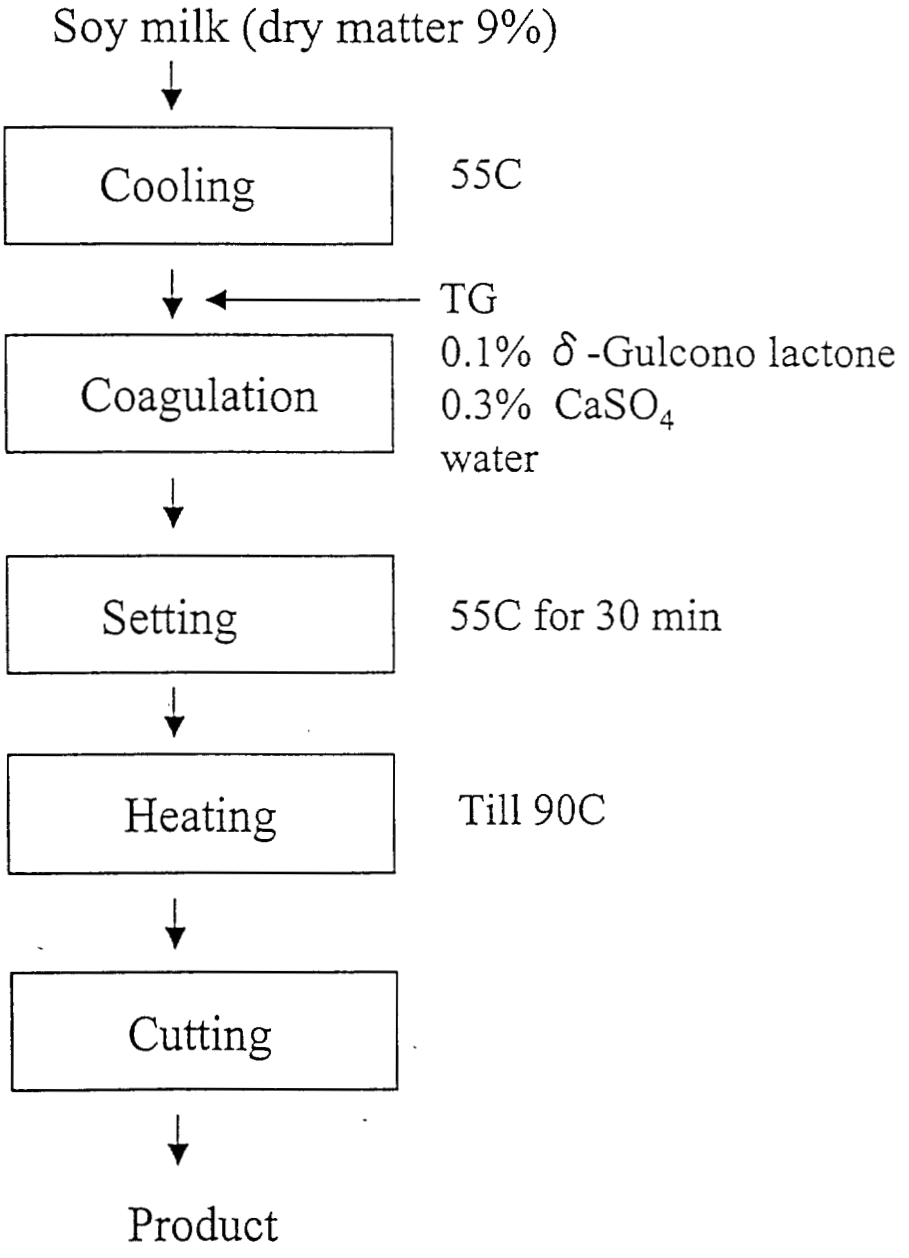


Breaking strength increases almost lineally with TG amount and reaches plateau around 3 U/gram protein. Serum separation is suppressed completely more than 3 U/ gram protein. The yogurt curd becomes brittle and rough texture when TG is added more than 3 U/ gram protein. TG addition of 2 to 3 U/gram protein is appropriate range for obtaining smooth and good texture. This range is also good enough to suppress serum separation. In this procedure, material milk was heated to inactivate TG activity before fermentation. When TG is added with starter culture during fermentation, larger effect can be obtain. In this case, 0.5 to 0.75 U/gram protein is large enough.

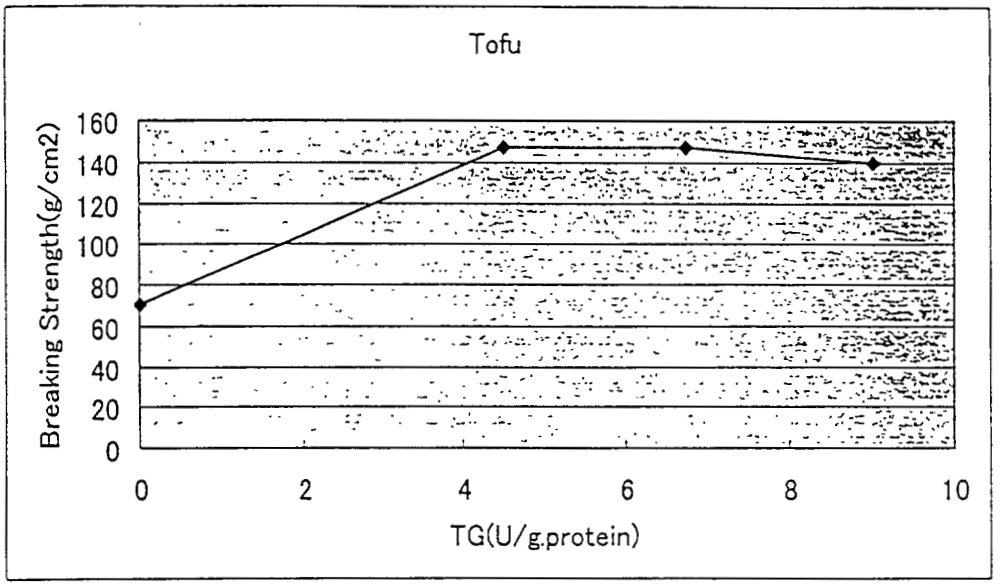
000099

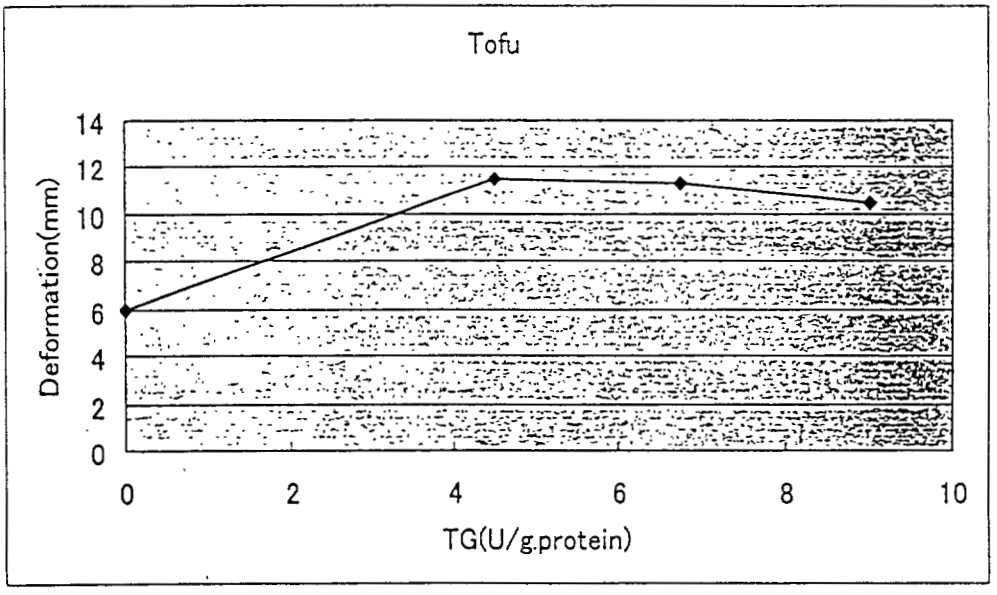
4. Soybean product (*tofu*)

Tofu manufacturing procedure



000100





Both breaking strength and deformation reaches almost plateau more than 4 U/gram protein. Tofu is required soft and smooth texture. Appropriate TG range is between 0.5 and 1.0 U/gram protein.

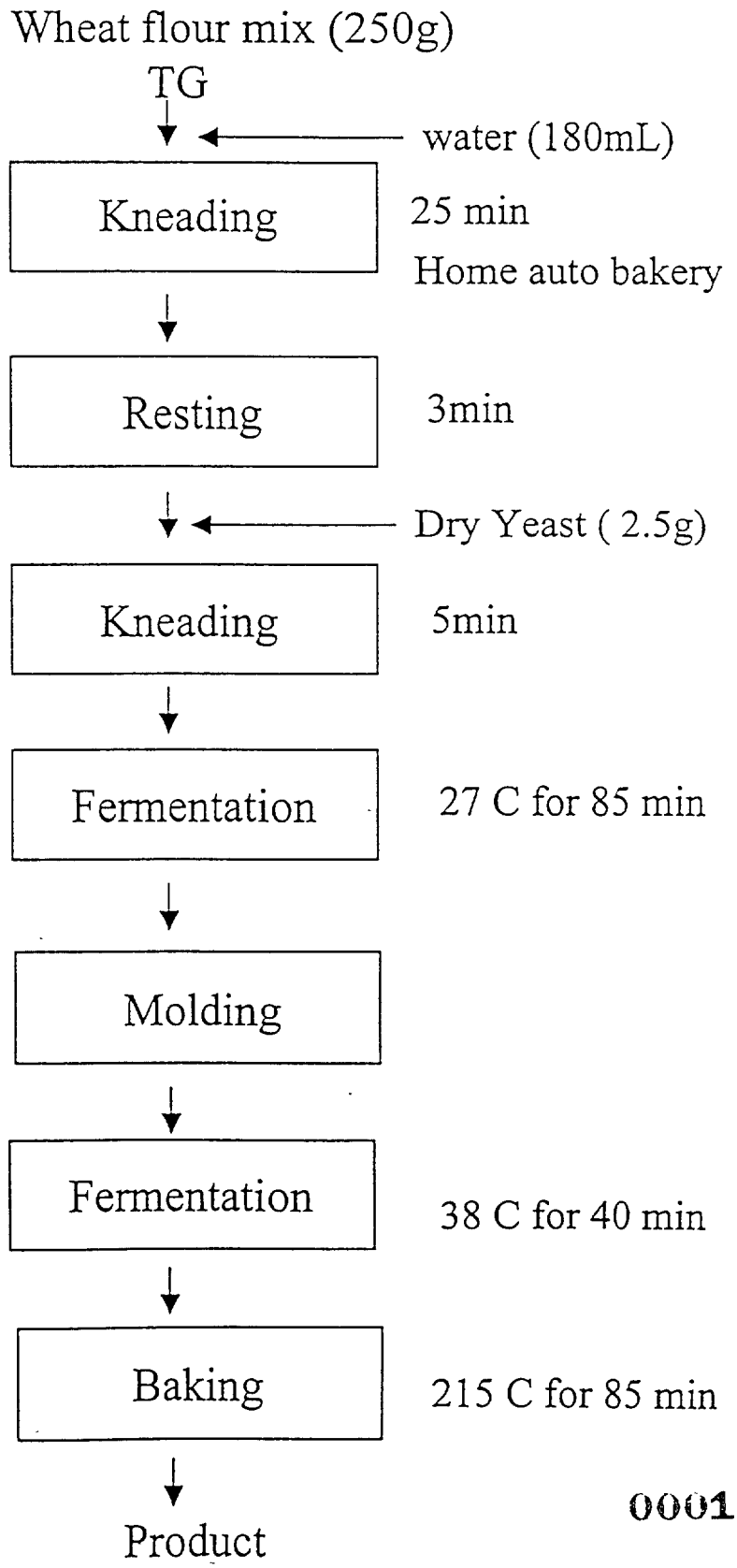
000101

5. Bakery (Bread)

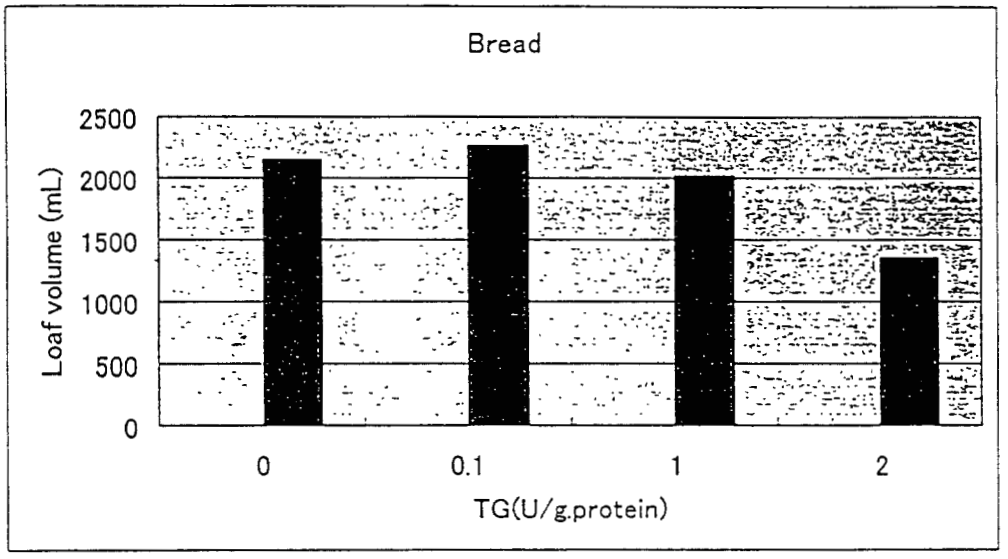
Bread manufacturing procedure

Table Ingredients for bread

Ingredients	Weight(%) in mix
Wheat flour (Protein 12%)	100
Sugar	6
Salt	2
Yeast food	0.2
Skim milk	1
Shortening	5



000102

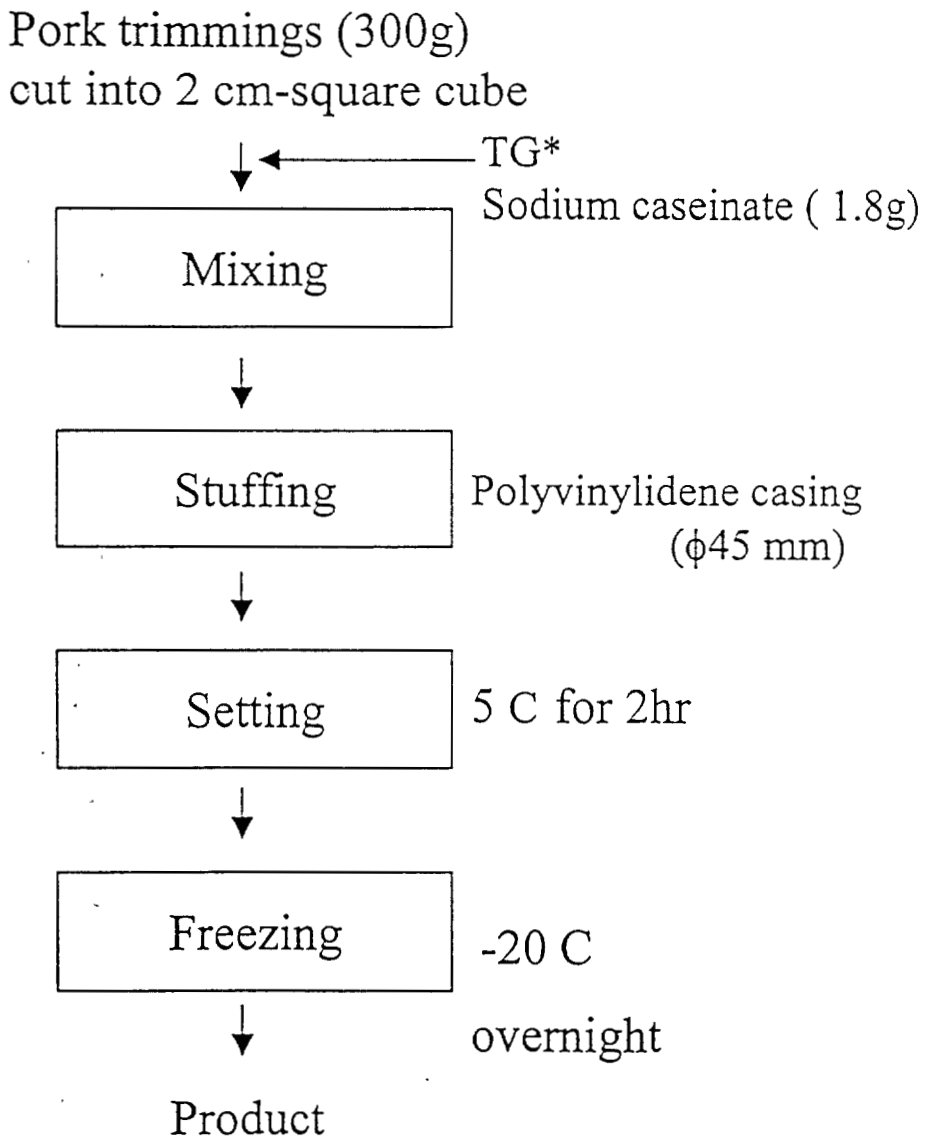


Loaf volume decreases with TG amount after its reaches maximum at 0.1U/gram protein. The dough becomes hard and rough with TG addition. Too hard and poor mouse feeling in final product is observed by sensory analysis when TG is added more than 0.1 U/gram protein (data is not shown). Appropriate TG amount is around 0.1U/gram protein.

000103

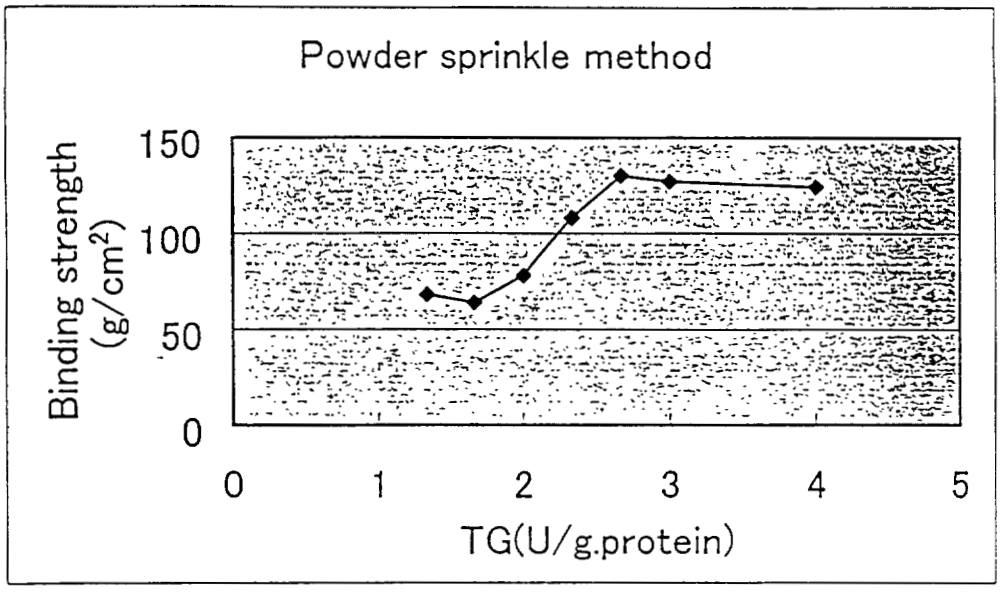
6. Restructured streak (Restructured pork steak)

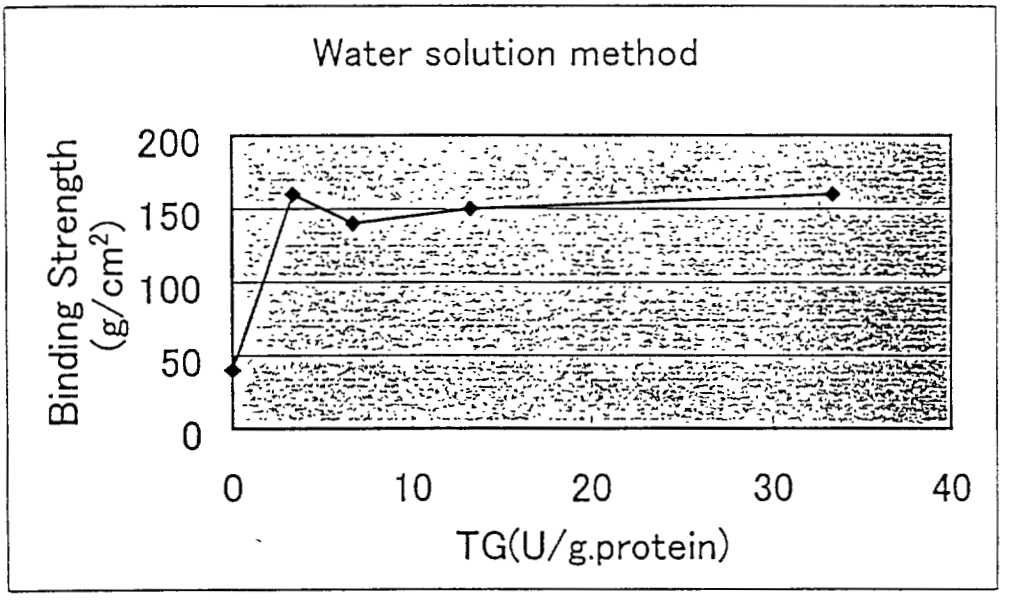
Restructured streak manufacturing procedure



* Water solution method: TG is dispersed in water and added to meat.
Powder sprinkle method: The meat is coated by TG as powder state.

000104





Feasible binding strength is more than 80 g/cm^2. Appropriate TG range is between 2.0 and 2.5 U/gram protein in powder sprinkle method. In case of water solution method, appropriate TG range is between 2.0 and 3.0 U/gram protein. It become plateau more than 3U and 5U/gram protein in powder sprinkle and water solution method.

000105

Table. Summary of appropriate TG range in various food items.

item	protein content (%)	TGase (U/gram protein)	TGase (ppm)*
Kamaboko	12.0	0.7-1.2	9.2-13.8
Sausage	13.0	1.5-2.3	20.0-30.0
Ham	14.0	0.7-1.5	10.0-20.0
Yogurt	3.5	2.0-3.0	7.0-10.5
Tofu	6.0	0.5-1.0	3.0-6.0
Bread	8.5	0.01-0.1	0.085-0.85
Restructured Steak powder sprinkle method	15.0	2.0-2.5	30.0-37.5
Restructured Steak water solution method	15.0	2.0-3.0	30.0-45.0

*Amount in final product.

000106

Appendix B

APPENDIX B

Information Related to Enzymes Considered GRAS by FDA and Whose Use is Limited to Good Manufacturing Practices (GMPs) Only

PART 184—DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

Subpart A—General Provisions

Sec.
184.1 Substances added directly to human food affirmed as generally recognized as safe (GRAS).

Subpart B—Listing of Specific Substances Affirmed as GRAS

184.1005 Acetic acid.
184.1007 Aconitic acid.
184.1009 Adipic acid.
184.1011 Alginic acid.
184.1012 α-Amylase enzyme preparation from Bacillus stearothermophilus.
184.1021 Benzoic acid.
184.1024 Bromelain.
184.1025 Caprylic acid.
184.1027 Mixed carbohydrase and protease enzyme product.
184.1033 Citric acid.
184.1034 Catalase (bovine liver).
184.1061 Lactic acid.
184.1063 Enzyme-modified lecithin.
184.1065 Linoleic acid.
184.1069 Malic acid.
184.1077 Potassium acid tartrate.
184.1081 Propionic acid.
184.1090 Stearic acid.
184.1091 Succinic acid.
184.1095 Sulfuric acid.
184.1097 Tannic acid.
184.1099 Tartaric acid.
184.1101 Diacetyl tartaric acid esters of mono- and diglycerides.
184.1115 Agar-agar.
184.1120 Brown algae.
184.1121 Red algae.
184.1133 Ammonium alginate.
184.1135 Ammonium bicarbonate.
184.1137 Ammonium carbonate.
184.1138 Ammonium chloride.
184.1139 Ammonium hydroxide.
184.1140 Ammonium citrate, dibasic.
184.1141a Ammonium phosphate, monobasic.
184.1141b Ammonium phosphate, dibasic.
184.1143 Ammonium sulfate.
184.1148 Bacterially-derived carbohydrase enzyme preparation.
184.1150 Bacterially-derived protease enzyme preparation.
184.1155 Bentonite.
184.1157 Benzoyl peroxide.
184.1165 n-Butane and iso-butane.
184.1185 Calcium acetate.
184.1187 Calcium alginate.
184.1191 Calcium carbonate.
184.1193 Calcium chloride.
184.1195 Calcium citrate.
184.1199 Calcium gluconate.
184.1201 Calcium glycerophosphate.
184.1205 Calcium hydroxide.
184.1206 Calcium iodate.
184.1207 Calcium lactate.
184.1210 Calcium oxide.
184.1212 Calcium pantothenate.
184.1221 Calcium propionate.
184.1229 Calcium stearate.
184.1230 Calcium sulfate.
184.1240 Carbon dioxide.
184.1245 Beta-carotene.
184.1250 Cellulase enzyme preparation derived from Trichoderma longibrachiatum.
184.1257 Clove and its derivatives.
184.1259 Cocoa butter substitute.
184.1260 Copper gluconate.
184.1261 Copper sulfate.
184.1262 Corn silk and corn silk extract.
184.1265 Cuprous iodide.
184.1271 L-Cysteine.
184.1272 L-Cysteine monohydrochloride.
184.1277 Dextrin.
184.1278 Diacetyl.
184.1282 Dill and its derivatives.
184.1287 Enzyme-modified fats.
184.1293 Ethyl alcohol.
184.1295 Ethyl formate.
184.1296 Ferric ammonium citrate.
184.1297 Ferric chloride.
184.1298 Ferric citrate.
184.1301 Ferric phosphate.
184.1304 Ferric pyrophosphate.
184.1307 Ferric sulfate.
184.1307a Ferrous ascorbate.
184.1307b Ferrous carbonate.
184.1307c Ferrous citrate.
184.1307d Ferrous fumarate.
184.1308 Ferrous gluconate.
184.1311 Ferrous lactate.
184.1315 Ferrous sulfate.
184.1316 Ficin.
184.1317 Garlic and its derivatives.
184.1318 Glucono delta-lactone.
184.1321 Corn gluten.
184.1322 Wheat gluten.
184.1323 Glyceryl monooleate.
184.1324 Glyceryl monostearate.
184.1328 Glyceryl behenate.
184.1329 Glyceryl palmitostearate.
184.1330 Acacia (gum arabic).
184.1333 Gum ghatti.
184.1339 Guar gum.
184.1343 Locust (carob) bean gum.
184.1349 Karaya gum (sterculia gum).
184.1351 Gum tragacanth.
184.1355 Helium.
184.1366 Hydrogen peroxide.
184.1370 Inositol.
184.1372 Insoluble glucose isomerase enzyme preparations.
184.1375 Iron, elemental.
184.1386 Isopropyl citrate.
184.1387 Lactase enzyme preparation from Candida pseudotropicalis.
184.1388 Lactase enzyme preparation from Kluyveromyces lactis.
184.1400 Lecithin.
184.1408 Licorice and licorice derivatives.
184.1409 Ground limestone.
184.1415 Animal lipase.
184.1420 Lipase enzyme preparation derived from Rhizopus niveus.
184.1425 Magnesium carbonate.
184.1426 Magnesium chloride.
184.1428 Magnesium hydroxide.
184.1431 Magnesium oxide.
184.1434 Magnesium phosphate.
184.1440 Magnesium stearate.
184.1443 Magnesium sulfate.
184.1443a Malt.
184.1444 Maltodextrin.
184.1445 Malt syrup (malt extract).
184.1446 Manganese chloride.
184.1449 Manganese citrate.
184.1452 Manganese gluconate.
184.1461 Manganese sulfate.
184.1472 Menhaden oil.
184.1490 Methylparaben.
184.1498 Microparticulated protein product.
184.1505 Mono- and diglycerides.
184.1521 Monosodium phosphate derivatives of mono- and diglycerides.
184.1530 Niacin.
184.1535 Niacinamide.
184.1537 Nickel.
184.1538 Nisin preparation.
184.1540 Nitrogen.
184.1545 Nitrous oxide.
184.1553 Peptones.
184.1555 Rapeseed oil.
184.1560 Ox bile extract.
184.1563 Ozone.
184.1583 Pancreatin.
184.1585 Papain.
184.1588 Pectins.
184.1595 Pepsin.
184.1610 Potassium alginate.
184.1613 Potassium bicarbonate.
184.1619 Potassium carbonate.
184.1622 Potassium chloride.
184.1625 Potassium citrate.
184.1631 Potassium hydroxide.
184.1634 Potassium iodide.
184.1635 Potassium iodate.
184.1639 Potassium lactate.
184.1643 Potassium sulfate.
184.1655 Propane.
184.1660 Propyl gallate.
184.1666 Propylene glycol.
184.1670 Propylparaben.
184.1676 Pyridoxine hydrochloride.
184.1685 Rennet (animal-derived) chymosin preparation (fermentation rived).
184.1695 Riboflavin.
184.1697 Riboflavin-5'-phosphate (sodium)
184.1698 Rue.
184.1699 Oil of rue.
184.1702 Sheanut oil.
184.1721 Sodium acetate.
184.1724 Sodium alginate.
184.1733 Sodium benzoate.
184.1736 Sodium bicarbonate.
184.1742 Sodium carbonate.
184.1751 Sodium citrate.
184.1754 Sodium diacetate.
184.1763 Sodium hydroxide.
184.1764 Sodium hypophosphite.
184.1768 Sodium lactate.
184.1769a Sodium metasilicate.
184.1784 Sodium propionate.
184.1792 Sodium sesquicarbonate.
184.1801 Sodium tartrate.
184.1804 Sodium potassium tartrate.
184.1807 Sodium thiosulfate.
184.1835 Sorbitol.
184.1845 Stannous chloride (anhydrous a dihydrated).
184.1848 Starter distillate.
184.1851 Stearyl citrate
184.1854 Sucrose.
184.1857 Corn sugar.
184.1859 Invert sugar.
184.1865 Corn syrup.
184.1866 High fructose corn syrup.
184.1875 Thiamine hydrochloride.
184.1878 Thiamine mononitrate.
184.1890 α-Tocopherols.
184.1901 Triacetin.
184.1903 Tributyrin.
184.1911 Triethyl citrate.
184.1914 Trypsin.
184.1923 Urea.
184.1924 Urease enzyme preparation fro Lactobacillus fermentum.
184.1930 Vitamin A.
184.1945 Vitamin B_{12}.
184.1950 Vitamin D.
184.1973 Beeswax (yellow and white).
184.1976 Candelilla wax.
184.1978 Carnauba wax.
184.1979 Whey.
184.1979a Reduced lactose whey.
184.1979b Reduced minerals whey.
184.1979c Whey protein concentrate.
184.1983 Bakers yeast extract.
184.1984 Zein.
184.1985 Aminopeptidase enzyme prepar tion derived from lactococcus lactis.

AUTHORITY: 21 U.S.C. 321, 342, 348, 371.

SOURCE: 42 FR 14653, Mar 15, 1977, unle otherwise noted.

000109

§ 184.1012 α-Amylase enzyme preparation from Bacillus stearothermophilus.

(a) α-Amylase enzyme preparation is obtained from the culture filtrate that results from a pure culture fermentation of a nonpathogenic and nontoxicogenic strain of *Bacillus stearothermophilus*. Its characterizing enzyme activity is α-amylase (1,4 α-D glucan glucanohydrolase (E.C. 3.2.1.1)).

(b) The ingredient meets the general and additional requirements for enzyme preparations in the "Food Chemicals Codex," 3d ed. (1981), pp. 107–110, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Office of Premarket Approval (HFS–200), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 1110 Vermont Ave. NW., suite 1200, Washington, DC, or the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practices. The affirmation of this ingredient as GRAS as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme, as defined in § 170.3(o)(9) of this chapter, in the hydrolysis of edible starch to produce maltodextrins and nutritive carbohydrate sweeteners.

(2) The ingredient is used at levels not to exceed current good manufacturing practices.

[60 FR 55789, Nov. 3, 1995]

000110

§ 184.1024 Bromelain.

(a) Bromelain (CAS Reg. No. 9001-00-7) is an enzyme preparation derived from the pineapples *Ananas comosus* and *A. bracteatus* L. It is a white to light tan amorphous powder. Its characterizing enzyme activity is that of a peptide hydrolase (EC 3.4.22.32).

(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d ed. (1981), p. 110, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC, or may be examined at the Office of Premarket Approval (HFS-200), Food and Drug Administration, 200 C St. SW., Washington, DC, and the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[60 FR 32910, June 26, 1995]

§ 184.1027 Mixed carbohydrase and protease enzyme product.

(a) Mixed carbohydrase and protease enzyme product is an enzyme preparation that includes carbohydrase and protease activity. It is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic strain of *B. licheniformis*.

(b) The ingredient meets the specifications of the Food Chemicals Codex, 3d Ed. (1981), p. 107, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme, as defined in § 170.3(o)(9) of this chapter, to hydrolyze proteins or carbohydrates.

(2) The ingredient is used in the following foods at levels not to exceed current good manufacturing practice: alcoholic beverages, as defined in § 170.3(n)(2) of this chapter, candy, nutritive sweeteners, and protein hydrolyzates.

[48 FR 240, Jan. 4, 1983]

000111

§ 184.1034 Catalase (bovine liver).

(a) Catalase (bovine liver) (CAS Reg. No. 9001–05–2) is an enzyme preparation obtained from extracts of bovine liver. It is a partially purified liquid or powder. Its characterizing enzyme activity is catalase (EC 1.11.1.6).

(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d ed. (1981), p. 110, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave., NW., Washington, DC 20418, or may be examined at the Office of Premarket Approval (HFS–200), Food and Drug Administration, 200 C St., SW., Washington, DC, and the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in §170.3(o)(9) of this chapter to decompose hydrogen peroxide.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[60 FR 32910, June 26, 1995]

§ 184.1148 Bacterially-derived carbohydrase enzyme preparation.

(a) Bacterially-derived carbohydrase enzyme preparation is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic and nontoxigenic strain of *Bacillus subtilis* or *B. amyloliquefaciens*. The preparation is characterized by the presence of the enzymes α-amylase (EC 3.2.1.1) and β-glucanase (EC 3.2.1.6), which catalyze the hydrolysis of *O*-glycosyl bonds in carbohydrates.

(b) The ingredient meets the general requirements and additional requirements in the monograph on enzyme preparations in the Food Chemicals Codex, 4th ed. (1996), pp. 128–135, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol Street, NW., Suite 700, Washington, DC. In addition, antibiotic activity is absent in the enzyme preparation when determined by an appropriate validated method such as the method "Determination of antibiotic activity" in the Compendium of Food Additive Specifications, vol. 2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992. Copies are available from Bernan Associates, 4611–F Assembly Dr., Lanham, MD 20706, or from The United Nations Bookshop, General Assembly Bldg., rm. 32, New York, NY 10017, or by inquiries sent to "http://www.fao.org". Copies may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC.

(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in §170.3(o)(9) of this chapter to hydrolyze polysaccharides (e.g., starch).

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[64 FR 19894, Apr. 23, 1999]

000112

§ 184.1150 Bacterially-derived protease enzyme preparation.

(a) Bacterially-derived protease enzyme preparation is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic and nontoxigenic strain of *Bacillus subtilis* or *B. amyloliquefaciens*. The preparation is characterized by the presence of the enzymes subtilisin (EC 3.4.21.62) and neutral proteinase (EC 3.4.24.28), which catalyze the hydrolysis of peptide bonds in proteins.

(b) The ingredient meets the general requirements and additional requirements in the monograph on enzyme preparations in the Food Chemicals Codex, 4th ed. (1996), pp. 128–135, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol Street, NW., Suite 700 Washington, DC. In addition, antibiotic activity is absent in the enzyme preparation when determined by an appropriate validated method such as the method "Determination of antibiotic activity" in the Compendium of Food Additive Specifications, vol. 2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992. Copies are available from Bernan Associates, 4611-F Assembly Dr., Lanham, MD 20706, or from The United Nations Bookshop, General Assembly Bldg., rm. 32, New York, NY 10017, or by inquiries sent to "http://www.fao.org". Copies may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[64 FR 19895, Apr. 23, 1999]

§ 184.1250 Cellulase enzyme preparation derived from Trichoderma longibrachiatum.

(a) Cellulase enzyme preparation is derived from a nonpathogenic, nontoxicogenic strain of *Trichoderma longibrachiatum* (formerly *T. reesei*). The enzyme, cellulase, catalyzes the endohydrolysis of 1,4-beta-glycosidic linkages in cellulose. It is obtained from the culture filtrate resulting from a pure culture fermentation process.

(b) The ingredient meets the general and additional requirements for enzyme preparations in the monograph specifications on enzyme preparations in the "Food Chemicals Codex," 4th ed. (1996), pp. 129 to 134, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Box 285, Washington, DC 20055 (Internet "http://www.nap.edu"), or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used in food as an enzyme as defined in § 170.3(o)(9) of this chapter for the breakdown of cellulose.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[64 FR 28361, May 26, 1999]

000113

§ 184.1316 Ficin.

(a) Ficin (CAS Reg. No. 9001-33-6) is an enzyme preparation obtained from the latex of species of the genus *Ficus*, which include a variety of tropical fig trees. It is a white to off-white powder. Its characterizing enzyme activity is that of a peptide hydrolase (EC 3.4.22.3).

(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d ed. (1981), p. 110, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave., NW., Washington, DC 20418, or may be examined at the Office of Premarket Approval (HFS-200), Food and Drug Administration, 200 C St., SW., Washington, DC, and the Office of the Federal Register, 800 North Capitol St., NW., suite 700, Washington, DC.

(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in §170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[60 FR 32910, June 26, 1995]

§ 184.1372 Insoluble glucose isomerase enzyme preparations.

(a) Insoluble glucose isomerase enzyme preparations are used in the production of high fructose corn syrup described in §184.1866. They are derived from recognized species of precisely classified nonpathogenic and nontoxicogenic microorganisms, including *Streptomyces rubiginosus*, *Actinoplanes missouriensis*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes*, and *Bacillus coagulans*, that have been grown in a pure culture fermentation that produces no antibiotics. They are fixed (rendered insoluble) for batch production with GRAS ingredients or may be fixed for further immobilization with either GRAS ingredients or materials approved under §173.357 of this chapter.

(b) The ingredient meets the general and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d Ed. (1981), p. 107, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408.

(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme, as defined in §170.3(o)(9) of this chapter, to convert glucose to fructose.

(2) The ingredient is used in high fructose corn syrup, at levels not to exceed current good manufacturing practice.

[48 FR 5720, Feb. 8, 1983, as amended at 61 FR 43450, Aug. 23, 1996]

000114

§ 184.1387 Lactase enzyme preparation from Candida pseudotropicalis.

(a) This enzyme preparation is derived from the nonpathogenic, nontoxicogenic yeast *C. pseudotropicalis*. It contains the enzyme lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23), which converts lactose to glucose and galactose. It is prepared from yeast that has been grown by a pure culture fermentation process.

(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d ed. (1981), pp. 107–110, which are incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitations other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme, as defined in § 170.3(o)(9) of this chapter, to convert lactose to glucose and galactose.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice. Current good manufacturing practice is limited to use of this ingredient to reduce the lactose content in milk and milk-derived food products where food standards do not preclude such use.

[61 FR 7704, Feb. 29, 1996]

§ 184.1388 Lactase enzyme preparation from Kluyveromyces lactis.

(a) This enzyme preparation is derived from the nonpathogenic, nontoxicogenic yeast *Kluyveromyces lactis* (previously named *Saccharomyces lactis*). It contains the enzyme B-galactoside galactohydrase (CAS Reg. No. CBS 683), which converts lactose to glucose and galactose. It is prepared from yeast that has been grown in a pure culture fermentation and by using materials that are generally recognized as safe or are food additives that have been approved for this use by the Food and Drug Administration.

(b) The ingredient meets the general and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d Ed. (1981), p. 107–110, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in §170.3(o)(9) of this chapter to convert lactose to glucose and galactose.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice. Current good manufacturing practice is to use this ingredient in milk to produce lactase-treated milk, which contains less lactose than regular milk, or lactose-reduced milk, which contains at least 70 percent less lactose than regular milk.

[49 FR 47387, Dec. 4, 1984]

000115

§ 184.1420 Lipase enzyme preparation derived from Rhizopus niveus.

(a) Lipase enzyme preparation contains lipase enzyme (CAS Reg. No. 9001-62-1), which is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic and nontoxigenic strain of *Rhizopus niveus*. The enzyme preparation also contains diatomaceous earth as a carrier. The characterizing activity of the enzyme, which catalyzes the interesterification of fats and oils at the 1- and 3-positions of triglycerides, is triacylglycerol lipase (EC 3.1.1.3).

(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the monograph on Enzyme Preparations in the "Food Chemicals Codex," 4th ed. (1996), pp. 133 and 134, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter for the interesterification of fats and oils.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[63 FR 24419, May 4, 1998]

§ 184.1583 Pancreatin.

(a) Pancreatin (CAS Reg. No. 8049-47-6) is an enzyme preparation obtained from porcine or bovine pancreatic tissue. It is a white to tan powder. Its characterizing enzyme activity that of a peptide hydrolase (EC 3.4.21.36).

(b) The ingredient meets the general requirements and additional requirements in the Food Chemicals Codex, 3d ed. (1981), p. 110, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Office of Premarket Approval (HFS-200), Food and Drug Administration, 200 C St. SW., Washington, DC, and the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[60 FR 32911, June 26, 1995]

000116

§ 184.1585 Papain.

(a) Papain (CAS Reg. No. 9001-73-4) is a proteolytic enzyme derived from *Carica papaya* L. Crude latex containing the enzyme is collected from slashed unripe papaya. The food-grade product is obtained by repeated filtration of the crude latex or an aqueous solution of latex or by precipitation from an aqueous solution of latex. The resulting enzyme preparation may be used in a liquid or dry form.

(b) The ingredient meets the specifications of the Food Chemicals Codex, 3d Ed. (1981), pp. 107-110, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter; processing aid as defined in § 170.3(o)(24) of this chapter; and texturizer as defined in § 170.3(o)(32) of this chapter.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

[48 FR 48806, Oct. 21, 1983]

§ 184.1595 Pepsin.

(a) Pepsin (CAS Reg. No. 9001-75-6) is an enzyme preparation obtained from the glandular layer of hog stomach. It is a white to light tan powder, amber paste, or clear amber to brown liquid. Its characterizing enzyme activity is that of a peptide hydrolase (EC 3.4.23.1).

(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 3d ed. (1981), p. 110, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Office of Premarket Approval (HFS-200), Food and Drug Administration, 200 C St. SW., Washington, DC, and the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[60 FR 32911, June 26, 1995]

000117

§ 184.1924 Urease enzyme preparation from Lactobacillus fermentum.

(a) This enzyme preparation is derived from the nonpathogenic, nontoxicogenic bacterium *Lactobacillus fermentum*. It contains the enzyme urease (CAS Reg. No. 9002-13-5), which facilitates the hydrolysis of urea to ammonia and carbon dioxide. It is produced by a pure culture fermentation process and by using materials that are generally recognized as safe (GRAS) or are food additives that have been approved for this use by the Food and Drug Administration (FDA).

(b) The ingredient meets the general and additional requirements for enzyme preparations in the "Food Chemicals Codex," 3d ed. (1981), pp. 107-110, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as GRAS as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used in wine, as defined in 27 CFR 2.5 and 4.10, as an enzyme as defined in § 170.3(o)(9) of this chapter to convert urea to ammonia and carbon dioxide.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice. Current good manufacturing practice is limited to use of this ingredient in wine to inhibit formation of ethyl carbamate.

[57 FR 60473, Dec. 21, 1992]

§ 184.1985 Aminopeptidase enzyme preparation derived from lactococcus lactis.

(a) Aminopeptidase enzyme preparation is derived from the nonpathogenic and nontoxicogenic bacterium *Lactococcus lactis* (previously named *Streptococcus lactis*). The preparation contains the enzyme aminopeptidase (CAS Reg. No. 9031-94-1; EC 3.4.11.1) and other peptidases that hydrolyze milk proteins. The preparation is produced by pure culture fermentation.

(b) The ingredient meets the specifications for enzyme preparations in the Food Chemicals Codex, 3d ed. (1981), pp. 107-110, which are incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Division of Petition Control (HFS-215), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 1110 Vermont Ave. NW., suite 1200, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitations other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme, as defined in § 170.3(o)(9) of this chapter, as an optional ingredient for flavor development in the manufacture of cheddar cheese, in accordance with § 133.113 of this chapter, and in the preparation of protein hydrolysates.

(2) The ingredient is used at levels not to exceed current good manufacturing practice.

[60 FR 54193, Oct. 20, 1995]

000118

U. S. Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Premarket Approval

Agency Response Letter
GRAS Notice No. GRN 000043

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
Washington, DC 20204

September 22, 2000

Ms. Lori Gregg
Novo Nordisk BioChem North America, Inc.
77 Perry Chapel Church Road
Box 576
Franklinton, NC 27525

Re: GRAS Notice No. GRN 000043

Dear Ms. Gregg:

The Food and Drug Administration (FDA) is responding to the notice, dated April 25, 2000, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)). FDA received your notice on April 28, 2000 and designated it as GRN No. 000043.

The subject of your notice is lipase enzyme preparation derived from *Aspergillus oryzae* carrying a gene encoding lipase from *Thermomyces lanuginosus*. The notice informs FDA of the view of Novo Nordisk that this lipase enzyme preparation is GRAS, through scientific procedures, for use in dough, baked goods, and the fats and oil industry at minimum levels necessary to achieve the desired effect. The lipase enzyme preparation would be used as a catalyst in the interesterification of glycerides and acidolysis between glycerides and fatty acids in fats and oils at a maximum level of one kilogram of lipase per ton of triglycerides. The lipase enzyme preparation would be used in the hydrolysis of primary ester bonds in triglycerides in dough and baked goods for the purpose of modifying lipid-gluten interactions at a maximum level of one to five grams per 100 kg of flour.

000119

In your notice, you describe: (1) a published review article about the safety of the host microorganism, *A. oryzae;* (2) scientific publications and recommendations issued by international organizations on the safety of enzymes used in food processing, including enzymes derived from genetically modified microorganisms; (3) published scientific articles that discuss the safety of the various components of the production organism, including the host organism, and the components of the genetic material that is

d into the host organism; (4) the basis for your conclusion that the presence of a gene encoding to the antibiotic ampicillin is not a concern; (5) chapters in several books that discuss the ɔ-ng process, which includes standard methods for the fermentation, processing, and he enzyme preparation; and (6) a published review of oral toxicity and genetic toxicity nducted with the subject lipase enzyme preparation.

g to your notice, the enzyme preparation meets the specifications for enzyme preparations in the Food Chemicals Codex (4th ed., 1996). The enzyme preparation also meets the ions for enzyme preparations provided by the Joint Expert Committee on Food Additives a joint committee of the Food and Agriculture Organization/World Health Organization).

the information provided by Novo Nordisk, as well as other information available to FDA, the ıs no questions at this time regarding Novo Nordisk's conclusion that lipase enzyme ɔn derived from a genetically modified strain of *A. oryzae* that contains a recombinant gene *T. lanuginosus* lipase is GRAS under the intended conditions of use. The agency has not, made its own determination regarding the GRAS status of the subject use of this enzyme ɔn. As always, it is your continuing responsibility to ensure that food ingredients that you e safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

ance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the on in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is for public review and copying on the Office of Premarket Approval's homepage on the Internet vm.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,
/s/
Alan M. Rulis, Ph.D.
Director
Office of Premarket Approval
Center for Food Safety and Applied Nutrition

---

t updated by saw 2000-SEP-28
ıst updated by saw/dms 2000-DEC-06

000120

- *Subject*: 63FR12421 Direct Food Substances Affirmed as Generally Recognized as Safe; Egg White Lysozyme
- *Date*: 13 Mar 1998 06:35:04 -0500
- *Organization*: Food and Drug Administration, HHS

Archive-Name: gov/us/fed/nara/fed-register/1998/mar/13/63FR12421
Posting-number: Volume 63, Issue 49, Page 12421

[Federal Register: March 13, 1998 (Volume 63, Number 49)]
[Proposed Rules]
[Page 12421-12426]
From the Federal Register Online via GPO Access [wais.access.gpo.gov]
[DOCID:fr13mr98-21]

==========

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 184

[Docket No. 89G-0393]

Direct Food Substances Affirmed as Generally Recognized as Safe;
Egg White Lysozyme

AGENCY: Food and Drug Administration, HHS.

ACTION: Tentative final rule.

SUMMARY: The Food and Drug Administration (FDA) is issuing a tentative final rule to amend its regulations to affirm that egg white lysozyme enzyme preparation, when labeled by the common or usual name ``egg white lysozyme" to identify its source, is generally recognized as safe (GRAS) for use in preventing late blowing of cheese caused by the bacterium Clostridium tyrobutyricum during cheese production. This action is in response to a petition submitted by Fordras S.A. (formerly SPA-Societa Prodotti Antibiotici S.p.A.). FDA has tentatively concluded

000121

that this use of the egg white lysozyme enzyme preparation is GRAS only when the ingredient statement for both bulk and packaged food that contains cheese manufactured using egg white lysozyme includes the common or usual name ``egg white lysozyme" to identify the source of the protein. To give interested persons an opportunity to comment on this condition of use required for GRAS status, FDA is issuing this tentative final rule.

DATES: Submit written comments by May 27, 1998.

ADDRESSES: Submit written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Linda S. Kahl, Center for Food Safety and Applied Nutrition (HFS-206), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-418-3101.

SUPPLEMENTARY INFORMATION:

I. Background

In accordance with the procedures described in Sec. 170.35 (21 CFR 170.35), SPA-Societa Prodotti Antibiotici S.p.A., now Fordras S.A., Milan, Italy, submitted a petition (GRASP 9G0355) requesting that egg white lysozyme used to inhibit the bacterium C. tyrobutyricum to prevent late blowing of cheese during production be affirmed as GRAS as a direct human food ingredient. FDA published the notice of filing for this petition in the Federal Register of October 27, 1989 (54 FR 43861), and gave interested persons until December 26, 1989, to submit written comments.

II. Standards for GRAS Affirmation

Under Sec. 170.30 (21 CFR 170.30), general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either: (1) Scientific procedures, or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety based upon scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation and ordinarily is based upon published studies, which may be corroborated by unpublished studies and other data and information (Sec. 170.30(b)). General recognition of safety through experience based on common use in food prior to January 1, 1958, may be determined without the quantity or quality of scientific procedures required for approval of a food additive regulation, but ordinarily is based upon generally available data and information concerning the pre-1958 history of use of the

000122

substance.
FDA has evaluated Fordras S.A.'s petition on the basis of scientific procedures to whether the petitioned use of egg white lysozyme enzyme preparation to prevent the late blowing of cheese caused by the bacterium C. tyrobutyricum during cheese production is GRAS. In evaluating the petition, FDA considered published and unpublished data and information relating to the identity of, characteristic properties of, and estimated dietary exposure to the enzyme component (i.e., lysozyme) of the petitioned enzyme preparation (Refs. 1 through 7). FDA also considered that the source of the petitioned enzyme preparation, egg white, has been safely consumed by humans as a source of food protein throughout recorded history, and, therefore, is GRAS (Sec. 170.30(d)), and that the methods used for extracting lysozyme from the egg white source do not ordinarily alter the chemical identity and characteristic properties of enzymes (Ref. 8). FDA also considered published scientific review articles (Refs. 1 and 2) and a generally available trade association bulletin (Ref. 7) discussing the use of egg white lysozyme enzyme preparation for its

[[Page 12422]]

technical effect of preventing late blowing of cheese contaminated with C. tyrobutyricum as well as generally available information documenting that this intended use of the petitioned enzyme preparation has been approved in several European countries (Refs. 9 through 13). Finally, FDA considered generally available and accepted information relating to processing aids used in the manufacture of the enzyme preparation and generally available and accepted specifications for food-grade enzyme preparations (Ref. 14).

III. Safety Evaluation

When present as a contaminant in milk used for cheesemaking, the pasteurization-resistant bacterium C. tyrobutyricum ferments lactate to produce carbon dioxide, hydrogen, and volatile organic acids. This fermentation causes a defect in cheese manufacture known as ``late blowing," which is typified by abnormal levels of open texture accompanied by undesirable odors and flavors. Late blowing can be a serious economic problem in the manufacture of several varieties of cheese (Refs. 1, 2, and 7).
The contamination by C. tyrobutyricum of milk used for cheesemaking, although reducible by good husbandry and hygienic milking practices, is unavoidable. Although treatment with certain chemical agents has been shown to be effective against the problems raised by this contamination, treatment with lysozyme enzyme preparation has been found to be the most effective method of managing the late blowing of cheese contaminated with C. tyrobutyricum (Refs. 1 and 2).

A. The Enzyme Component

000123

Enzymes are proteins or conjugated proteins (i.e., a protein that contains a nonamino acid moiety such as a carbohydrate) produced by plants, animals, and microorganisms that function as biochemical catalysts (American Heritage Dictionary of the English Language). Most enzymes are very specific in their ability to catalyze only certain chemical reactions; this high degree of specificity and strong catalytic activity are the most important functional properties of enzymes (Ref. 15).

The Commission on Enzymes of the International Union of Biochemistry has devised a systematic strategy for naming enzymes. This system combines a naming system and a numbering system. For most enzymes, the systematic name is derived from the names of the substrate, product, and type of reaction. The systematic number is based on the class and subclasses to which the enzyme belongs. The systematic name of lysozyme is peptidoglycan N-acetylmuramoylhydrolase. Its systematic number is EC No. 3.2.1.17 and its Chemical Abstracts Service Registry Number (CAS Reg. No.) is 9001-63-2.

Lysozyme was first discovered by A. Fleming, who identified lysozyme as an antibacterial enzyme present in nasal mucus membrane (Ref. 3). Subsequently, it was learned that the antibacterial activity of lysozyme occurs because of its ability to catalyze the hydrolysis of the structural polysaccharide peptidoglycan present in cell walls of certain bacteria (Ref. 2). Lysozyme activity has been shown to be present in bacteria, fungi, plants, and almost all animal tissues, with the highest levels found in secretions (including milk, mucus, saliva, and tears) and eggs. Lysozyme is believed to function in all of these organisms and tissues as an endogenous antimicrobial substance (Refs. 1 and 2).

Lysozyme was the first enzyme to have the details of its three-dimensional structure published (Ref. 4), and it has become one of the best characterized of all enzymes, serving as an example for studies of enzyme mechanism and molecular evolution (Refs. 5 and 6). Lysozymes from various organisms are very similar to one another. Egg white lysozyme differs very little in structure, amino acid sequence and composition, catalytic mechanism, and substrate specificity from the enzyme found in human milk, saliva, mucus, and tears (Refs. 3 and 6).

The petitioner provided two published scientific review articles (Refs. 1 and 2) that discuss the use of egg white lysozyme in cheese and other food. The petitioner also provided a generally available trade association bulletin (Ref. 7) that focuses on the use of egg white lysozyme for its technical effect of preventing late blowing in cheese. This bulletin describes the late blowing defect and how it arises, traditional chemical control measures (other than the use of lysozyme) to reduce the problem, and the increasing interest in using lysozyme as a replacement for traditional chemical control measures. In addition, the petitioner provided generally available information documenting that this intended use of the petitioned enzyme preparation has been approved in several countries, including Denmark, France, Germany, Italy, and Spain (Refs. 9 through 13).

000124

FDA considered the estimated dietary exposure to lysozyme for the

proposed use in cheese (Refs. 16 and 17). Lysozyme accounts for approximately 3.5 percent of the total protein of domestic hen egg whites (Ref. 7). Whole eggs contain lysozyme at a level of approximately 3,300 parts per million (ppm). The petitioner reported that cheese manufactured using egg white lysozyme enzyme preparation contains a maximum of 400 ppm of lysozyme, or at least 8 times less than eggs on a weight basis. FDA has estimated a long-term mean intake of lysozyme to be 74 milligrams per person per day (mg/p/d) for consumers of eggs and 3.8 mg/p/d for consumers of cheese; the respective 90th percentile intakes are estimated to be 163 mg/p/day and 8.1 mg/p/day. Egg whites from which lysozyme is extracted will be subsequently consumed in other food uses. Thus, there will be no long-term net increase in lysozyme intake by the general population because egg whites without lysozyme will replace egg whites in current use that contain lysozyme (Ref. 16). On a per eating occasion basis, lysozyme intake for cheese consumers may be 16 mg on average, or 22 mg at the 90th percentile level. For comparison, a per eating occasion lysozyme intake for egg consumers may be 264 mg on average, or 416 mg at the 90th percentile level. Thus, lysozyme intake per eating occasion due to cheese consumption may constitute 5 to 6 percent of lysozyme intake due to egg consumption (Ref. 17).

In general, issues relevant to a safety evaluation of proteins such as the enzyme component of an enzyme preparation are potential toxicity and allergenicity (Ref. 18). Proteins derived from egg whites do not raise toxicity concerns because egg whites have been safely consumed by humans as a source of food throughout recorded history without any reports of toxicity. However, proteins derived from egg whites do raise allergenicity concerns because, as with many common foods, there have been reports that consumption of egg whites can cause an allergic reaction in certain individuals, particularly children (Ref. 19). Therefore, FDA considered the question of whether the lysozyme component of egg whites is allergenic.

In evaluating this question, FDA considered a report of an in vitro study of the binding of antibodies to specific egg proteins, where the antibodies were derived from the serum of patients known to be allergic to eggs (Ref. 20). This report suggests that lysozyme was an allergen for some individuals who became sensitive to egg whites. Although this study does not establish that ingestion of egg white lysozyme in cheese will actually cause a clinically

[[Page 12423]]

significant allergic reaction in such sensitive individuals, FDA is not aware of any data or information that would refute the study's inference that egg white lysozyme may be allergenic. Accordingly, FDA is proposing labeling, as discussed below, to alert the sensitive population to the presence of egg white lysozyme in cheese.

A related question is whether egg white lysozyme, when present in cheese, is capable of inducing an allergenic response in susceptible individuals who have not previously consumed egg whites, e.g., because

000125

their customary diet excludes eggs. This question is no different than for any other food containing egg white when consumed by individuals with unknown susceptibility to eggs. The proposed label declaration would provide such individuals with the same protection as that provided by other egg-containing products with ingredient labeling. Thus, individuals who experience an allergic reaction to lysozyme-containing cheese could identify egg white lysozyme as a possible cause of the reaction.

B. Enzyme Source, Manufacturing Methods, and Processing Aids

Commercial preparations of lysozyme are derived from domestic hen egg whites using ion exchange methods and selective precipitation to isolate a highly purified protein fraction that contains mainly lysozyme but also may contain small amounts of other egg white proteins. Consistent with the agency's finding in its GRAS affirmation of microparticulated protein product (55 FR 6384, February 23, 1990), FDA finds that egg whites have been safely consumed by humans throughout recorded history and, therefore, are GRAS (Sec. 170.30(d)). The agency evaluated the methods used to isolate the enzyme lysozyme from egg whites. These methods are based on generally available and accepted principles of protein purification (Ref. 8). Such methods, if appropriately selected, do not ordinarily alter the chemical identity and characteristic properties of enzymes. Therefore, these methods do not materially change the quality, utility, functionality, or safety of enzymes. Moreover, the retention of the antibacterial activity that is characteristic of egg white lysozyme when egg white-derived lysozyme enzyme preparation is used in cheese evidences that lysozyme in the manufactured enzyme preparation remains unaltered from the lysozyme in egg whites. This is corroborative evidence of the fact that the methods used to isolate lysozyme from egg whites do not materially change the quality, utility, functionality or safety of the enzyme lysozyme.
Enzyme preparations used in food processing are usually not chemically pure but contain, in addition to the enzyme component, materials that derive from the enzyme source. As mentioned above, egg white lysozyme enzyme preparation may contain small amounts of other egg white proteins. A related question is whether such proteins that may be present in the enzyme preparation are allergenic. Even if present, other source-derived proteins would not be a concern because the proposed label declaration for egg white lysozyme would alert individuals who are sensitive to egg whites to the possible presence of other proteins derived from egg whites.
In addition to source-derived materials, enzyme preparations used in food processing usually contain materials that derive from the manufacturing methods used to generate the finished enzyme preparation. The egg white lysozyme enzyme preparation that is the subject of this document complies with the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 4th ed. (Ref. 14). The egg white lysozyme enzyme preparation that is the subject of this document may contain substances that are added to the

000126

enzyme preparation, such as preservatives, stabilizers or diluents, and trace amounts of processing aids that are used in its preparation. These substances must be acceptable for general use in foods (Refs. 14 and 15).

C. Labeling as a Condition of Use

Egg whites are known to be an allergenic food source, particularly in children (Ref. 19). There is a literature report (Ref. 20) indicating that lysozyme may in fact have been an allergen for some individuals who became sensitive to egg whites. Although the reported in vitro study does not establish that ingestion of egg white lysozyme in cheese will actually cause a clinically significant allergic reaction in such sensitive individuals, FDA is not aware of any data or information that would refute the study's inference that egg white lysozyme may be allergenic. Therefore, FDA concludes that there is insufficient information in the current record to determine whether the ingestion of egg white lysozyme elicits an allergenic response when consumed by individuals who are sensitive to egg whites. Accordingly, as discussed below, FDA is proposing labeling to alert such individuals to the presence of egg white lysozyme in cheese. Such labeling also would alert the sensitive population to the possible presence of source-derived proteins other than lysozyme in the enzyme preparation. Under section 409(c)(1) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 348(c)(1)), FDA is authorized, in approving the use of a food additive, to list the conditions under which the additive may be safely used. These conditions may include any labeling requirements that the agency deems necessary to ensure the safe use of the additive. Similarly, under Sec. 184.1(b)(3) (21 CFR 184.1(b)(3)), in affirming a substance as GRAS, FDA is authorized to set forth the particular conditions of use, including labeling, under which there is general recognition among qualified experts that the use of the substance is safe. After careful review of the evidence on the use of egg white lysozyme enzyme preparation in preventing late blowing in cheese, FDA has tentatively concluded that such use is GRAS only when the conditions of its use include a declaration on the label or labeling of the presence of egg white lysozyme in both bulk and packaged food containing such treated cheese. Therefore, this tentative final rule (Sec. 184.1550(c)(1)) establishes that the declaration of egg white lysozyme enzyme preparation by the common or usual name ``egg white lysozyme" is a condition of use required for GRAS status, so that consumers who are allergic to egg white products can be alerted to the presence of the egg white-derived enzyme in treated cheese.

D. Summary and Conclusions

The petitioner provided published data and information relating to the identity of, characteristic properties of, and estimated dietary exposure to the enzyme component (Refs. 1 through 7). The source of the petitioned enzyme preparation, egg white, has been safely consumed by

000127

humans as a source of food protein throughout recorded history, and, therefore, is GRAS (Sec. 170.30(d)). The petitioner provided generally available information showing that the methods used for extracting lysozyme from the egg white source do not ordinarily alter the chemical identity and characteristic properties of enzymes (Ref. 8). Moreover, there is corroborating evidence that the extraction of egg white lysozyme does not change its chemical identity or characteristics because the antibacterial activity of egg white lysozyme is retained. FDA concludes that the methods used to manufacture egg white lysozyme enzyme preparation do not change the safety for food use of the enzyme lysozyme and that toxicological

[[Page 12424]]

studies are not necessary to establish the safety of lysozyme or other source-derived proteins that may remain in the manufactured enzyme preparation. FDA also concludes that there will be no net increase in dietary exposure of the general population to the commonly consumed enzyme lysozyme due to the proposed use in cheese because lysozyme will simply be transferred from eggs to cheese (Ref. 16).

The petitioner also provided generally available and accepted information relating to processing aids used in the manufacture of the enzyme preparation and generally available and accepted specifications for food grade enzyme preparations (Ref. 14). FDA concludes that substances added to the egg white lysozyme enzyme preparation or potential residues of processing aids used in the manufacturing process do not present a basis for concern about the safety of the egg white lysozyme enzyme preparation.

The petitioner provided published scientific review articles (Refs. 1 and 2) and a generally available trade bulletin (Ref. 7) that discuss the use of the egg white lysozyme enzyme preparation in cheese and other food, including its use for the intended effect of preventing late blowing of cheese contaminated with C. tyrobutyricum. The petitioner also provided generally available information documenting that this intended use of lysozyme has been approved in several European countries (Refs. 9 through 13). FDA concludes that generally available and accepted data and information establish that lysozyme will achieve the intended technical effect of preventing late blowing in cheese contaminated with C. tyrobutyricum.

Finally, information in the petition and otherwise available to FDA raises the question of whether the lysozyme component of egg whites is allergenic. FDA is proposing labeling to alert individuals who may be sensitive to egg whites to the presence of egg white lysozyme in cheese, including the possible presence of other source-derived proteins that may be present in the enzyme preparation.

IV. Comments

FDA received two comments in response to the filing notice. One comment expressed agreement that lysozyme is GRAS for use in preventing

000128

late blowing in cheese and supported the affirmation of GRAS status by the agency.

One comment stated that use of lysozyme as a food preservative may lead to selection of lysozyme-resistant strains of the bacterial food poisoning agents Listeria monocytogenes and C. botulinum, rendering one of the body's main defense mechanisms useless against resistant strains. The comment likened the potential selection of lysozyme-resistant strains of bacteria to the selection of penicillin-resistant bacteria as a result of its widespread use. The comment pointed out that the body could not readily substitute the lysozyme naturally present in secretions such as tears and saliva for another antimicrobial.

The mechanism of action of lysozyme involves hydrolysis of the structural peptidoglycan present in cell walls of susceptible bacteria. Therefore, development of resistance to lysozyme would require that a bacterium develop a variant of peptidoglycan that is resistant to the action of lysozyme. Development of such a variant peptidoglycan is, in principle, possible. However, as already discussed, lysozyme activity has been shown to be present in bacteria, fungi, plants, and almost all animal tissues. If such relative ubiquity has not resulted in the clinically significant selection of lysozyme-resistant bacteria to date, the use of lysozyme in those cheeses that are susceptible to late blowing is unlikely to favor selection of lysozyme-resistant bacteria and adversely affect the public health. Moreover, FDA is not considering lysozyme for use as a widespread food preservative. Rather, FDA is considering the narrow question of whether the use of lysozyme in preventing late blowing in cheese is generally recognized as safe. FDA disagrees that this limited use in cheese is analogous to the widespread use of antibiotics such as penicillin and the subsequent# selection of antibiotic-resistant bacterial strains. Therefore, FDA concludes that the use of lysozyme in preventing late blowing in cheese does not raise concerns about the selection of lysozyme-resistant strains of L. monocytogenes or C. botulinum.

V. Specifications

The agency finds that, because the potential impurities in the egg white lysozyme preparation that may originate from the source or manufacturing process do not raise any basis for concern about the safe use of the preparation, the general requirements and additional requirements for enzyme preparations in the monograph on Enzyme Preparations in the Food Chemicals Codex, 4th ed. (1996), which are being incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51, are adequate as minimum criteria for food-grade egg white lysozyme enzyme preparation. Lysozyme assay can be performed using a method entitled ``Lysozyme hydrochloride, Microbiological Determination," which is included in the petition (Ref. 21) or by using any appropriate validated method.

000129

VI. Conclusions

The agency has evaluated all available information and finds, based upon the published information about the manufacturing methods used in the preparation of egg white lysozyme enzyme preparation, and published data and information about the identity and characteristic properties of egg white lysozyme, that the enzyme component of egg white lysozyme enzyme preparation is unaltered from the lysozyme found in the commonly consumed food, eggs. The agency also finds, based upon generally available and accepted information, that when the preparation is manufactured in accordance with Sec. 184.1550(c), the source, egg whites, and the manufacturing process will not introduce impurities into the preparation that may render its use unsafe. Further, the agency finds, based upon published information, that egg white lysozyme enzyme preparation will achieve its intended technical effect of preventing late blowing in cheese contaminated with C. tyrobutyricum. Therefore, the agency tentatively concludes, based upon the evaluation of published data and information, corroborated by unpublished data and information, that the egg white lysozyme enzyme preparation described in the regulation set out below is GRAS for use by the general population in preventing late blowing in cheese.
To give interested persons an opportunity to comment on the proposed label declaration that is a condition of use required for GRAS status, FDA is issuing this tentative final rule under 21 CFR 10.40(f)(6). FDA will review any comments that are relevant to this condition of use and that are received within the 75 day comment period and will respond accordingly to these comments in the Federal Register.

VII. Environmental Considerations

The agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch

[[Page 12425]]

(address above) between 9 a.m. and 4 p.m., Monday through Friday.

VIII. Analysis of Economic Impacts

A. Benefit-Cost Analysis

FDA has examined the impacts of this tentative final rule under Executive Order 12866. Executive Order 12866 directs Federal agencies to assess the costs and benefits of available regulatory alternatives, and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental,

000130

public health and safety effects; distributive impacts; and equity). According to Executive Order 12866, a regulatory action is ``significant" if it meets any one of a number of specified conditions; including having an annual effect on the economy of $100 million, adversely affecting in a material way a sector of the economy, competition, or jobs, or if it raises novel legal or policy issues. FDA finds that this tentative final rule is not a significant regulatory action, as defined by Executive Order 12866. In addition, it has been determined that this final rule is not a major rule for the purpose of congressional review.

The primary benefit of this action is to remove uncertainty about the regulatory status of the petitioned substance. FDA is tentatively affirming the GRAS status of egg white lysozyme in cheese only when the ingredient statement of the bulk and packaged food that contains the cheese includes the common or usual name of the substance, i.e., ``egg white lysozyme." The labeling requirement will add a small cost to the future use of the petitioned substance, and therefore, is not a significant action under the Executive Order 12866.

FDA has examined the impacts of this tentative final rule under the Unfunded Mandates Reform Act of 1995 (UMRA) (Pub. L. 104-4). A written statement under section 202(a) of the UMRA is not required for this rule because the rule does not impose a mandate that results in an expenditure of $100 million or more by State, local, and tribal governments in the aggregate, or by the private sector, in any 1 year.

B. Regulatory Flexibility Act

FDA has evaluated this tentative final rule under the Regulatory Flexibility Act. The Regulatory Flexibility Act (5 U.S.C. 601-612) requires Federal agencies to consider alternatives that would minimize the economic impact of their regulations on small entities.

FDA believes that this tentative final rule is not likely to have a significant economic impact on a substantial number of small entities. However, the agency seeks comment on this tentative conclusion. First, FDA is tentatively affirming the GRAS status of egg white lysozyme in cheese only when the ingredient statement of the bulk and packaged food that contains the cheese includes the common or usual name of the substance, i.e., ``egg white lysozyme." This labeling requirement will impose only minimal costs to the future use of the petitioned substance. Second, FDA has information that the petitioner does not currently sell egg white lysozyme in the United States (Refs. 22 and 23). Moreover, FDA is not aware of any manufacture or use of cheese containing egg white lysozyme in the United States. If no small entities are currently manufacturing or using cheese containing egg white lysozyme, the proposed labeling requirements would not impose any cost to small entities. However, because FDA does not have any information on whether other entities in the United States are manufacturing or using cheese containing egg white lysozyme, FDA is unable to conclude, in this tentative final rule, that there will be no significant economic impact on a substantial number of small entities.

000131

Therefore, the agency seeks comment on the manufacture or use, by any small entity, of cheese containing egg white lysozyme. In its final rule, the agency will, based on any relevant comments received, determine whether there is a significant economic impact on a substantial number of small entities.

IX. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Carini, S., G. Mucchetti, and E. Neviani, ``Lysozyme: Activity Against Clostridia and Use in Cheese Production--A Review," Microbiologie Aliments Nutrition, 3:299-320, 1985.
2. Procter, V. A., and F. E. Cunningham, ``The Chemistry of Lysozyme and Its Use as a Food Preservative and a Pharmaceutical," CRC Critical Reviews in Food Science and Nutrition, 26:359-395, 1988.
3. Fleming, A., ``On a Remarkable Bacteriolytic Element Found in Tissues and Secretions," Proceedings of the Royal Society, 93:306-317, 1922.
4. Blake, C. C. F., D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, ``Structure of Hen Egg-White Lysozyme," Nature, 206:757-783, 1965.
5. Kuhara, S., E. Ezaki, T. Fukamizo, and K. Hayashi, ``Estimation of the Free Energy Change of Substrate Binding in Lysozyme-Catalyzed Reactions," Journal of Biochemistry, 92:121-127, 1982.
6. Malcolm, B., K. Wilson, B. Matthews, J. Kirsch, and A. Wilson, ``Ancestral Lysozymes Reconstructed, Neutrality Tested, and Thermostability Linked to Hydrocarbon Packing," Nature, 345:86-89, 1990.
7. ``The Use of Lysozyme in the Prevention of Late Blowing in Cheese," Bulletin of the International Dairy Foundation, No. 216, 1987.
8. Chaplin, M. F., and C. Bucke, Enzyme Technology, Cambridge University Press, New York, NY, pp. 66-72, 1990.
9. Denmark Ministry of Agriculture Circular on Approved Enzymes for Cheese, Journal nr. 10.1.5-18/83, January 13, 1984.
10. French Republic Ministry of Agriculture Service Note 5236, April 21, 1987.
11. Federal Republic of Germany, Bundesgesetzblatt Nr. 56, pp. 2103-2107, November 22, 1985.
12. Official Gazette of Italian Republic, General Series n. 23, October 4, 1986.
13. Spanish Ministry of Health and Consumption, General Direction of Public Health, General Sanitary Register, File n. 8826, June 20, 1983.
14. Monograph on ``Enzyme Preparations," Food Chemicals Codex, National Academy Press, Washington, DC, 4th ed., pp. 133-134, 1996.

000132

15. Pariza, M. W. and E. M. Foster, ``Determining the Safety of Enzymes Used in Food Processing," Journal of Food Protection, 46:453-468, 1983.
16. Memorandum dated March 20, 1990, from Food and Color Additives Review Section, FDA, to Direct Additives Branch, FDA, ``Use of Lysozyme to Prevent the `Late Blowing' of Cheese."
17. Memorandum dated August 5, 1996, from Chemistry Review Branch, FDA, to Biotechnology Policy Branch, FDA.
18. Kessler, D. A., M. R. Taylor, J. H. Maryanski, E. L. Flamm, and L. S. Kahl, ``The Safety of Foods Developed by Biotechnology," Science, 256:1747-1749 and 1832, 1992.
19. Crespo, J. F., C. Pasqual, A. Ferrer, A. W. Burke, J. M. Diaz Pena, and M. M. Esteban, ``Egg White-specific IgE Level as a Tolerance Marker in the Follow-up of Egg Allergy," Allergy Proceedings, 15:73-76, 1994.
20. Anet, J., J. F. Back, R. S. Baker, D. Barnett, R. W. Burley, and M. E. H. Howden, ``Allergens in the White and Yolk of Hen's Egg; a Study of IgE Binding by Egg Proteins," International Archives of Allergy and Applied Immunology, 77:364-371, 1985.
21. Lysozyme Hydrochloride, Microbiological Determination.
22. Letter dated November 25, 1996, from John B. Dubeck, Keller and Heckman, to Linda Kahl, FDA.
23. Letter dated February 28, 1997, from John F. Foley, Keller and Heckman, to Linda Kahl, FDA.

List of Subjects in 21 CFR Part 184

Food ingredients, Incorporation by reference.
Therefore, under the Federal Food, Drug, and Cosmetic Act and under

[[Page 12426]]

authority delegated to the Commissioner of Food and Drugs and redelegated to the Director, Center for Food Safety and Applied Nutrition, it is proposed that 21 CFR part 184 be amended as follows:

PART 184--DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

1. The authority citation for 21 CFR part 184 continues to read as follows:

Authority: 21 U.S.C. 321, 342, 348, 371.

2. Section 184.1550 is added to subpart B to read as follows:

Sec. 184.1550 Egg white lysozyme.

(a) Egg white lysozyme (CAS Reg. No. 9001-63-2) is the enzyme peptidoglycan N-acetylmuramoylhydrolase (EC No. 3.2.1.17) obtained by

000133

extraction from egg whites. The enzyme catalyzes the hydrolysis of peptidoglycan in the cell walls of certain bacteria including Clostridium tyrobutyricum.
(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the monograph on Enzyme Preparations in the Food Chemicals Codex, 4th ed. (1996), which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, and may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.
(c)(l) The ingredient is used in cheeses, as defined in Sec. 170.3(n)(5) of this chapter, in accordance with Sec. 184.1(b)(3) at levels not to exceed current good manufacturing practice.
(2) The affirmation of the use of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following conditions of use:
(i) The ingredient is used as an enzyme as defined in Sec. 170.3(o)(9) of this chapter.
(ii) Current good manufacturing practice utilizes a level of the ingredient sufficient to prevent the late blowing of cheeses caused by the bacterium Clostridium tyrobutyricum during cheese production.
(iii) The ingredient statement for both bulk and packaged food that contains cheese manufactured using egg white lysozyme shall include the common or usual name ``egg white lysozyme" to identify the source of the protein.

Dated: March 3, 1998.
L. Robert Lake,
Director, Office of Policy, Planning and Strategic Initiatives, Center for Food Safety and Applied Nutrition.
[FR Doc. 98-6571 Filed 3-12-98; 8:45 am]
BILLING CODE 4160-01-F

Generated by MHonArc 2.1.0, enhanced by BYTE

[Federal Register: April 23, 1999 (Volume 64, Number 78)]
[Rules and Regulations]
[Page 19887-19895]
From the Federal Register Online via GPO Access [wais.access.gpo.gov]
[DOCID:fr23ap99-13]

==========

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 184

[Docket No. 84G-0257]

Carbohydrase and Protease Enzyme Preparations Derived From Bacillus Subtilis or Bacillus Amyloliquefaciens; Affirmation of GRAS Status as Direct Food Ingredients

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is affirming that carbohydrase enzyme preparations derived from either Bacillus subtilis or B. amyloliquefaciens and protease enzyme preparations derived from either B. subtilis or B. amyloliquefaciens are generally recognized as safe (GRAS) for use as direct food ingredients. This action is a partial response to a petition filed by the Ad Hoc Enzyme Technical Committee (now the Enzyme Technical Association).

DATES: The regulation is effective April 23, 1999. The Director of the Office of the Federal Register approves the incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51 of certain publications listed in 21 CFR 184.1148 and 184.1150, effective April 23, 1999.

FOR FURTHER INFORMATION CONTACT: Linda S. Kahl, Center for Food Safety and Applied Nutrition (HFS-206), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-418-3101.

SUPPLEMENTARY INFORMATION:

Table of Contents

I. Introduction
II. Standards for GRAS Affirmation
III. Background
    A. Identity and Technical Effect
    B. Methods of Manufacture
IV. Safety Evaluation
    A. Pre-1958 History of Use in Food
    1. Bacillus Subtilis
    2. Bacillus Amyloliquefaciens
    B. Corroborating Evidence of Safety
    1. The Enzyme Components

000135

2. **Enzyme** Sources, Manufacturing Methods, and Processing Aids
V. Comments
VI. Conclusions
VII. Environmental Considerations
VIII. Analysis for Executive Order 12866
IX. Regulatory Flexibility Analysis
X. Paperwork Reduction Act of 1995
XI. Effective Date
XII. References

I. Introduction

In accordance with the procedures described in Sec. 170.35 (21 CFR 170.35), the Ad Hoc **Enzyme** Technical Committee (now the **Enzyme** Technical Association), c/o Miles Laboratories, Inc., 1127 Myrtle St., Elkhart, IN 46514, submitted a petition (GRASP 3G0016) requesting that the following **enzyme** preparations be affirmed as **GRAS** for **use** in food: (1) Animal-derived **enzyme** preparations: Catalase (bovine liver); lipase, animal; pepsin; rennet; rennet, bovine; and trypsin; (2) plant-derived **enzyme** preparations: Bromelain; malt; and papain; (3) microbially-derived **enzyme** preparations: Lipase, catalase, glucose oxidase, and carbohydrase from Aspergillus niger, var.; mixed carbohydrase and protease from Bacillus subtilis, var.; carbohydrase from Rhizopus oryzae; and carbohydrase from Saccharomyces species.

FDA published a notice of filing of this petition in the Federal Register of April 12, 1973 (38 FR 9256), and gave interested persons an opportunity to submit comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. The petition was amended by notices published in the Federal Register of June 12, 1973 (38 FR 15471), proposing affirmation that microbially-derived **enzyme** preparations (carbohydrase, lipase, and protease) from A. oryzae are **GRAS** for **use** in food; in the Federal Register of August 29, 1984 (49 FR 34305), proposing affirmation that the **enzyme** preparations ficin, obtained from species of the genus Ficus (fig tree), and pancreatin, obtained from bovine and porcine pancreas, are **GRAS** for **use** in food; in the Federal Register of June 23, 1987 (52 FR 23607), proposing affirmation that the protease **enzyme** preparation from A. niger is **GRAS** for **use** in food; and in the Federal Register of August 5, 1996 (61 FR 40648), proposing affirmation that carbohydrase and protease **enzyme** preparations from B. amyloliquefaciens are **GRAS** for **use** in food. In the June 23, 1987, notice, FDA also noted the petitioner's assertion that pectinase **enzyme** preparation from A. niger and lactase **enzyme** preparation from A. niger are included under carbohydrase **enzyme** preparation from A. niger, and that invertase **enzyme** preparation from Saccharomyces cerevisiae and lactase **enzyme** preparation from Kluyveromyces marxianus are both included under carbohydrase **enzyme** preparation from species of the genus Saccharomyces. The agency further noted that, therefore, pectinase **enzyme** preparation from A. niger, lactase **enzyme** preparation from A. niger, invertase **enzyme** preparation from S. cerevisiae, and lactase **enzyme** preparation from K. marxianus were to be considered part of the petition. Interested persons were given an opportunity to submit comments to the Dockets Management Branch (address above) on each amendment.

After the petition was filed, the agency published, as part of its comprehensive safety review of **GRAS** substances, two **GRAS** affirmation regulations that covered three of the **enzyme** preparations from animal and plant sources included in the petition. These two regulations are: (1) Sec. 184.1685 Rennet (animal derived) (21 CFR 184.1685), which was published in the Federal Register of November 7, 1983 (48 FR 51151) and

http://frwebgate3.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=708415209+0+0+0&W 03/02/2001

includes the petitioned **enzyme** preparations rennet and bovine rennet; and (2) Sec. 184.1585 Papain (21 CFR 184.1585), which was published in the Federal Register of October 21, 1983 (48 FR 48805). Thus,

[[Page 19888]]

rennet, bovine rennet, and papain are already affirmed as **GRAS** and need not be addressed further.

In a notice published in the Federal Register of September 20, 1993 (58 FR 48889), the agency announced that the petitioner had requested that the following **enzyme** preparations be withdrawn from the petition without prejudice to the filing of a future petition: (1) Pancreatin used for its lipase activity, (2) pancreatin used for its amylase activity, and (3) amylase derived from unmalted barley extract. In that notice, the agency stated that, in light of the petitioner's request, any future action by FDA on the petition would not include a determination of the **GRAS** status of these three **enzyme** preparations.

In a final rule published in the Federal Register of June 26, 1995 (60 FR 32904), the agency affirmed as **GRAS** the following **enzyme** preparations derived from animal sources: Catalase (bovine liver), animal lipase, pepsin, trypsin, and pancreatin (as a source of protease activity). In that same final rule, the agency also affirmed as **GRAS** the following **enzyme** preparations derived from plant sources: Bromelain, ficin, and malt.

This final rule addresses the following bacterially-derived **enzyme** preparations: (1) carbohydrase **enzyme** preparation from B. subtilis; (2) protease **enzyme** preparation from B. subtilis; (3) carbohydrase **enzyme** preparation from B. amyloliquefaciens; and (4) protease **enzyme** preparation from B. amyloliquefaciens. \1\ The other microbial **enzyme** preparations in the petition will be dealt with separately in a future issue of the Federal Register.

\1\ Although the petition requested **GRAS** affirmation for mixed carbohydrase and protease **enzyme** preparation from B. subtilis, the petitioner subsequently agreed that this **enzyme** preparation be evaluated as two separate **enzyme** preparations, carbohydrase **enzyme** preparation from B. subtilis and protease **enzyme** preparation from B. subtilis. **Enzyme** preparations that contain mixtures of carbohydrases and proteases can be used either for their carbohydrase activity or for their protease activity, and they are usually sold according to their intended **use**. FDA requested the petitioner's agreement to this change to reflect the distinct uses of mixed carbohydrase and protease **enzyme** preparations in food depending on whether a particular preparation is being used for its carbohydrase activity or for its protease activity.

II. Standards for **GRAS** Affirmation

Under Sec. 170.30 (21 CFR 170.30) and 21 U.S.C. 321(s), general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either scientific procedures or, in the case of a substance used in food prior to January 1, 1958, experience based on common **use** in food. General recognition of safety based upon scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive and ordinarily is based upon published studies, which may be corroborated

by unpublished studies and other data and information (Sec. 170.30(b)). General recognition of safety through experience based on common use in food prior to January 1, 1958, may be determined without the quantity or quality of scientific procedures required for approval of a food additive, and ordinarily is based upon generally available data and information concerning the pre-1958 use of the substance (Sec. 170.30(c)).

For the enzyme preparations from B. subtilis and B. amyloliquefaciens that are the subject of this document, the Enzyme Technical Association bases its request for affirmation of GRAS status on a history of safe food use prior to 1958. As discussed in the preamble to the proposed rule for the most recent amendment to Sec. 170.30, general recognition of safety through experience based on common use in food requires a consensus on the safety of the substance among the community of experts who are qualified to evaluate the safety of food ingredients (50 FR 27294 at 27295, July 2, 1985).

III. Background

A. Identity and Technical Effect

Enzymes are proteins that originate from living cells and produce chemical change by catalytic action (Random House Dictionary of the English Language, 1987). Most enzymes are very specific in their ability to catalyze only certain chemical reactions; this high degree of specificity and strong catalytic activity are the most important functional properties of enzymes (Ref. 1).

Commercial enzyme preparations such as those that are the subject of this document usually contain several enzymes that have catalytic activities other than those for which they are sold--i.e., other than their characterizing enzyme activities. As discussed in more detail in section III.B of this document, the methods of manufacture for a specific commercial enzyme preparation are tailored to maximize the characterizing enzyme activity. The other enzymes that are present in the preparation generally are present at low levels.

Carbohydrases, which are also known as glycosidases, are enzymes whose catalytic activity is the hydrolysis (i.e., splitting) of O-glycosyl bonds in carbohydrates. The carbohydrase enzyme preparations that are the subject of this document each contain two or more carbohydrases, including: (1) <greek-a>-amylase, which hydrolyzes <greek-a>-1,4-glucan bonds in polysaccharides (e.g., starch) yielding monosaccharides, linear oligosaccharides and branched oligosaccharides (dextrins), and (2) <greek-b>-glucanase, which hydrolyzes 1,3 and some 1,4 linkages in <greek-b>-D-glucans (polysaccharides that are common in cereals such as oats, barley, and rye), yielding oligosaccharides and glucose (Refs. 2 and 3). Because the major carbohydrase in the carbohydrase enzyme preparations derived from B. subtilis or B. amyloliquefaciens is <greek-a>-amylase, the primary use of these enzyme preparations is the hydrolysis of starch in processes such as the preparation of starch syrups and the fermentation of beer (Refs. 3 through 5).

Proteases are enzymes whose catalytic activity is the hydrolysis of peptide bonds in proteins, yielding peptides and amino acids. The protease enzyme preparations that are the subject of this document each contain two or more proteases, including subtilisin and neutral proteinase (Refs. 2 and 3). The primary use of the protease enzyme preparations derived from B. subtilis or B. amyloliquefaciens is in the preparation of protein hydrolysates and the tenderizing of meat (Refs. 3 through 5).

Table 1 lists the characterizing enzyme activities and associated

International Union of Biochemistry **Enzyme** Commission (EC) numbers of the carbohydrase and protease **enzyme** preparations derived from B. subtilis or B. amyloliquefaciens.

#Table 1.--**Enzyme** Activities and EC Numbers Associated With **Enzyme** Preparations Derived From B. Subtilis or B. Amyloliquefaciens

Enzyme Preparation	Characterizing **Enzyme** Activity	EC Number
Carbohydrase	<greek-a>-Amylase	3.2.1.1
	<greek-b>-Glucanase	3.2.1.6
Protease	Subtilisin	3.4.21.62
	Neutral Proteinase	3.4.24.28

B. Methods of Manufacture

All microbial strains, including bacterial strains, used to manufacture **enzyme** preparations are started from a

[[Page 19889]]

pure laboratory culture and grown, or ``fermented,'' in a sterile liquid nutrient medium or sterile moistened semisolid medium. Accepted microbiological techniques are used to exclude contaminating organisms and to avoid development of substrains from within the culture itself (Ref. 6). Although specific conditions of fermentation vary from manufacturer to manufacturer, common fermentation procedures are: (1) The submerged culture method, which uses closed fermenters equipped with agitators, aeration devices, and jackets or coils for temperature control; and (2) the semisolid culture method, which uses horizontal rotating drums or large chambers fitted with trays (Refs. 5 and 6). During fermentation by either method, the pH, temperature, appearance or disappearance of certain ingredients, purity of culture, and **level** of **enzyme** activity must be carefully controlled. The fermentation is harvested at the point where laboratory tests indicate that maximum production of **enzyme** activity has been attained.

In practice, the processes by which microbial-derived **enzyme** preparations are produced vary widely. Each single strain of microorganism produces a large number of enzymes (Ref. 5). The absolute and relative amounts of various individual enzymes produced vary markedly among species and even among strains of the same species. They also vary depending upon the composition of medium on which the microorganism grows, and upon the fermentation conditions. The petitioner states that for a specific **enzyme** preparation the production strain, medium composition, and fermentation conditions are optimized to maximize the desired **enzyme** activity (Refs. 7 and 8).

The carbohydrase and protease enzymes from B. subtilis and B. amyloliquefaciens are excreted into the fermentation medium (Refs. 9 through 11). In the semisolid culture method, an **enzyme** that is present in the fermentation medium is extracted either directly from the moist material, or later after the culture mass has been dried. In the submerged culture method, the microorganisms and other insolubles are removed from the fermentation medium by decanting, filtering, or centrifuging, and therefore an extraction step is not required. In either method, further processing steps may involve clarification, evaporation, precipitation, drying, and grinding (Refs. 6 and 9 through 12).

IV. Safety Evaluation

A. Pre-1958 History of Use in Food

Enzyme preparations have been safely used for many years in the production and processing of food, for example, in the baking, dairy, and brewing industries (e.g., see Refs. 1, 4, and 13).

1. Bacillus Subtilis

The petitioner has provided generally available information, including published reviews, showing that carbohydrase and protease enzyme preparations derived from B. subtilis were commonly used in food prior to 1958 (Refs. 4 and 5). This information is summarized in Table 2.

Table 2.--Applications of Bacterial Carbohydrase and Protease Enzyme Preparations in Food Prior to 1958

Enzyme preparation	Food categories	Technical effect or industry application	References
Carbohydrase	Beer	Mashing¹	4 and 5
	Syrup for cocoa and chocolate	Reduction of viscosity	4 and 5
	Sugar	Recovery from scrap candy	4 and 5
	Distilled beverages	Mashing	4
	Precooked cereals	Modification of cereal starches to improve characteristics	
Protease	Beer	Chillproofing	4
	Condiments	Not reported	5
	Milk	Protein hydrolysis	5

\1\ Mashing is the conversion of starch to sugars.

In the published article by Underkofler et al. (Ref. 5), the authors use the general terms ``bacterial amylase'' and ``bacterial protease'' to refer to bacterially-derived carbohydrase and protease enzyme preparations used in food at the time of the article. However, the article also includes a table in which the source bacterium for bacterially-derived enzyme preparations is identified as B. subtilis.

In the published article by Underkofler and Ferracone (Ref. 4), the authors use the general terms ``bacterial carbohydrase'' and ``bacterial protease'' to refer to bacterially-derived carbohydrase and protease enzyme preparations used in food at the time of the article. Unlike the Underkofler et al. article, however, the Underkofler and Ferracone article does not identify the source bacterium for these enzyme preparations. Although it is not possible to determine conclusively whether the descriptor ``bacterial'' in the Underkofler and Ferracone article refers to B. subtilis, the use of this term by the same principal author in two scientific articles published in consecutive years to describe the source of protease and carbohydrase enzyme preparations used in the food industry, coupled with the identification of the source bacterium for these enzyme preparations as B. subtilis in the Underkofler et al. article, makes it likely that the source bacterium referred to by Underkofler and Ferracone was in fact

http://frwebgate3.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=708415209+0+0+0&W 03/02/2001

B. subtilis.

The food uses shown in Table 2, using terminology from the cited reference(s), were documented in articles that were published before or during 1958; the cited references demonstrate that the **use** of these **enzyme** preparations in a variety of foods was widely recognized by 1958. Therefore, the agency concludes that carbohydrase and protease **enzyme** preparations derived from B. subtilis were in common **use** in food prior to January 1, 1958.

2. Bacillus Amyloliquefaciens

According to the petitioner (Refs. 8 and 14 through 16), the species B. amyloliquefaciens was not classified under the name B. amyloliquefaciens until it was taxonomically separated from the species B. subtilis in the late 1980's (Refs. 17 and 18). Therefore, the petitioner asserts, references in contemporaneous scientific literature to pre-1958 food **use** of **enzyme** preparations from B. amyloliquefaciens occur under the name B. subtilis.

With respect to carbohydrase components of the petitioned **enzyme**

[[Page 19890]]

preparations, the petitioner cites scientific literature describing a distinctive group of bacteria, within the group originally considered to be B. subtilis, that are known to possess a high **level** of <greek-a>-amylase activity and are currently designated as B. amyloliquefaciens (Refs. 19 through 22). The petitioner also cites a scientific review article (Ref. 23) that states that the source organism for commercial preparations of <greek-a>-amylase from B. amyloliquefaciens was called B. subtilis prior to its current designation as B. amyloliquefaciens. With respect to the protease components of the petitioned **enzyme** preparations, the petitioner cites a statement in the same scientific review article (Ref. 23) that most bacterial protease preparations produced before 1960 were derived from B. amyloliquefaciens.

As FDA noted in the preamble to another final rule affirming an **enzyme** preparation as **GRAS** (58 FR 27197 at 27199, May 7, 1993), the taxonomic placement and name of an organism may change as a result of scientific advances. If internationally accepted rules of nomenclature are observed, references to a particular organism can be followed historically in the scientific literature. Thus, changes in the taxonomic placement of an organism should not affect the ability to identify scientific references to the organism, including scientific references to its toxigenicity, pathogenicity, or **use** in the production of food or enzymes.In reviewing the petition, FDA has evaluated whether the scientific information documenting pre-1958 food **use** of bacterially-derived carbohydrase and protease **enzyme** preparations pertains to carbohydrase and protease **enzyme** preparations from B. amyloliquefaciens. Although it is not possible to determine conclusively whether any one reference to B. subtilis in the scientific literature refers to the species now referred to as B. amyloliquefaciens, the totality of the scientific evidence supports a determination that some carbohydrase and some protease **enzyme** preparations that were described in scientific literature documenting their common **use** in food before 1958 as derived from B. subtilis were in fact derived from B. amyloliquefaciens. Therefore, the agency concludes that carbohydrase and protease **enzyme** preparations derived from B. amyloliquefaciens were in common **use** in food prior to January 1, 1958.

B. Corroborating Evidence of Safety

Because enzymes are highly efficient catalysts, they are needed in

only minute quantities to perform their function. When used in accordance with current good manufacturing practice (CGMP), the amounts added to food represent only a minute fraction of the total food mass. FDA estimates dietary **exposure** to **enzyme** preparations derived from B. subtilis or B. amyloliquefaciens at 200 mg/person/day (Ref. 24). This **estimate** is exaggerated because the agency used the total consumption of microbially-derived **enzyme** preparations in food as an approximation for the consumption of **enzyme** preparations derived from B. subtilis or B. amyloliquefaciens. Thus, the **estimate** relies on the worst-case assumption that all microbially-derived **enzyme** preparations that are consumed in food are derived from B. subtilis or B. amyloliquefaciens. This assumption is extremely conservative because there are numerous microbially-derived **enzyme** preparations that are **GRAS** for **use** in food (see, e.g., 21 CFR 184.1012, 184.1027, 184.1387, 184.1388, 184.1924, and 184.1985).

1. The **Enzyme** Components

Enzymes, including carbohydrase and protease enzymes in the **enzyme** preparations that are the subject of this document, are naturally occurring proteins that are ubiquitous in living organisms. A wide variety of enzymes has always been present in human food. Many naturally occurring enzymes remain active in unprocessed food and therefore are consumed as active enzymes. For example, active enzymes are present in fresh fruits and vegetables and are not inactivated unless the fruits or vegetables are cooked (Refs. 1 and 25).

Enzymes derived from microorganisms have been used as components of foods that have been safely consumed as part of the diet throughout human history (Ref. 26). For example, such common foods as bread and yogurt are produced using enzymes derived from microorganisms (Refs. 26 and 27).

The carbohydrase and protease enzymes in the **enzyme** preparations that are the subject of this document are substantially equivalent \2\ to carbohydrase and protease enzymes from other microorganisms that FDA has evaluated and found to be safe and that are routinely consumed as part of a normal diet in the United States. For example, FDA has affirmed the **use** of a mixed carbohydrase and protease **enzyme** preparation derived from Bacillus licheniformis is **GRAS** (see 21 CFR 184.1027). In addition, carbohydrases derived from various fungi (e.g., Rhizopus niveus, Rhizopus oryzae, and A. niger) are approved for **use** as secondary direct food additives (see 21 CFR 173.110, 173.130, and 173.120, respectively).

\2\ A 1996 report of the joint Food and Agriculture Organization and World Health Organization (FAO/WHO) consultation group (Ref. 28) stated that ``[s]ubstantial equivalence embodies the concept that if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e. the food or food component can be concluded to be as safe as the conventional food or food component). Account should be taken of any processing that the food or food component may undergo as well as the intended **use** and the intake by the population.'' As discussed more fully in FDA's proposal to amend the agency's regulations pertaining to substances that are generally recognized as safe (62 FR 18938 at 18944, April 17, 1997), international expert groups such as the FAO/WHO consultation group and the Organization for Economic Co-operation and Development (OECD) consultation group have recommended that the concept of ``substantial equivalence'' be applied to the safety assessment of foods and substances intentionally added to food.

000142

---

http://frwebgate3.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=708415209+0+0+0&W 03/02/2001

In general, issues relevant to a safety evaluation of proteins such as the **enzyme** component of an **enzyme** preparation are potential toxicity and allergenicity. Pariza and Foster (Ref. 1) note that very few toxic agents have enzymatic properties, and those that do (e.g., diphtheria toxin and certain enzymes in the venom of poisonous snakes) catalyze unusual reactions that are not related to the types of catalysis that are common in food processing and that are the subject of this document. Further, as the agency has noted in the context of guidance to industry regarding the safety assessment of new plant varieties, enzymes do not generally raise safety concerns (57 FR 22984 at 23000, May 29, 1992). Exceptions include enzymes that catalyze the formation of toxic substances or substances that are not ordinarily digested and metabolized. The catalytic activities of the enzymes that are the subject of this document are well known; they split proteins or carbohydrates into smaller subunits that are readily metabolized by the human body and that do not have toxic properties.

According to Pariza and Foster (Ref. 1), there have been no confirmed reports of allergies or primary irritations in consumers caused by enzymes used in food processing. There have been, however, some reports of allergies and primary irritations from skin contact with enzymes or inhalation of dust from concentrated enzymes (for example, proteases used in the manufacture of laundry detergents) (Refs. 29 through 31). These reports relate primarily to workers in production plants (Ref. 30) and are not relevant to an evaluation of

[[Page 19891]]

the safety of ingestion of such enzymes in food.

The 1977 report of the Select Committee on **GRAS** substances concerning the plant **enzyme** papain (Ref. 29) supports the view that the ingestion of an active protease at levels found in food products is not likely to affect the human gastrointestinal tract, where many proteases already exist at levels adequate to digest food:

In common with other proteolytic enzymes, papain digests the mucosa and musculature of tissues in contact with the active **enzyme** for an appreciable period. Because there is no food **use** of papain that could result in the **enzyme** preparation occurring in sufficient amount in foods to produce these effects, this property does not pose a dietary hazard.

FDA concludes that generally available and accepted data and information corroborate the safety of the **enzyme** components of the **enzyme** preparations that are the subject of this document by establishing that these **enzyme** components are identical or substantially equivalent to enzymes that are known to have been safely consumed in the diet for many years. FDA also concludes that generally available and accepted data and information corroborate that the **enzyme** components of the **enzyme** preparations that are the subject of this document are nontoxigenic and nonallergenic when ingested.

2. **Enzyme** Sources, Manufacturing Methods, and Processing Aids

Enzyme preparations used in food processing are usually not chemically pure; in addition to the **enzyme** component(s), they may contain other components derived from the production organism and the fermentation medium, residual amounts of processing aids, and substances added as stabilizers, preservatives, or diluents. The agency has concluded that the **enzyme** components of the carbohydrase and protease **enzyme** preparations derived from B. subtilis or B. amyloliquefaciens do not raise safety concerns; therefore, the remaining safety issue is whether other components of the **enzyme** preparations are toxic or raise other safety concerns.

a. Antibiotics. Some microorganisms are capable of producing antibiotics, which are a special class of metabolites that can inhibit the growth of, or kill, other microorganisms. Some microorganisms have genetic traits that make them resistant to one or more antibiotics such as penicillin, tetracycline, and kanamycin. These traits or markers are often located on plasmids (extrachromosomal pieces of deoxyribonucleic acid (DNA) that are easily transferred to other microorganisms in the environment (e.g., in the gastrointestinal tract). The presence of antibiotics in the food supply would be expected to favor the growth of microorganisms resistant to the antibiotic, and thus could accelerate the spread of antibiotic resistance among microorganisms, including human pathogens, rendering them resistant to therapy with antibiotic drugs. Therefore, experts have recommended that microbial-derived **enzyme** preparations that are intended for food **use** not contain clinically important antibiotics (Refs. 1 and 32).

Accordingly, FDA has evaluated the potential for carbohydrase or protease **enzyme** preparations derived from B. subtilis or B. amyloliquefaciens to contain antibiotics as contaminants derived from the bacterial source. Although Bacillus species are capable of producing a number of linear or cyclic polypeptide antibiotics following the exponential phase of growth as part of the process of spore formation (Ref. 33), the production of antibiotics can be repressed by selection of strains that produce low or undetectable levels of antibiotics as well as by strict control of the growth conditions. In addition, the **enzyme** preparations can be tested for the presence of antibiotic activity by routine methods (Ref. 34) to ensure that they do not contain antibiotics. Because of safety concerns about the presence of antibiotics in substances added to food, a condition of agency affirmation of **GRAS** status for the **enzyme** preparations that are the subject of this document is that the **enzyme** preparations not contain antibiotics.

b. Toxicity and pathogenicity. A published scientific review article (Ref. 23) states that Bacillus species, with the exception of the B. cereus group (which does not include B. subtilis or B. amyloliquefaciens) do not produce toxins. Another published scientific review article on the safety of B. subtilis and B. amyloliquefaciens (Ref. 35) notes that B. subtilis is consumed in large quantities in the Japanese food natto. Further, according to a monograph on microbial enzymes that was prepared under the auspices of the agency-initiated review of **GRAS** substances conducted during the 1970's, there had been no reported problems of pathogenicity or toxicity with **enzyme** preparations derived from B. subtilis for **use** in food as of the time of that review (Ref. 12).

More recently, de Boer and Diderichsen (Ref. 35) searched the scientific literature for references that might implicate B. subtilis or B. amyloliquefaciens as a cause of human disease. These authors characterized B. subtilis as an opportunistic microorganism with no pathogenic potential to humans. Although they reported that cultures from some patients with opportunistic infections have revealed the presence of B. subtilis along with other microorganisms, they attributed the presence of B. subtilis in these cultures to the virtual ubiquity of this microorganism in the environment (e.g., B. subtilis commonly occurs in the soil and can be isolated in the home environment from sites such as the kitchen and bathroom). De Boer and Diderichsen also noted that only patients treated with immunosuppressive drugs appeared to be susceptible to such infections. Moreover, viable cells, which are not present in finished **enzyme** preparations, would be a prerequisite for any opportunistic infection in an immunocompromised patient. De Boer and Diderichsen also reported that their search for references on B. amyloliquefaciens infections revealed no such cases.

As discussed in section IV.A.2 of this document, any references to B. amyloliquefaciens prior to the late 1980's would be expected to occur under the name B. subtilis.

A few reports have implicated B. subtilis as a potential source of food poisoning when present as a contaminant in food (Refs. 36 and 37). However, a particular strain of virtually any microorganism may, under certain circumstances, mutate to become an opportunistic pathogen. Therefore, FDA considered these reports in the context of: (1) The information summarized in the monograph on microbial enzymes (Ref. 12); (2) the scientific review article describing Bacillus species other than those in the B. cereus group as nontoxigenic (Ref. 23); (3) the documented consumption of B. subtilis bacteria in the Japanese food natto (Ref. 35); and (4) the characterization by de Boer and Diderichsen of B. subtilis as an opportunistic microorganism with no pathogenic potential to humans (Ref. 36). Based on this information, FDA concludes that nontoxigenic and nonpathogenic strains of B. subtilis are widely available and have been safely used in a variety of food applications. Because an **enzyme** preparation derived from a toxigenic or pathogenic source would not be **GRAS**, a condition of agency affirmation of **GRAS** status for the **enzyme** preparations that are the subject of this document is that the bacterial strains used as a source of these **enzyme** preparations be nontoxigenic and nonpathogenic.

c. Manufacturing methods and processing aids. **Enzyme** preparations

[[Page 19892]]

that are manufactured in accordance with CGMP using the methods described in section III.B of this document meet the general requirements and additional requirements in the monograph on **enzyme** preparations in the Food Chemicals Codex, 4th ed. (Ref. 3). Such **enzyme** preparations are produced using substances that are acceptable for **use** in foods and under culture conditions that ensure a controlled fermentation, thus preventing the introduction of extraneous microorganisms that could be the source of toxic materials and other toxic substances (Ref. 3).

FDA concludes that generally available and accepted data and information corroborate the safety of carbohydrase and protease **enzyme** preparations derived from nontoxigenic and nonpathogenic strains of B. subtilis or B. amyloliquefaciens and manufactured in accordance with CGMP by establishing that any added substances or impurities derived from the **enzyme** source or introduced during the manufacturing of such **enzyme** preparations would not be expected to present health concerns.

V. Comments

FDA received seven comments in response to the filing notice and none in response to the amendment notices. Of these, FDA received two comments from food manufacturers, two from trade associations, one from a manufacturer of enzymes for **use** in animal feed, one from a pharmaceutical manufacturer, and one from a consumer group. Six comments supported the petition for **GRAS** affirmation, stating that the **enzyme** preparations included in the petition have a long history of **use** in foods such as cheese, bread, and corn syrup.

One comment stated that B. subtilis has a history of **use** in animal feed and requested **GRAS** affirmation for this **use**. However, the petition is for the **use** of certain **enzyme** preparations in human food, and not in animal feed. Therefore, the agency finds that this comment is not relevant to the petition.

One comment asserted that **enzyme** preparations should not be considered **GRAS**. The comment further asserted that the **use** of **enzyme**

000145

preparations should be declared on the label of foods and that consumers should be warned about hazards inherent in their **use**. The comment stated that **enzyme** preparations are rarely purified to any significant degree and contain a variety of cellular constituents and metabolic debris. The comment further argued that, although **enzyme** preparations are used at low levels and are inactivated after the treatment of food, they may elicit allergic reactions and other biological activities which could be detrimental to human health. In support of this statement, the comment cited a published scientific article (Ref. 38) that reported that **enzyme** preparations from B. subtilis caused temporary weight loss and aggravated infection in mice when injected into the abdominal cavity and caused hemolysis and hemagglutination of sheep erythrocytes in in vitro studies.

FDA has evaluated the comment and the article it cited. For the following two reasons, FDA concludes that the study cited by the comment is not relevant to food uses of the bacterial **enzyme** preparations that are the subject of this document.

First, the paper did not identify the composition of the B. subtilis **enzyme** preparations tested. The preparations were intended for **use** in laundry detergents; such nonfood grade **enzyme** preparations need not conform to specifications for **enzyme** preparations used in food processing. For example, nonfood grade **enzyme** preparations may include processing aids that are not acceptable for food **use**. Because of such differences, the results from the testing of laundry cleaning **enzyme** preparations have little value in the safety assessment of food-processing **enzyme** preparations.

Second, in the cited study, adverse effects were observed in mice after the intraperitoneal administration of B. subtilis autolysates. However, **exposure** to **enzyme** preparations in food occurs by ingestion and not by injection. The difference in the route of **exposure** is particularly significant for assessing the significance of immunological effects. With intraperitoneal administration, the components of the immune system are directly exposed to a high **level** of the test compound. This contrasts with **exposure** to **enzyme** preparations in food, whereby low levels of the **enzyme** preparations are ingested and undergo hydrolysis by digestive enzymes before any interaction with the immune system. Pariza and Foster (Ref. 1) note that there are no confirmed reports of allergic reactions in consumers caused by enzymes used in food processing.

Moreover, a report of the Joint Food and Agriculture Organization/ World Health Organization Expert Committee on Food Additives (JECFA) corroborates the safety of food uses of **enzyme** preparations from B. subtilis (Ref. 39). This report concluded that results from a 90-day feeding study in rats showed no adverse effects. The test diet was meat protein-based and supplemented with a protease **enzyme** preparation from B. subtilis at a 1-percent **level** (equivalent to approximately 1 gram of **enzyme** preparation per kilogram of body weight per day). This **level** is more than 300 times greater than the highest **level** that would be expected in the human diet (200 mg/person/day, or 3.3 mg/kg body weight per day for a 60 kg person), as estimated in section IV.B of this document.

With respect to the comment's assertion that **enzyme** preparations should be declared on the label of foods in which they are used, the agency notes that under certain circumstances, applicable regulations already require **use** of an **enzyme** preparation in a food to be declared on the label, depending upon the nature of the **enzyme** preparation's **use** and technical effect in the food. Section 403(i)(2) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 343(i)(2)) requires that all ingredients of multi-ingredient foods be listed on the label of the food. By regulation, FDA has exempted certain ingredients that are used

000146

only as processing aids from this requirement. Section 101.100(a)(3)(ii)(a) and (a)(3)(ii)(c) (21 CFR 101.100(a)(3)(ii)(a) and (a)(3)(ii)(c)) provides an exemption from the ingredient listing requirement for processing aids that are added to a food for their technical or functional effect during processing, but are either removed from the food before packaging or are present in the finished food at insignificant levels and do not have any technical or functional effect in the finished food. Although many **enzyme** preparations are used as processing aids in food (e.g., amylase preparations used in the manufacture of glucose syrup and protease preparations used in the manufacture of protein hydrolysates), other **enzyme** preparations that are added during processing (e.g., protease preparations used in tenderizing meat) are not processing aids as defined in Sec. 101.100(a)(3)(ii) because they remain active in the finished food product. For example, enzymes used in the manufacture of swiss and cheddar cheese remain active in the finished cheese, enhancing body, flavor, and aroma (49 FR 29242, July 19, 1984). Because such effects in the finished food remove the enzymes from the ingredient listing exemption for processing aids in Sec. 101.100(a)(3)(ii)(c), the **use** of such enzymes must be declared on the label. Therefore, whether a label declaration is needed for the **use** of an **enzyme** preparation in a food will depend upon its function and effect in the food.

[[Page 19893]]

VI. Conclusions

The petitioner has provided generally available evidence demonstrating that carbohydrase and protease **enzyme** preparations from B. subtilis were in common **use** in food prior to 1958. FDA has determined, under Sec. 170.30(a) and (c)(1), that this information provides an adequate basis upon which to conclude that the safety of these **enzyme** preparations for **use** in food is generally recognized among the community of experts qualified by scientific training and experience to evaluate the safety of food ingredients.

The petitioner has also provided generally available evidence demonstrating that the bacterium now known as B. amyloliquefaciens was formerly included within the B. subtilis classification. Based on its analysis of the data submitted, the agency concludes that the evidence of common **use** in food pertains to carbohydrase and protease **enzyme** preparations from the bacterium now known as B. amyloliquefaciens as well as to carbohydrase and protease **enzyme** preparations from B. subtilis.

This evidence of common **use** in food prior to 1958 is corroborated by information that the enzymes themselves and the sources from which they are derived are nontoxic and nontoxicogenic, and that manufacturing will not introduce impurities that would adversely affect the safety of the finished **enzyme** preparations. Moreover, the carbohydrase and protease **enzyme** preparations from B. subtilis and B. amyloliquefaciens are substantially equivalent to enzymes naturally present in foods that have been safely consumed in the human diet for many years.

Having evaluated the information in the petition, along with other available information related to the **use** of these **enzyme** preparations, the agency concludes that carbohydrase **enzyme** preparation and protease **enzyme** preparation derived from either B. subtilis or B. amyloliquefaciens are **GRAS** under conditions of **use** consistent with CGMP. The agency is basing its conclusion on evidence of a substantial history of safe consumption of the **enzyme** preparations in food by a

000147

http://frwebgate3.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=708415209+0+0+0&W 03/02/2001

significant number of consumers prior to 1958, corroborated by the other evidence summarized in section IV.B of this document.

FDA is affirming that the **use** of these bacterially-derived carbohydrase and protease **enzyme** preparations in food is **GRAS** with no limits other than CGMP (21 CFR 184.1(b)(1)). To clarify the identity of each **enzyme** preparation, the agency is including in Secs. 184.1148(a) and 184.1150(a) the EC numbers of the enzymes that supply the characterizing **enzyme** activities of each preparation. In order to make clear that the affirmation of the **GRAS** status of these **enzyme** preparations is based on the evaluation of specific uses, the agency is including in Secs. 184.1148(c) and 184.1150(c) the technical effect and the specific substances on which each **enzyme** preparation acts, although the data show no basis for a potential risk from any foreseeable **use** of these **enzyme** preparations.

For simplicity, FDA is affirming the **GRAS** status of both carbohydrase **enzyme** preparations in a single combined regulation that describes the source of the **enzyme** as B. subtilis or B. amyloliquefaciens, rather than affirming the **GRAS** status of carbohydrase derived from B. subtilis separately from that of carbohydrase derived from B. amyloliquefaciens. Likewise, FDA is affirming the **GRAS** status of both protease **enzyme** preparations in a single combined regulation that describes the source of the **enzyme** as B. subtilis or B. amyloliquefaciens.

To ensure that the **enzyme** preparations are of suitable purity for **use** in food, FDA is including in the regulations the general requirements and additional requirements for **enzyme** preparations in the monograph ``**Enzyme** Preparations'' in the Food Chemicals Codex, 4th ed. (1996) as general specifications for these **enzyme** preparations. Furthermore, to ensure that the **use** of these **enzyme** preparations does not promote the development of antibiotic resistance, the agency is specifying that the **enzyme** preparations must be free of antibiotic activity as determined by a suitable method (e.g., the method described in Ref. 34).

VII. Environmental Considerations

The agency has determined under 21 CFR 25.32(f) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

VIII. Analysis for Executive Order 12866

FDA has examined the impacts of this final rule under Executive Order 12866. Executive Order 12866 directs Federal agencies to assess the costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety effects; distributive impacts; and equity). According to Executive Order 12866, a regulatory action is significant if it meets any one of a number of specified conditions, including having an annual effect on the economy of $100 million, adversely affecting in a material way a sector of the economy, competition, or jobs, or raising novel legal or policy issues. FDA finds that this final rule is not a significant regulatory action as defined by Executive Order 12866. In addition, the agency has determined that this final rule is not a major rule for the purpose of Congressional review.

000148

The primary benefit of this action is to remove uncertainty about the regulatory status of the petitioned substances. No compliance costs are associated with this final rule because no new activity is required

and no current or future activity is prohibited by this rule.

IX. Regulatory Flexibility Analysis

FDA has examined the impacts of this final rule under the Regulatory Flexibility Act. The Regulatory Flexibility Act (5 U.S.C. 601-612) requires agencies to consider alternatives that would minimize the economic impact of their regulations on small entities. No compliance costs are associated with this final rule because no new activity is required and no current or future activity is prohibited. Accordingly, under the Regulatory Flexibility Act (5 U.S.C. 605(b)), the agency certifies that this final rule will not have a significant economic impact on a substantial number of small entities.

X. Paperwork Reduction Act of 1995

This final rule contains no collections of information. Therefore, clearance by the Office of Management and Budget under the Paperwork Reduction Act of 1995 is not required.

XI. Effective Date

As this rule recognizes an exemption from the food additive definition in the Federal Food, Drug, and Cosmetic Act, and from the approval requirements applicable to food additives, no delay in effective date is required by the Administrative Procedure Act, 5 U.S.C. 553(d). The rule will therefore be effective immediately (5 U.S.C. 553(d)(1)).

XII. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons

[[Page 19894]]

between 9 a.m. and 4 p.m., Monday through Friday.

1. Pariza, M. W., and E. M. Foster, ``Determining the Safety of Enzymes Used in Food Processing,'' Journal of Food Protection, vol. 46, pp. 453-468, 1983.

2. Scott, D., ``Enzymes, Industrial,'' Encyclopedia of Chemical Technology, edited by H. F. Mark et al., John Wiley and Sons, New York, 3d ed., vol. 9, pp. 173-224, 1978.

3. Monograph on ``Enzyme Preparations,'' Food Chemicals Codex, National Academy Press, Washington, DC, 4th ed., pp. 131 and 133-134, 1996.

4. Underkofler, L. A., and W. J. Ferracone, ``Commercial Enzymes--Potent Catalyzers that Promote Quality,'' Food Engineering, vol. 29, pp. 123, 125-126, 130, and 133, 1957.

5. Underkofler, L. A., R. R. Barton, and S. S. Rennet, ``Microbiological Process Report--Production of Microbial Enzymes and Their Applications,'' Applied Microbiology, vol. 6, pp. 212-221, 1958.

6. Beckhorn, E. J., M. D. Labee, and L. A. Underkofler, ``Production and Use of Microbial Enzymes for Food Processing,'' Journal of Agricultural and Food Chemistry, vol. 13, pp. 30-34, 1965.

7. Comments of Ad Hoc Enzyme Technical Committee regarding FDA's draft final regulations, entitled ``Enzymes Proposed for Affirmation as GRAS,'' with a letter dated December 21, 1984, from Roger D.

000149

Middlekauff, Ad Hoc **Enzyme** Technical Committee, to Kenneth A. Falci, FDA.

8. Response of the **Enzyme** Technical Association to the letter dated December 13, 1985, of Lawrence Lin regarding GRASP 3G0016, received February 18, 1986.

9. Smythe, C. V., ``Microbiological Production of Enzymes and Their Practical Applications,'' Economic Botany, vol. 5, pp. 126-144, 1951.

10. Reed, G., ``Enzymes, Industrial,'' Encyclopedia of Chemical Technology, edited by R. E. Kirk and D. F. Othmer, Interscience Encyclopedia, Inc., New York, 1st supplemental vol., pp. 294-312, 1957.

11. Fogarty, W. M., editor, Microbial Enzymes and Biotechnology, Applied Science Publishers, New York, NY, pp. 1-11, 22-25, 34-35, 111-113, 115-118, 162-173, 259-260, 282-286, 1983.

12. Rogers, R.W., ``Monograph on Microbial Enzymes,'' Informatics, Inc., vol. 1, pp. 1-16, 1977.

13. Reed, G., ``Industrial Enzymes--Now Speed Natural Processes,'' Food Engineering, vol. 24, pp. 105-109, 1952.

14. Response of the **Enzyme** Technical Association to the letter dated June 26, 1986, of Lawrence J. Lin regarding GRASP 3G0016, received with a letter dated October 3, 1986, from Roger D. Middlekauff of the **Enzyme** Technical Association, to Lawrence J. Lin, FDA.

15. Letter dated August 17, 1995, from Gary L. Yingling, **Enzyme** Technical Association, to Alan M. Rulis, FDA.

16. Letter dated April 16, 1996, from Alice J. Caddow, **Enzyme** Technical Association, to Linda Kahl, FDA.

17. Priest, F. G., M. Goodfellow, L. A. Shute, and R. C. W. Berkeley, ``Bacillus amyloliquefaciens sp. nov., nom. rev.,'' International Journal of Systematic Bacteriology, vol. 37, pp. 69-71, 1987.

18. Priest, F. G., M. Goodfellow, and C. Todd, ``A numerical classification of the genus Bacillus,'' Journal of General Microbiology, vol. 134, pp. 1847-1882, 1988.

19. Baptist, J. N., M. Mandel, and R. J. Gherna, ``Comparative zone electrophoresis of enzymes in the genus Bacillus,'' International Journal of Systematic Bacteriology, vol. 28, pp. 229-244, 1978.

20. Gordon, R. E., W. C. Haynes, and C. Hor-Nay Pang, ``The genus Bacillus,'' U.S. Department of Agriculture, 1973.

21. Welker, N. E., and L. L. Campbell, ``Unrelatedness of Bacillus amyloliquefaciens and Bacillus subtilis,'' Journal of Bacteriology, vol. 94, pp. 1124-1130, 1967.

22. Welker, N. E., and L. L. Campbell, ``Comparison of the alpha-amylase of Bacillus amyloliquefaciens and Bacillus subtilis,'' Journal of Bacteriology, vol. 94, pp. 1131-1135, 1967.

23. Aunstrup, K., ``Production, Isolation, and Economics of Extracellular Enzymes,'' Applied Biochemistry and Bioengineering, vol. 2, pp. 27-69, 1979.

24. Memorandum dated June 6, 1985, from John Modderman, Food Additive Chemistry Evaluation Branch, to L. Lin, **GRAS** Review Branch. ``Enzymes proposed for **GRAS** affirmation based on history of **use**.''

25. De Becze, G. I., ``Food Enzymes,'' Critical Reviews in Food Technology, vol. 1, pp. 479-518, 1970.

26. Phaff, H. J., M. W. Miller, and E. M. Mrak, ``The Life of Yeasts, Their Nature, Activity, Ecology, and Relation to Mankind,'' Harvard University Press, Cambridge, MA, pp. 1-6, 133-149, 1966.

27. Wilcox, G. ``Manufacture of Yogurt'' from ``Eggs Cheese and Yogurt Processing,'' Noyes Data Corp., p. 269, 1971.

000150

28. ``Biotechnology and Food Safety,'' Food and Agriculture Organization, Rome, 1996.
29. ``Evaluation of the Health Aspects of Papain as a Food Ingredient,'' Select Committee on GRAS Substances, Washington, DC, available through U.S. Department of Commerce, National Technical Information Service, Order No. PB-274-174, 1977.
30. Fulwiler, R. D., ``Detergent Enzymes--An Industrial Hygiene Challenge,'' American Industrial Hygiene Association Journal, vol. 32, pp. 73-81, 1971.
31. ``Enzyme-containing Laundering Compounds and Consumer Health,'' National Research Council/National Academy of Sciences, National Technical Information Service, Washington, DC, Order No. PB-204-118, 1971.
32. Reanney, D., ``Extrachromosomal Elements as Possible Agents of Adaptation and Development,'' Bacteriological Reviews, vol. 40, pp. 552-590, 1976.
33. Fogarty, W. M., P. J. Griffine, and A. M. Joyce, ``Enzymes of Bacillus species--Part 1,'' Process Biochemistry, vol. 9, pp. 11-13, 15, 17-18, 24, 1974.
34. ``Determination of Antibiotic Activity,'' Compendium of Food Additive Specifications, vol. 2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992.
35. De Boer, A. S., and B. Diderichsen, ``On the Safety of Bacillus subtilis and Bacillus amyloliquefaciens: A Review,'' Applied Microbiology and Biotechnology, vol. 36, pp. 1-4, 1991.
36. Sneath, P. H. A., ``Endospore-forming Gram-Positive Rods and Cocci,'' in Bergey's Manual of Systematic Bacteriology, edited by P. H. A. Sneath et al., Williams & Wilkins, vol. 2, pp. 1104-1139, Baltimore, 1986.
37. Gilbert, R. J, P. C. B. Turnbull, J. M. Parry, and J. M. Kramer, ``Bacillus cereus and other Bacillus Species: Their Part in Food Poisoning and other Clinical Infections,'' in The Endospore-forming Bacteria, edited by Berkeley and Goodfellow, Academic Press, London, pp. 297-314, 1981.
38. Dubos, R., ``Toxic Factors in Enzymes Used in Laundry Products,'' Science, vol. 173, pp. 259-260, 1971.
39. ``Toxicological Evaluation of Some Enzymes, Modified Starches, and Certain Other Substances,'' 15th report of the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives, WHO Technical Report Series, FAO Nutrition Meetings Report Series, pp. 3-10, 1972.

List of Subjects in 21 CFR Part 184

Food additives, Food ingredients, Incorporation by reference.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, and redelegated to the Director, Center for Food Safety and Applied Nutrition, 21 CFR part 184 is amended as follows:

PART 184--DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

1. The authority citation for 21 CFR part 184 continues to read as follows:

Authority: 21 U.S.C. 321, 342, 348, 371.

2. Section 184.1148 is added to subpart B to read as follows:

Sec. 184.1148 Bacterially derived carbohydrase **enzyme** preparation.

(a) Bacterially derived carbohydrase **enzyme** preparation is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic and nontoxigenic strain of Bacillus subtilis or B. amyloliquefaciens. The preparation is characterized by the presence of the enzymes <greek-a>-amylase (EC 3.2.1.1) and <greek-b>-glucanase (EC 3.2.1.6), which catalyze the hydrolysis of O-glycosyl bonds in carbohydrates.

(b) The ingredient meets the general requirements and additional requirements in the monograph on **enzyme** preparations in the Food Chemicals Codex, 4th ed. (1996), pp. 128-135, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are

[[Page 19895]]

available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol Street, NW., Suite 700, Washington, DC. In addition, antibiotic activity is absent in the **enzyme** preparation when determined by an appropriate validated method such as the method ``Determination of antibiotic activity'' in the Compendium of Food Additive Specifications, vol. 2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992. Copies are available from Berman Associates, 4611-F Assembly Dr., Lanham, MD 20706, or from The United Nations Bookshop, General Assembly Bldg., rm. 32, New York, NY 10017, or by inquiries sent to ``http://www.fao.org''. Copies may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC.

(c) In accordance with Sec. 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as **GRAS** as a direct food ingredient is based upon the following current good manufacturing practice conditions of **use**:

(1) The ingredient is used as an **enzyme** as defined in Sec. 170.3(o)(9) of this chapter to hydrolyze polysaccharides (e.g., starch).

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

3. Section 184.1150 is added to subpart B to read as follows:

Sec. 184.1150 Bacterially-derived protease **enzyme** preparation.

(a) Bacterially derived protease **enzyme** preparation is obtained from the culture filtrate resulting from a pure culture fermentation of a nonpathogenic and nontoxigenic strain of Bacillus subtilis or B. amyloliquefaciens. The preparation is characterized by the presence of the enzymes subtilisin (EC 3.4.21.62) and neutral proteinase (EC 3.4.24.28), which catalyze the hydrolysis of peptide bonds in proteins.

(b) The ingredient meets the general requirements and additional requirements in the monograph on **enzyme** preparations in the Food Chemicals Codex, 4th ed. (1996), pp. 128-135, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321,

Washington, DC, or at the Office of the Federal Register, 800 North Capitol Street, NW., Suite 700 Washington, DC. In addition, antibiotic activity is absent in the **enzyme** preparation when determined by an appropriate validated method such as the method ``Determination of antibiotic activity'' in the Compendium of Food Additive Specifications, vol. 2, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Organization of the United Nations, Rome, 1992. Copies are available from Bernan Associates, 4611-F Assembly Dr., Lanham, MD 20706, or from The United Nations Bookshop, General Assembly Bldg., rm. 32, New York, NY 10017, or by inquiries sent to ``http://www.fao.org''. Copies may be examined at the Center for Food Safety and Applied Nutrition's Library, 200 C St. SW., rm. 3321, Washington, DC.

(c) In accordance with Sec. 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as **GRAS** as a direct food ingredient is based upon the following current good manufacturing practice conditions of **use**:

(1) The ingredient is used as an **enzyme** as defined in Sec. 170.3(o)(9) of this chapter to hydrolyze proteins or polypeptides.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

Dated: March 26, 1999.

L. Robert Lake,

Director, Office of Policy, Planning and Strategic Initiatives, Center for Food Safety and Applied Nutrition.

[FR Doc. 99-10011 Filed 4-22-99; 8:45 am]

BILLING CODE 4160-01-F

000153

Appendix C

APPENDIX C

Panel Members for Transglutaminase GRAS

February 15, 2001

Dr. Jay Goodman
Michigan State University
B-440 Life Sciences Building
East Lansing, MI 48824-1020

Dr. Philip Portoghese
University of Minnesota
308 Harvard St., SE
Minneapolis, MN 55455

Dr. William Waddell
14300 Rose Wycombe Lane
Prospect, KY 40059

Dr. Bernard Wagner
38 Mohawk Road
Short Hills, NJ 07078

Dr. Vernon Young
Massachusetts Institute of Technology
77 Massachusetts Avenue, Room E17-434
Cambridge, MA 02139

Panel Coordinator:

Dr. Bruce Bernard, President
SRA International, Inc.
1920 L Street, NW, Suite 420
Washington, D.C. 20036

SRA International, Inc.

3 January 2002

Dr. Linda Kahl
Office of Food Additive Safety (HFS-206)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Re: GRAS Notification for Transglutaminase

Dear Dr. Kahl:

On behalf of Ajinomoto USA (AJ), SRA International Inc. (SRA) is providing this Generally Recognized as Safe (GRAS) Exemption Claim. AJ is hereby notifying FDA that the use of transglutaminase in food (in general) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FFDCA) because AJ has determined that such use is GRAS. Specifically, with regard to this claimed exemption:

1. Name and Address of Notifier

   Ajinomoto USA
   1120 Connecticut Avenue, NW Suite 416
   Washington, DC 20036

2. Common/Usual Name of the Notified Substance

   Transglutaminase

3. Applicable Conditions of Use

   a. Applicable Foods
   The notified substance, transglutaminase, may be added to any protein-containing food to bring about the cross-linking protein residues.

   b. Levels of Use
   The notified substance is to be employed at the lowest levels necessary to achieve the desired technical effects (i.e. governed by Good Manufacturing Practices (GMP).

1920 L Street, N.W., Suite 420, Washington, D.C. 20036
Telephone: (202) 728-1400 Telefax: (202) 331-3393

c. Desired Technical Effect
The sole effect of the notified substance is to cross-link glutamine and lysine residues through acyl-transfer of the γ-carboxyamide group of protein). As the number of cross-linked glutamine-lysine residues increase, changes occur in the size and structure of the food proteins present resulting in a meaningful and measurable modification in some physical properties of the food (e.g. breaking strength, texture, moisture retention). These modified physical properties translate to changes in sensory attributes (e.g., appearance).

d. Consuming Populations
The notified substance may be consumed by all subgroups within the U.S.

4. Basis for GRAS Determination

The use of the notified substance in all protein containing foods consistent with GMP is GRAS through scientific procedures.

5. Availability of Data and Information

The data and information that are the basis for the notifier's GRAS determination are available for the FDA to review and copy during reasonable times at the offices of SRA International, Inc. 1920 L Street, N.W. Suite 420, Wash. DC, 20036 or will be sent to FDA upon request.

Sincerely,

Bruce K. Bernard, Ph.D.
President, SRA International Inc.
Authorized Representative for
Ajinomoto USA

BKB/km
CC: AJ (USA)

SRA International, Inc.

3 January 2002

Dr. Linda Kahl
Office of Food Additive Safety (HFS-206)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Re: GRAS Notification for Transglutaminase

Dear Dr. Kahl:

On behalf of Ajinomoto USA (AJ), SRA International Inc. (SRA) is providing this Generally Recognized as Safe (GRAS) Exemption Claim. AJ is hereby notifying FDA that the use of transglutaminase in food (in general) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FFDCA) because AJ has determined that such use is GRAS. Specifically, with regard to this claimed exemption:

1. Name and Address of Notifier

   Ajinomoto USA
   1120 Connecticut Avenue, NW Suite 416
   Washington, DC 20036

2. Common/Usual Name of the Notified Substance

   Transglutaminase

3. Applicable Conditions of Use

   a. Applicable Foods
   The notified substance, transglutaminase, may be added to any protein-containing food to bring about the cross-linking protein residues.

   b. Levels of Use
   The notified substance is to be employed at the lowest levels necessary to achieve the desired technical effects (i.e. governed by Good Manufacturing Practices (GMP).

1920 L Street, N.W., Suite 420, Washington, D.C. 20036
Telephone: (202) 728-1400 Telefax: (202) 331-3393

c. Desired Technical Effect
The sole effect of the notified substance is to cross-link glutamine and lysine residues through acyl-transfer of the γ-carboxyamide group of protein). As the number of cross-linked glutamine-lysine residues increase, changes occur in the size and structure of the food proteins present resulting in a meaningful and measurable modification in some physical properties of the food (e.g., breaking strength, texture, moisture retention). These modified physical properties translate to changes in sensory attributes (e.g., appearance).

d. Consuming Populations
The notified substance may be consumed by all subgroups within the U.S.

4. Basis for GRAS Determination

The use of the notified substance in all protein containing foods consistent with GMP is GRAS through scientific procedures.

5. Availability of Data and Information

The data and information that are the basis for the notifier's GRAS determination are available for the FDA to review and copy during reasonable times at the offices of SRA International, Inc. 1920 L Street, N.W. Suite 420, Wash. DC 20036 or will be sent to FDA upon request.

Sincerely,

Bruce K. Bernard, Ph.D.
President, SRA International Inc.
Authorized Representative for
Ajinomoto USA

BKB/km
CC: AJ (USA)

SRA International, Inc.

3 January 2002

Dr. Linda Kahl
Office of Food Additive Safety (HFS-206)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Re: GRAS Notification for Transglutaminase

Dear Dr. Kahl:

On behalf of Ajinomoto USA (AJ), SRA International Inc. (SRA) is providing this Generally Recognized as Safe (GRAS) Exemption Claim. AJ is hereby notifying FDA that the use of transglutaminase in food (in general) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FFDCA) because AJ has determined that such use is GRAS. Specifically, with regard to this claimed exemption:

1. Name and Address of Notifier

   Ajinomoto USA
   1120 Connecticut Avenue, NW Suite 416
   Washington, DC 20036

2. Common/Usual Name of the Notified Substance

   Transglutaminase

3. Applicable Conditions of Use

   a. Applicable Foods
      The notified substance, transglutaminase, may be added to any protein-containing food to bring about the cross-linking protein residues.

   b. Levels of Use
      The notified substance is to be employed at the lowest levels necessary to achieve the desired technical effects (i.e. governed by Good Manufacturing Practices (GMP).

1920 L Street, N.W., Suite 420, Washington, D.C. 20036
Telephone: (202) 728-1400 Telefax: (202) 331-3393

c. Desired Technical Effect
The sole effect of the notified substance is to cross-link glutamine and lysine residues through acyl-transfer of the γ-carboxyamide group of protein). As the number of cross-linked glutamine-lysine residues increase, changes occur in the size and structure of the food proteins present resulting in a meaningful and measurable modification in some physical properties of the food (e.g. breaking strength, texture, moisture retention). These modified physical properties translate to changes in sensory attributes (e.g., appearance).

d. Consuming Populations
The notified substance may be consumed by all subgroups within the U.S.

4. Basis for GRAS Determination

The use of the notified substance in all protein containing foods consistent with GMP is GRAS through scientific procedures.

5. Availability of Data and Information

The data and information that are the basis for the notifier's GRAS determination are available for the FDA to review and copy during reasonable times at the offices of SRA International, Inc. 1920 L Street, N.W. Suite 420, Wash. DC 20036 or will be sent to FDA upon request.

Sincerely,

Bruce K. Bernard, Ph.D.
President, SRA International Inc.
Authorized Representative for
Ajinomoto USA

BKB/km
CC: AJ (USA)

SUBMISSION END

000162

[redacted]

Reference List for Industry Submission, GRN 000095

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000081 - 000085	Lee, H.G; Lanier, T.C.; Hamann, D.D.; Knopp, J.A.	Transglutaminase Effects on Low Temperature Gelation of Fish Protein Sols	1997	Journal of Food Science	Volume 62, Number 1, pgs 20-24
000086 - 000089	Kuraishi, Chiya; Sakamoto, Jiro; Yamazaki, Katsutoshi; Susa, Yasuyuki; Kuhara, Chiho; Soeda, Takahiko	Production of Restructured Meat using Micriobial Transglutaminase without Salt or Cooking	1997	Journal of Food Science	Volume 62, Number 3, pgs 488-490, 515

NA- Not applicable

SRA International, Inc.

AM

FAX

To: Lane Rightberger PhD **From:** Bruce Bernard

Company:

Fax: ~~[illegible]~~ 202. 418. 3131 **Date:** 2 April 02

Phone: 202. 418 3032 **Pages:** 35

Re: CBAS NOT. # 0000 95 **CC:**

☐ **Urgent** ☒ **For Review** ☐ **Please Comment**

Comments:

Per your request.
Thank you.

(b)(6)

P.S. Please confirm receipt.

1920 L Street, N.W., Suite 420, Washington, D.C. 20036
Telephone: (202) 728-1400 Telefax: (202) 331-3393

SRA International, Inc.

2 April 2002

Lane Highbarger, Ph.D.
CFSAN, FDA, HFS –255
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: FDA GRAS Notification Number 000095
Transglutaminase from Streptoverticillium mobaraense

Dear Dr. Highbarger:

Per our 12 February 2002 telephone conversation, there were four (4) areas for which the U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (FDA, CFSAN) was seeking additional information. We have previously provided the requested information for one of these items. Enclosed please find information on the remaining three (3) items.

By way of summary, below please find an enumeration of the four (4) items together with a short explanation of their disposition.

ITEMS IDENTIFIED BY THE AGENCY

1. **Typographical Error**

Subject: The Agency noted typographical errors on p.23 of our submission (i.e. use of g [grams] instead of mg [milligrams]).

Resolution: Page 23 was corrected and the revised version received by the Agency on 12 February. It is our understanding that this item is resolved.

1920 L Street, NW, Suite 420, Washington, DC 20036
Telephone: (202) 728 1400 Telefax: (202) 331-3393

2. **Nature of the Added Peptide**

Subject: The Agency would like more information provided regarding the nature of the peptide that is added to the 'revised process' (employed for the oxygen-stable transglutaminase only).

Resolution: A variety of information is provided as an attachment to this letter (Appendix A).

Name and Manufacturer

The additive is known as 'DMV Glutamine Peptide' and is marketed by DMV International Nutritionals (1712 Daltown Plaza, Frasar, NY. 607.746.6473). The FDA knows this company.

Current Use in Human Food

It is currently used in a variety of nutritional products including clinical nutrition, sports and functional foods. In point of fact, DMV Glutamine Peptide is used in the enteral nutrition product "Novasource" manufactured by Novartis (Appendix A, A1).

Regulatory Status

EU Regulations:
Reviewed in 1996 (MAFF)/UK – "Glutamine peptide is not considered a novel food" (Appendix A, A1).

US Regulations:
21 CFR § 184.1553 – peptones (protein hydrolysates) are Generally Recognized as Safe (GRAS) (Appendix A, A2).

Food Chemicals Codex (Fourth Ed. and First Supplement to Fourth Ed.):
DVM Glutamine Peptide meets all the Codex requirements for a hydrolyzed protein (Appendix A, A3-A10).

Manufacturing information

Process:
Wheat protein is hydrolyzed using both acidification and enzymatic processes. A process flow diagram is provided in Appendix A (A11).

Certificate of Manufacture:
The manufacturing process complies will all FDA food regulations (Appendix A, A12).

Absence of GMO (Certificate):
The peptide and all ingredients employed are produced without the use of genetically modified organisms (Appendix A, A13).

Product Characterization

Hydrolysis characteristics:
Analysis of the product demonstrates that the average molecular weight is 770D, the degree of hydrolysis is 11%, the AN/TN-ratio is 13 and free amino acids account for 2% (Appendix A, A14).

Molecular weight profile:
Analysis of the hydrolysate demonstrates that >60% has a MW of <500. The entire distribution is presented in Appendix A (A14).

Chemical Analysis:
Analysis of the product demonstrates that peptide bonded glutamine accounts for ≥ 27% of the product (Appendix A, A15).

Amino Acid Profile:
Results of analyses are presented in Appendix A (A15).

Elemental Analysis:
Results of analyses are presented in Appendix A (A15).

Microbiological Analysis:
Results of analyses are presented in Appendix A (A15).

Unhydrolyzed protein (gluten) content: using an AOAC approved method (AOAC 991.19), the peptide was determined to contain less than 40 ppm of unhydrolyzed gluten (Appendix A, A16).

3. **Request for Additional Name**

Subject: Due to the unknown potential for an allergic reaction from the addition of unhydrolyzed wheat protein to the fermentation step of transglutaminase production, the Agency requested that the product produced using the 'revised process' (oxygen-stable TG) be labeled in a manner that identifies wheat derived protein as a possible constituent.

Resolution: The members of the GRAS Panel and the manufacturer were independently contacted regarding the issue. While the manufacturer agreed to a name change and ingredient label change, the Panel was concerned about the scientific implication of this change. After extensive discussion they approved the following addition to the GRAS certificate:

The following will be added to the GRAS certificate:

"CONDITION OF SAFE USE

While some experts believe that an allergic reaction to wheat protein is a 'threshold phenomenon', the GRAS Panel agrees that conclusive scientific information quantifying this threshold level is not currently available. In the absence of these data, the Panel is unable to determine if a level of ≤ 3.0 ppb,* which is the maximum level of hydrolyzed wheat protein that would result from the use of transglutaminase as stated, is below that threshold.

Under these conditions, the Panel recommends two labeling requirements as a condition of safe use.

1. The manufacturer has been requested to employ a different name for this product in order to distinguish it from the TG product that does not contain glutamine peptide.

2. The manufacturer has been requested to identify, in the ingredient section of the label, that the product contains "...hydrolyzed wheat protein...".

* Based on analyses of glutamine peptide product in use, the actual maximum concentration of wheat protein present will be < 1.0 ppb (Appendix A, A17).

RESPONSE OF THE MANUFACTURER

The manufacturer was contacted and agreed to comply with both of the Panel's labeling requests in the following manner:

1. The label for the product containing glutamine will be given the name Stabilized Transglutaminase, abbreviated STG, to distinguish it from the product that does not contain glutamine (i.e. Transglutaminase, abbreviated TG).

2. The ingredient section of the label for the product containing glutamine (STG) will state that the product contains '...transglutaminase (contains hydrolyzed wheat protein)...' whereas the label for the non-stabilized product will state contains 'transglutaminase'.

In addition, the manufacturer will advise its clients that the use of STG in foods, requires that the ingredient labeling of those foods must include a statement that this food contains glutamine peptide (hydrolyzed wheat protein).

Attached please find signatures from the Panel members supporting the requirements and accepting the manufacturer's suggestions for meeting those requirements (Appendix B, B1-B10).

4. **Request for Telephone Numbers of GRAS Panel Members**

Subject: The Agency requests the telephone numbers of the GRAS Panel Members. The Agency wishes to directly obtain their opinions regarding the addition of the wheat-derived protein.

Resolution: The names and telephone numbers are provided below:

Jay Goodman, Ph.D.	517.353.936
Philip Portoghese, Ph.D.	612.624.9174
William Waddell, M.D. Ph.D.	502.228.4220
Bernard Wagner, M.D.	973.467.8132
Vernon Young, Ph.D.	617.253.5801

We trust that this submission provides all the information the Agency requires to complete its evaluation of our GRAS Notification submission. If additional information is required, please contact us at the telephone number listed on the first page of this submission.

Sincerely,

(b)(6)

Bruce K. Bernard, President
President, SRA International Inc.

BKB/km
Attach. A, A1-A17
B, B1-B10
CC: R. Bursey

Appendix A

Product Description - DMV Glutamine Peptide

Glutamine Peptide is a wheat protein hydrolysate manufactured under carefully controlled conditions using food grade enzymes. Based on the application needs, three types of glutamine peptides have been developed.

1. Glutamine Peptide

Applications: Glutamine Peptide is specially developed for supplementation in dry and moist foods.
Benefits: It has a high content of natural, peptide bonded glutamine (28%) and features a good price / performance ratio. It has a very good sensoric profile and extremely low in bitterness as observed in humans.

Market

Human Nutrition
a. Clinical Nutrition Market Segment
b. Sports and Functional Food Market Segment

Both in Japan and Europe glutamine enriched TEN-formulas have been introduced in the market using Glutamine Peptide as the glutamine source.

Pet Nutrition
a. Value added products for general nutrition
b. Concept of Functional foods

In Japan and Europe Glutamine to lower the risk for viral infections and improve work activity in Racing horses is being vigorously explored.

Regulatory Information

EU Regulations

Mid 1996 Glutamine Peptide was reviewed by the Ministry of Agriculture, Fisheries and Food (MAFF)/UK resulting in the following statement.... " Glutamine Peptide would not fall within the scope of the impending EC Regulation on Novel Foods and Processes". Glutamine Peptide is therefore not considered a novel food.

US Regulations

21 CFR section 184.1553 of the FDA regulations declares peptones (protein hydrolysates) as Generally Recognized as Safe (GRAS). Glutamine Peptide may be regarded as GRAS. In addition, Glutamine Peptide does not contain genetically modified ingredients.

U.S. Food and Drug Administration - Center for Devices and Radiological Health

Code of Federal Regulations
Title 21 - Food and Drugs
Revised as of April 1, 2001

[Code of Federal Regulations]
[Title 21, Volume 3]
[Revised as of April 1, 2001]
From the U.S. Government Printing Office via GPO Access
[CITE: 21CFR184.1553]

[Page 524-525]

TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES (CONTINUED)

PART 184--DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE--Table of

Subpart B--Listing of Specific Substances Affirmed as GRAS

Sec. 184.1553 Peptones.

(a) Peptones are a variable mixture of polypeptides, oligopeptides, and amino acids that are produced by partial hydrolysis of casein, animal tissue, soy protein isolate, gelatin, defatted fatty tissue, egg albumin, or lactalbumin (whey protein). Peptones are produced from these proteins using proteolytic enzymes that either are considered to be generally recognized as safe (GRAS) or are regulated as food additives. Peptones are also produced by denaturing any of the proteins listed in this paragraph with safe and suitable acids or heat.

(b) FDA is developing food-grade specifications for peptones in cooperation with the National Academy of Sciences. In the interim, these ingredients must be of a purity suitable for their intended use.

(c) In accordance with Sec. 184.1(b)(1), these ingredients are used in food with no limitation other than current good manufacturing practice. The affirmation of these ingredients as GRAS as direct human food ingredients is based upon the following current good manufacturing practice conditions of use:

(1) These ingredients are used as nutrient supplements as defined in Sec. 170.3(o)(20) of this chapter; as processing aids as defined in Sec. 170.3(o)(24) of

[[Page 525]]

this chapter; and as surface-active agents as defined in Sec. 170.3(o)(29) of this chapter.

(2) These ingredients are used in food at levels not to exceed current good manufacturing practice.

(d) Prior sanctions for these ingredients different from the uses established in this section do not exist or have been waived.

[49 FR 25430, June 21, 1984, as amended at 50 FR 49536, Dec. 3, 1985]

000175

FOURTH EDITION

FOOD CHEMICALS CODEX

Effective July 1, 1996

COMMITTEE ON FOOD CHEMICALS CODEX

Food and Nutrition Board
Institute of Medicine
National Academy of Sciences

NATIONAL ACADEMY PRESS
Washington, D.C. 1996

000176

Parsley Seed Oil

DESCRIPTION

The oil obtained by steam distillation of the ripe seed of *Petroselinum crispum* (Fam. *Umbelliferae*). It is a yellow to light brown liquid having a rather harsh odor. It is soluble in most fixed oils and in mineral oil. It is slightly soluble in propylene glycol, but it is insoluble in glycerin.

Functional Use in Foods Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum in the section on *Infrared Spectra* (*Series A: Essential Oils*), using the same test conditions as specified therein.
Acid Value Not more than 4.0.
Angular Rotation Between −4° and −10°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.513 and 1.522 at 20°.
Saponification Value Between 2 and 10.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.040 and 1.080.

TESTS

Acid Value Determine as directed for *Acid Value* under *Fats and Related Substances*, Appendix VII.
Angular Rotation Determine in a 100-mm tube as directed under *Optical (Specific) Rotation*, Appendix IIB.
Heavy Metals Shake 10 mL of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, Appendix IIB Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed for *Saponification Value* under *Essential Oils and Flavors*, Appendix VI, using about 5 g, accurately weighed.
Solubility in Alcohol Proceed as directed in the general method, Appendix VI. One mL dissolves in 6 mL of 80% alcohol, occasionally with slight haziness.
Specific Gravity Determine by any reliable method (see *General Provisions*).

Packaging and Storage Store in full, preferably glass, tin-lined, or other suitably lined containers in a cool place protected from light.

Partially Hydrolyzed Proteins

Enzyme-Hydrolyzed (Source) Protein; Partially Hydrolyzed (Source) Protein; (Source) Peptone; Enzyme-Modified (Source) Protein; Partial Enzymatic Digest of (Source) Protein; Partial Acid Digest of (Source) Protein

INS: 429

DESCRIPTION

Partially Hydrolyzed Proteins are composed of peptides and polypeptides resulting from the partial or incomplete hydrolysis (breakdown) of peptide bonds present in edible proteinaceous materials catalyzed by heat, food-grade proteolytic enzymes, and/or suitable food-grade acids. Their degree of hydrolysis typically range from 3% to 85% on the basis of peptide bond cleavage. During processing, the proteinaceous raw material may be treated with safe and suitable alkaline materials. The edible proteinaceous materials used as raw materials are derived from casein and other milk products such as whey protein; from animal tissue, including gelatin, defatted animal tissue, and egg albumen; and from soy protein products, yeast, wheat protein products, or other suitable and safe plant sources. Partially Hydrolyzed Proteins may be available in liquid, paste, powder, or granular form.

Note: Depending on the protein source and the degree of hydrolysis, Partially Hydrolyzed Proteins may present an allergenic risk to sensitized individuals.

Functional Use in Foods Binder; dough conditioner; emulsifier and emulsifier salt; flavoring agent; flavor enhancer; nutrient supplement; fermentation aid; processing aid; surface-active agent; formulation aid; texturizer.

REQUIREMENTS

Calculate all analyses on the dried basis. In a suitable tared container, evaporate liquid and paste samples to dryness on a steam bath, then, as for the powdered and granular forms, dry to constant weight at 105° (see *General Provisions*).
Labeling Indicate the source of protein, including type.
Assay (Total Nitrogen; TN) Not less than 7.0%.
α-Amino Nitrogen (AN) Not less than 90.0% and not more than 110.0% of the amount claimed on the label.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Not less than 2.0% and not more than 62.0%, when calculated on an ammonia nitrogen-free basis.
Ammonia Nitrogen (NH_3-N) Not more than 1.5%.
Ash (Total) Not more than 40.0%.
Glutamic Acid Not more than 20.0% as $C_5H_9NO_4$ and not more than 35.0% of the total amino acids.
Heavy Metals (as Pb) Not more than 10 mg/kg.
Lead Not more than 3 mg/kg.

TESTS

Assay (Total Nitrogen) Proceed as directed under *Nitrogen Determination*, Appendix IIIC.
α-Amino Nitrogen Proceed as directed under *α-Amino Nitrogen* in the monograph for *Acid Hydrolysates of Proteins*.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Calculate by dividing the percent α-amino nitrogen (*AN*) by the percent total nitrogen (*TN*) as corrected for ammonia nitrogen (NH_3-N) according to the formula

$$100\ [(AN - NH_3\text{-N})/(TN - NH_3\text{-N})].$$

Ammonia Nitrogen Proceed as directed under *Ammonia Nitrogen* in the monograph for *Acid Hydrolysates of Proteins*.
Ash (Total) Proceed as directed in the general method, Appendix IIC, using a 1-g sample.
Glutamic Acid Proceed as directed under *Glutamic Acid* in the monograph for *Acid Hydrolysates of Proteins*.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, Appendix IIIB, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 3 μg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers.

Peanut Oil (Unhydrogenated)

DESCRIPTION

A pale yellow oil with a bland flavor obtained from the kernel of the peanut plant *Arachis hypogaea* by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21° to 27°, but solidifies to a gel-like consistency at refrigerator temperatures (2° to 4°). It is free from visible foreign matter at 21° to 27°, but sometimes clouds at temperatures above 21°.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

REQUIREMENTS

Identification Unhydrogenated Peanut Oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, Appendix VII:

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<0.2	6–15	<1.0	1.3–6.5	36–72	13–45

Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0
Weight % (Range):	<2.0	<1.0–2.5	0.5–2.1	1.5–4.8	<0.1	1.0–2.5

Color (AOCS-Wesson) Not more than 5.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 84 and 100.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 2.0%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Color Proceed as directed for *Color* (AOCS-Wesson) under *Fats and Related Substances*, Appendix VII.
Free Fatty Acids Proceed as directed under *Free Fatty Acids*, Appendix VII, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Proceed as directed under *Iodine Value*, Appendix VII.
Lead Determine as directed under *Method II* in the *Atomic Absorption Spectrophotometric Graphite Furnace Method* under *Lead Limit Test*, Appendix IIIB, using a 3-g sample.
Linolenic Acid Proceed as directed under *Fatty Acid Composition*, Appendix VII.
Peroxide Value Proceed as directed under *Peroxide Value* in the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.
Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, Appendix VII.
Water Proceed as directed under *Water Determination*, Appendix IIB. However, in place of 35 to 40 mL of methanol, use 50 mL of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Pectins

INS: 440 CAS: [9000-69-5]

DESCRIPTION

Pectins consist mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium, and ammonium salts. It is obtained by extraction in an aqueous medium of appropriate edible plant material, usually citrus fruits or apples. No organic precipitants shall be used other than methanol, ethanol, and isopropanol. In some types, a portion of the methyl esters may have been converted to primary amides by treatment with ammonia under alkaline conditions. It usually occurs as a white, yellowish, light grayish, or light brownish powder. The commercial product is normally diluted with sugars for standardization purposes. In addition to sugars, pectins may be mixed with suitable food-grade salts required for pH control and desirable setting characteristics.

FIRST SUPPLEMENT
TO THE
FOURTH EDITION

FOOD CHEMICALS CODEX

Effective December 1, 1997

COMMITTEE ON FOOD CHEMICALS CODEX
Food and Nutrition Board
Institute of Medicine
National Academy of Sciences

NATIONAL ACADEMY PRESS
Washington, DC 1997

2 / *Monograph Specifications*

Revision: Add *3-Chloropropane-1,2-diol* and *1,3-Dichloro-2-propanol* specifications; correct *Potassium* and *Sodium* tests.

Acid Hydrolysates of Proteins

Acid-Hydrolyzed Proteins; Hydrolyzed Vegetable Protein (HVP); Hydrolyzed Plant Protein (HPP); Hydrolyzed (Source) Protein Extract; Acid-Hydrolyzed Milk Protein

DESCRIPTION

Acid Hydrolysates of Proteins are composed primarily of amino acids, small peptides (peptide chains of five or fewer amino acids), and salts resulting from the essentially complete hydrolysis of peptide bonds in edible proteinaceous materials catalyzed by heat and/or food-grade acids. Cleavage of peptide bonds typically ranges from a low of 85% to essentially 100%. In processing, the protein hydrolysates may be treated with safe and suitable alkaline materials. The edible proteinaceous materials used as raw materials are derived from corn, soy, wheat, yeast, peanuts, rice, or other safe and suitable vegetable or plant sources, or from milk. Individual products may be in liquid, paste, powder, or granular form.

Functional Use in Foods Flavoring agent; flavor enhancer; adjuvant.

REQUIREMENTS

Calculate all analyses on the dried basis. In a suitable tared container, evaporate liquid and paste samples to dryness on a steam bath, then, as for the powdered and granular forms, dry to constant weight at 105° (see the *General Provisions*).

Assay (Total Nitrogen; TN) Not less than 4.0% total nitrogen.
α-Amino Nitrogen (AN) Not less than 3.0%.
α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Not less than 62.0% and not more than 85.0%, when calculated on an ammonia nitrogen-free basis.
Ammonia Nitrogen (NH_3-N) Not more than 1.5%.
3-Chloropropane-1,2-diol (3-CPD) Not more than 1 mg/kg, calculated on the dried basis.
1,3-Dichloro-2-propanol (DCP) Not more than 0.05 mg/kg, calculated on the dried basis.
Glutamic Acid Not more than 20.0% as $C_5H_9NO_4$ and not more than 35.0% of the total amino acids.
Heavy Metals (as Pb) Not more than 10 mg/kg.
Insoluble Matter Not more than 0.5%.
Lead Not more than 5 mg/kg.
Potassium Not more than 30.0%.
Sodium Not more than 20.0%.

TESTS

Assay (Total Nitrogen) Proceed as directed under *Nitrogen Determination*, Appendix IIIC.
α-Amino Nitrogen Transfer 7 to 25 g, accurately weighed, into a 500-mL volumetric flask with the aid of several 50-mL portions of warm ammonia-free water, dilute to volume with water, and mix. Neutralize 200 mL of the solution with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide, using phenolphthalein TS as indicator, and add 10 mL of freshly prepared phenolphthalein–formol solution (50 mL of 40% formaldehyde containing 1 mL of 0.05% phenolphthalein in 50% alcohol neutralized exactly to pH 7 with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide). Titrate with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide to a distinct red color, add a small, but accurately measured, volume of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide in excess, and back titrate to neutrality with 0.2 *N* hydrochloric acid. Conduct a blank titration using the

000180

same reagents, with 20 mL of water in place of the test solution. Each mL of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide is equivalent to 2.8 mg of α-amino nitrogen.

α-Amino Nitrogen/Total Nitrogen (AN/TN) Percent Ratio Calculate by dividing the percent α-amino nitrogen (*AN*) by the percent total nitrogen (*TN*) as corrected for ammonia nitrogen (NH_3-*N*) according to the formula

$$100[(AN - NH_3\text{-}N)/(TN - NH_3\text{-}N)].$$

Ammonia Nitrogen (**Caution:** Provide adequate ventilation.) (Note: All reagents should be nitrogen free, where available, or otherwise very low in nitrogen content.) Transfer from 700 mg to 2.2 g of the sample to a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass, wrapping the sample, if solid or semi-solid, in nitrogen-free filter paper to facilitate the transfer if desired.

Add about 200 mL of water, and mix. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets, or a 2 in 5 sodium hydroxide solution, down the inside of the flask so that it forms a layer under the solution, using a sufficient amount (usually about 25 g of solid NaOH) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-mL flask. Add from 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and then heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination, substituting 2 g of sucrose for the sample, and make any necessary correction (see the *General Provisions*). Each mL of 0.5 *N* acid consumed is equivalent to 7.003 mg of ammonia nitrogen.

> Note: If it is known that the substance to be determined has a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each mL of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.

Calculate the percent ammonia nitrogen by dividing the weight of ammonia nitrogen, in mg, by the weight of the sample, in mg, times 100.

3-Chloropropane-1,2-diol (3-CPD)

3-CPD Stock Solution Transfer 12.5 mg, accurately weighed, of reagent-grade 3-chloropropane-1,2-diol (3-CPD) into a 100-mL volumetric flask, dilute to volume with ethyl acetate, and mix.

Dilute 3-CPD Solution Dilute 5 mL of *3-CPD Stock Solution* to 100 mL with ethyl acetate to yield a solution containing 6.25 µg/mL.

Internal Standard Solution Transfer 50 mg of 1-chlorotetradecane into a 50-mL volumetric flask, and dilute to volume with ethyl acetate. Dilute 1 mL of this solution to 100 mL with ethyl acetate to yield a solution containing 10 µg/mL.

Standard Solutions

A. Pipet 2 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute to volume with ethyl acetate, and mix. The resulting *Standard Solution* contains 0.5 µg/mL of 3-CPD.

B. Pipet 8 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute to volume with ethyl acetate, and mix. The resulting *Standard Solution* contains 2.0 µg/mL of 3-CPD.

C. Pipet 16 mL of *Dilute 3-CPD Solution* and 2.5 mL of *Internal Standard Solution* into a 25-mL volumetric flask, dilute this mixture to volume with ethyl acetate, and mix. The resulting *Standard Solution* contains 4.0 µg/mL of 3-CPD.

Chromatographic System Use a gas chromatograph equipped with an electrolytic conductivity detector operated in the halogen mode. The gas chromatograph is fitted with either a capillary injector operated in the splitless mode or a purged, packed injector with a glass insert. Use a 30-m × 0.53-mm id fused silica column coated with 1-µm Supelcowax 10 or an equivalent bonded carbowax column. The column is fitted with a 50-cm retention gap of 0.53 mm deactivated, fused silica. Use helium as the carrier gas at a flow-rate of 8 mL/min. Set the column temperature to 170° for 5 min, then raise the temperature at a rate of 5°/min to 250°, and hold it at that temperature for 10 min. The injector temperature is maintained at 225°.

Use hydrogen as the reactant gas at a flow-rate of 30 mL/min, and use 1-propanol as the solvent at a flow-rate through the cell of 0.5 mL/min or the manufacturer's specified flow-rate for the optimum operation of the electrolytic conductivity detector. The reactor temperature should be 900°, with a base temperature of 275°. Minimize contamination of the reaction tube by venting flow from the column at all times, except for the time during which compounds of interest elute.

Calibration Inject 1 µL each of standard solutions *A*, *B*, and *C* into the gas chromatograph. Calculate the response area ratios of 3-CPD to the *Internal Standard* for each standard solution. Plot the response area ratios versus the µg of 3-CPD in each standard solution to obtain the standard curve.

Procedure Adjust an accurately weighed sample of Acid Hydrolysates of Proteins, as needed, with 20% aqueous sodium chloride to obtain a solution with a solids content of 36%. Weigh a 20-g aliquot of the solution directly into a 20-mL Extrelut column (EM Science, Gibbstown, NJ, or equivalent), and allow it to equilibrate for 15 min. Elute the column with 150 mL of ethyl acetate, collecting the eluent in a 250-mL, short-neck, round-bottom flask with a 24/40 joint. Concentrate the eluent to a volume of approximately 3 mL using a rotary evaporator at 50°. Add 0.5 mL of *Internal Standard Solution* to the eluent, transfer this mixture to a 4-dram screw-cap vial, and dilute to a volume of 5.0 mL. Inject 1 µL into the gas chromatograph, measure its response area ratio of 3-CPD to the *Internal Standard*, and determine from the standard curve the µg of 3-CPD in the 20-g aliquot taken.

1,3-Dichloro-2-propanol (DCP)

Eluent Transfer 850 mL of chromatographic-grade pentane and 150 mL of chromatographic-grade diethyl ether into a suitable container, and mix well.

DCP Stock Solution Transfer 50 mg, accurately weighed, of reagent-grade 1,3-dichloro-2-propanol (DCP) into a 50-mL volumetric flask, dilute to volume with *Eluent*, and mix.

Dilute DCP Solution Stepwise and quantitatively dilute the *DCP Stock Solution* with *Eluent* to obtain a final solution containing 1 µg/mL of DCP.

Internal Standard Solution Transfer 50 mg of trichlorobenzene into a 50-mL volumetric flask, dilute to volume with *Eluent*, and mix. Use a 1 in 1000 dilution of this solution as the *Internal Standard Solution*.

Standard Solutions Into separate 50-mL volumetric flasks pipet 1-, 2-, 3-, and 4-mL portions of *Dilute DCP Solution*. To each add 1.0 mL of *Internal Standard Solution*, dilute to volume with *Eluent*, and mix.

Sample Preparation Dissolve 5.0 g, accurately weighed, of Acid Hydrolysates of Proteins in a minimum volume of 20% aqueous sodium chloride solution. Transfer this solution quantitatively to an Extrelut column (EM Science, Gibbstown, NJ, or equivalent). After 15 min, elute the column with three 20-mL portions of *Eluent*, collecting all the eluate. Carefully evaporate the eluate to less than 4 mL. Add 1.0 mL of *Internal Standard Solution*, and dilute with *Eluent*, as necessary, to bring the final volume to 5.0 mL.

Chromatographic System Use a gas chromatograph equipped with a split injector and a nickel electron-capture detector. The gas chromatograph is fitted with a 50-m × 0.2-mm id fused silica column coated with dimethylpolysiloxane (Carbowax 20M, or equivalent). Use nitrogen as the carrier gas at a flow-rate of 8 mL/min. Before use, precondition the column by heating it at 200° and the detector at 300° for 24 h. Set the injector temperature at 250° and the electron-capture detector at 300°, and program the column temperature as follows: Maintain for 10 min at 115°, raise rapidly at 30°/min to 200°, and maintain at 200° for 12 min.

Calibration Inject 1.0 µL of each of the four *Standard Solutions* into the gas chromatograph. Calculate the response area ratios of DCP to the *Internal Standard Solution* for each *Standard Solution*. Plot the response area ratios versus the µg of DCP in each *Standard Solution* to obtain the standard curve.

Procedure Similarly, inject 1.0 µL of *Sample Preparation*. Measure its response ratio, and determine from the standard curve the µg of DCP in the sample taken.

Glutamic Acid

Apparatus Use an ion-exchange amino acid analyzer, equipped with sulfonated polystyrene columns, in which the effluent from the sample is mixed with ninhydrin reagent and the absorbance of the resultant color is measured continuously and automatically at 570 and 440 nm by a recording photometer.

Standard Solution Weigh 1250 ± 2 mg of glutamic acid, reagent grade, and place in a 500-mL volumetric flask. Fill the flask half-full with water, and add 5 mL of hydrochloric acid to help dissolve the amino acid, dilute to volume with water, and mix. Prepare the standard for analysis by diluting 1 mL of this solution with 4 mL of 0.2 *N* sodium citrate, pH 2.2, buffer. This *Standard Solution* contains 0.5 mg of glutamic acid per mL (C_S).

Sample Preparation Accurately weigh 5 mg of the sample and dilute to exactly 5 mL with 0.2 *N* sodium citrate, pH 2.2, buffer. Remove any insoluble material by centrifugation or filtration.

Procedure Using 2-mL aliquots of the *Standard Solution* and *Sample Preparation*, proceed as directed according to the apparatus manufacturer's instructions. From the chromatograms thus obtained, match the retention times produced by the *Standard Solution* with those produced by the *Sample Solution*, and identify the peak produced by glutamic acid. Record the area of the glutamic acid peak from the sample as A_U, and that from the standards as A_S.

Calculations Calculate the concentration, C_A, in mg/mL, of glutamic acid in the *Sample Preparation* by the formula

$$A_U \times C_S/A_S,$$

in which C_S is the concentration, in mg/mL, of the glutamic acid in the *Standard Solution*.

Calculate the percentage of glutamic acid, on the basis of total amino acids, by the formula

$$100C_A/6.25N_T,$$

in which N_T is the percentage of total nitrogen determined in the *Assay*.

Calculate the percentage of glutamic acid in the sample by the formula

$$100 \times C_A/S_W,$$

in which S_W is the weight of the sample taken, in mg.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Matter Transfer about 5 g, accurately weighed, into a 250-mL Erlenmeyer flask, add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry at 105° for 1 h, cool, and weigh.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 5 µg of lead ion (Pb) in the control.

Potassium

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Standard Solution Transfer 38.20 mg of reagent-grade potassium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Transfer 5.0 mL of this solution to a 1000-mL volumetric flask, dilute to volume with deionized water, and mix. Each mL contains 1.0 µg of K.

Sample Solution Transfer 1.00 ± 0.05 g of previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 1000-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix. Use a 1 to 300 dilution as the *Sample Solution*.

Procedure Determine the absorbance of each solution at 766.5 nm, following the manufacturer's instruction for optimum

operation of the spectrophotometer. The absorbance produced by the *Sample Solution* does not exceed that of the *Standard Solution.*

Sodium

Spectrophotometer Use any suitable atomic absorption spectrophotometer.

Standard Solution Transfer 25.42 mg of reagent-grade sodium chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with deionized water, and mix. Transfer 5.0 mL of this solution to a 1000-mL volumetric flask, dilute to volume with deionized water, and mix. Each mL of the final *Standard Solution* contains 0.5 µg of Na. Using water as the solvent, prepare a 1 in 100 dilution of this solution to obtain the final working *Standard Solution.*

Sample Solution Transfer 1.00 ± 0.05 g of previously dried sample, accurately weighed, into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 100-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix. Using water as the solvent, prepare a 1 in 4000 dilution of this solution to obtain the final *Sample Solution.*

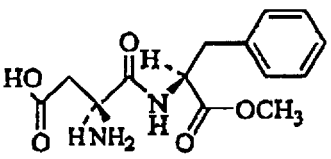
Procedure Determine the absorbance of each solution at 589.0 nm, following the manufacturer's instructions for optimum operation of the spectrophotometer. The absorbance produced by the *Sample Solution* does not exceed that of the *Standard Solution.*

Packaging and Storage Store in well-closed containers.

Revision: Delete *Transmittance* specification.

Aspartame

N-L-α-Aspartyl-L-phenylalanine 1-Methyl Ester; APM



$C_{14}H_{18}N_2O_5$ Formula wt 294.31

INS: 951 CAS: [22839-47-0]

DESCRIPTION

A white, odorless, crystalline powder having a sweet taste. It is sparingly soluble in water and slightly soluble in alcohol. The pH of a 0.8% solution is between about 4.5 and 6.0.

Functional Use in Foods Sweetener; sugar substitute; flavor enhancer.

REQUIREMENTS

Identification The infrared absorption spectrum of a potassium bromide dispersion of Aspartame exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aspartame Reference Standard.

Assay Not less than 98.0% and not more than 102.0% of $C_{14}H_{18}N_2O_5$, calculated on the dried basis.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Not more than 1.5%.

Heavy Metals (as Pb) Not more than 10 mg/kg.

Loss on Drying Not more than 4.5%.

Other Related Substances Not more than 2.0%.

Residue on Ignition Not more than 0.2%.

Specific Rotation $[\alpha]_D^{20°}$: Between +14.5° and +16.5°, calculated on the dried basis.

TESTS

Assay Transfer about 300 mg of the sample, accurately weighed, to a 150-mL beaker, dissolve in 1.5 mL of formic acid (96%), and add 60 mL of glacial acetic acid. Add crystal violet TS, and titrate immediately with 0.1 *N* perchloric acid to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 *N* perchloric acid is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

> **Note:** Use 0.1 *N* perchloric acid previously standardized to a green endpoint. A blank titration exceeding 0.1 mL may be due to excessive water content and may cause loss of visual endpoint sensitivity.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid

Mobile Phase Weigh 5.6 g of potassium phosphate monobasic into a 1-L flask, add 820 mL of water, and dissolve. Adjust the pH to 4.3 using phosphoric acid, add 180 mL of methanol, and mix. Filter through a 0.45-µm disk, and de-gas.

Diluting Solvent Add 200 mL of methanol to 1800 mL of water, and mix.

Impurity Standard Preparation Transfer about 25 mg of USP 5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Reference Standard, accurately weighed, into a 100-mL volumetric flask. Add 10 mL of methanol, and dissolve. Dilute to volume with water, and mix. Pipet 15 mL of this solution into a 50-mL volumetric flask, dilute to volume with *Diluting Solvent*, and mix. Use a freshly prepared solution.

Sample Preparation Transfer about 50 mg of the Aspartame sample, accurately weighed, to a 10-mL volumetric flask. Dilute to volume with *Diluting Solvent*, and mix. Use a freshly prepared solution.

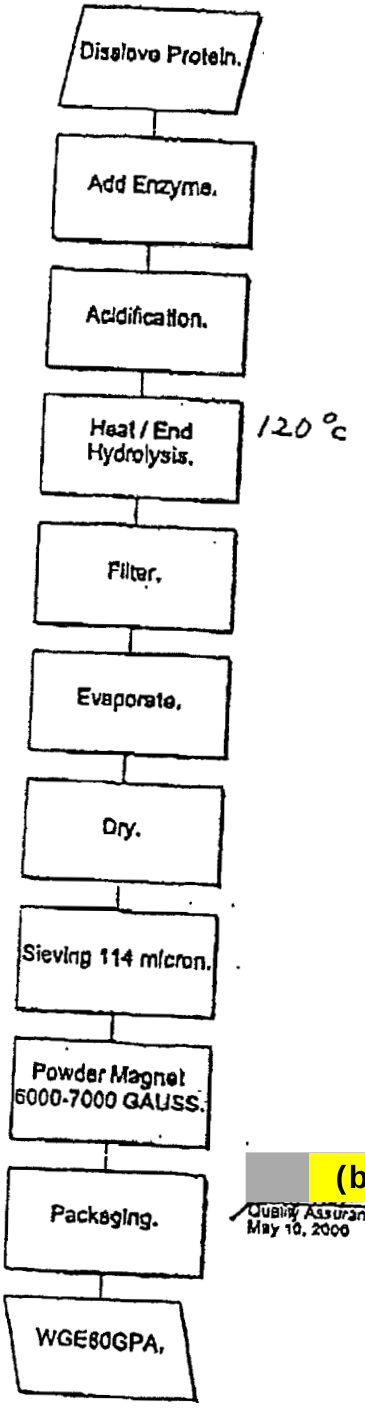
Chromatographic System Use a suitable high-pressure liquid chromatograph equipped with a detector measuring at 210 nm and a 250- × 4.6-mm column packed with octadecyl silanized silica (10-µm Partisil ODS-3, or equivalent) and operated under isocratic conditions at 40°. The flow rate of the *Mobile Phase* is about 2 mL/min.

System Suitability The area responses of three replicate injections of the *Impurity Standard Preparation* show a relative standard deviation of not more than 2.0%.

DMV INTERNATIONAL

Nutritionals

WGE80GPA - Process Flow Diagram
Fraser



(b)(6)

Quality Assurance Manager
May 10, 2000

DMV International Nutritionals
an operating unit of DMV Inc.

1712 Delawn Plaza, Fraser, New York 13753
Telephone 607 / 746-6473, 746-3082
Administrative Telefax 607 / 746-2710
Production Telefax 607 / 746-2585

DMV JAPAN

CERTIFICATE

To AJINOMOTO CO., INC.

Glutamine peptide WGE80GPA is manufactured under food grade conditions and complies with all FDA food regulations and is food grade.

(b)(6)

Norihito Iwabuchi
Branch manager
DMV JAPAN
Branch of Campina Melkunie BV
March 17, 2000

Branch of Campina Melkunie bv
Kudan Vigas Bldg. 3F, 1-10-9 Kudan-Kita
Chiyoda-ku, Tokyo 102-0073
Tel.: 03-5276-0441, Fax: 03-5276 0445

A-12

000185

Milk products, Lactose, Whey protein concentrates, Toppings, Coffee creamers, For concentrates, Milk powders
All offers for the sale and delivery of products by Campina Melkunie bv, and all agreements with Campina Melkunie bv with respects thereto, are subject to the general conditions of Campina Melkunie bv as filed on 1 January 1999 with the registry of the District Court in Middelburg under the number 1/89. A copy of these conditions will be sent on request free of charge.

CAMPINA MELKUNIE

17070-0194

DMV INTERNATIONAL

Nutritionals

CERTIFICATE

TO WHOM IT MAY CONCERN:

DMV International Nutritionals does not use ingredients, enzymes nor technical aids in the manufacture of **WGE80GPA**, which have been produced using genetically modified organisms.

Nevertheless, we are aware of the increasing use of these organisms for the manufacturing of pharmaceutical ingredients, and for enzyme production, such as chymosine, as well as increasingly in certain agricultural products such as soy, corn, and wheat.

We, therefore, cannot guarantee wholly in the complete production chain, starting at the farm and ending at DMV International Nutritionals premises, that these organisms are not used.

(b)(6)

Steve Braun
Technical Director
September 8, 1999

DMV International Nutritionals
an operating unit of DMV Inc.

1712 Deltown Plaza, Fraser, New York 13753
Telephone 607 / 746-6473, 746-3082
Administrative Telefax 607 / 746-2710
Production Telefax 607 / 746-2585

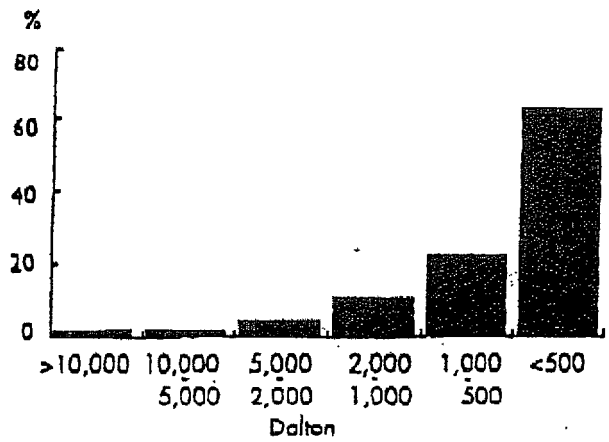
Product Information
WGE 80 GPA
Glutamine Peptide

Applications
Glutamine Peptide is specially developed for supplementation of nutritional beverages such as sports, functional drinks and enteral nutrition supplements, but can also be applied for glutamine enrichment of tablet and instant drink formulations.

Product description
Glutamine Peptide is a low pH, clarified wheat protein hydrolysate manufactured under carefully controlled conditions using food grade enzymes.

Properties
Benefits of glutamine supplementation in nutrition focus on an improved immune response, faster recovery after exercise or injury, prevention of fatigue and the overtraining syndrome, maintenance/restoration of the nitrogen balance and glycogen replenishment.
Glutamine Peptide has a number of benefits: it has a high content of natural, peptide bonded glutamine and is completely stable in solutions, even at high temperatures (pasteurization, retort- and UHT-sterilization) and low pH. In addition, Glutamine Peptide is easy to dissolve, yields completely clear solutions and has a very good sensoric profile (low in bitterness).

Hydrolysis characteristics
- Molecular weight profile:



- Average molecular weight ($\overline{MW}$) 770 D
- Degree of hydrolysis (DH) 11 %
- AN/TN-ratio (x 100) 13
- Free Amino Acids (FAA). 2 %

- **for use in nutritional beverages such as sports, functional and enteral drink formulations geared towards support of the immune system and recovery**
- **natural source of peptide bonded glutamine**
- **heat and acid stable source of glutamine**
- **completely soluble, high clarity in solution**
- **bland taste, very low bitterness**

Packaging
This product is packaged in a multi-wall paper bag with a double polyethylene liner with a net content of 20.0 kg.

Storage Conditions
Protein hydrolysates are hygroscopic and can absorb odours. Therefore, adequate protection is essential. Temperatures below 25°C, relative humidities (R.H.) below 65 %, and an odour-free environment will extend the storage life.

Shelf life
In the original sealed packaging, Glutamine Peptide has a shelf life of at least 2 years.

Food Legislation
All the ingredients to make Glutamine Peptide are food grade.

Literature
Additional information will be supplied on request. Technical bulletins are available which explain the background of the various product parameters mentioned in this data sheet and our methods of analysis used.

CHEMICAL

Total Nitrogen (TN)		13.5 %
Protein (TN x 5.80*), as is		78.3 %
Protein (TN x 5.80*), dry basis		82.4 %
Peptide bonded glutamine	27 %	
Free glutamine	< 1 %	
Fat		0.2 %
Carbohydrates, total		13.5 %
Ash (800°C)		3.0 %
Moisture		5.0 %
pH (6 % solution; 20°C)		3.9

*) conversion factor calculated as a function of degree of hydrolysis; for further explanation, please refer to separate technical bulletin.

MICROBIOLOGICAL

Standard Plate Count	max. 5,000/g
Coliform	max. 10/g
E. coli	neg. in 11 g
Salmonella	neg. in 100 g
Yeast and Mould	max. 100/g

PHYSICAL/SENSORIC

Colour, powder	white
Colour, solution	faint white
Solubility	200 g/l
Clarity (2% solution)	clear
Bulk density (tapped)	400 g/l

HYDROLYSIS CHARACTERISTICS

AN/TN-ratio (x 100)		13
Degree of hydrolysis (DH)		11 %
Molecular weight profile:	> 10,000 D	1 %
	10,000 - 5,000 D	1 %
	5,000 - 2,000 D	5 %
	2,000 - 1,000 D	11 %
	1,000 - 500 D	21 %
	< 500 D	61 %

Average molecular weight (MW)	770 D
Free Amino Acids (FAA)	2 %

AMINO ACID PROFILE:		**PER G OF PROTEIN**
Alanine	(Ala)	23 mg
Arginine	(Arg)	28 mg
Aspartic acid	(Asp)	25 mg
Cystine	(Cys)	16 mg
Glutamic acid[1]	(Glu)	408 mg
Glutamine	(Gln)	345 mg
Glycine	(Gly)	34 mg
Histidine	(His)	16 mg
Isoleucine	(Ile)	32 mg
Leucine	(Leu)	64 mg
Lysine	(Lys)	13 mg
Methionine	(Met)	14 mg
Phenylalanine	(Phe)	53 mg
Proline	(Pro)	137 mg
Serine	(Ser)	44 mg
Threonine	(Thr)	23 mg
Tryptophan	(Trp)	4 mg
Tyrosine	(Tyr)	30 mg
Valine	(Val)	36 mg
Total		**1,000 mg**

[1] including glutamine

Total Branched Chain Amino Acids (BCAA) 132 mg

ELEMENTAL PROFILE:		**PER 100 G PRODUCT**
Sodium	(Na)	550 mg
Potassium	(K)	550 mg
Calcium	(Ca)	80 mg
Magnesium	(Mg)	60 mg
Phosphorus	(P)	310 mg
Chloride	(Cl)	250 mg
Iron	(Fe)	2 mg

DMV INTERNATIONAL

Nutritionals

TO WHOM IT MAY CONCERN:

DMV INTERNATIONAL Nutritionals has analyzed WGE80GPA, Enzymatically Hydrolyzed Wheat Gluten, for gluten content. The method of analysis, AOAC 991.19, is a colorimetric monoclonal antibody enzyme immunoassay. Using AOAC 991.19, WGE80GPA has tested less than 40 PPM gluten.

(b)(6)

Steven D. Braun, Ph.D.
Technical Director
DMV INTERNATIONAL Nutritionals
August 26, 1999

000189

DMV International Nutritionals an operating unit of DMV Inc.

1712 Daltown Plaza, Fraser, New York 13753
Telephone 607 / 746-6479, 746-3082
Administrative Telefax 607 / 746-2710
Production Telefax 607 / 746-2585

Appendix B

SRA International, Inc.

29 March 2002

Jay I. Goodman, Ph.D.
Michigan State University
Dept. of Pharmacology and Toxicology
B-440 Life Sciences Building
East Lansing, MI 48824-1317

Re: Addition to GRAS certificate

Dear Dr. Goodman:

You were a member of the expert panel, which on 15 February 2001 determined the above-identified enzyme to be GRAS under specified conditions of intended use.

At the request of the U.S. Food and Drug Administration, you recently approved a modification to the original GRAS certificate. The FDA requires the signatures of each panel member on this change to the GRAS certificate. Therefore, below please find the statement you approved and a space for your signature. We appreciate your assistance in this matter.

The following will be added to the GRAS certificate:

CONDITION OF SAFE USE

While some experts believe that an allergic reaction to wheat protein is a 'threshold phenomenon', the GRAS Panel agrees that conclusive scientific information quantifying this threshold level is not currently available. In the absence of these data, the Panel is unable to determine if a level of ≤ 3.0 ppb, which is the maximum level of hydrolyzed wheat protein that would result from the use of transglutaminase as stated, is below that threshold.

Under these conditions, the Panel recommends two labeling requirements as a condition of safe use.

1. The manufacturer has been requested to employ a different name for this product in order to distinguish it from the TG product that does not contain glutamine peptide.

2. The manufacturer has been requested to identify, in the ingredient section of the label, that the product contains "...hydrolyzed wheat protein...".

RESPONSE OF THE MANUFACTURER

The manufacturer agrees to comply with both of the Panel's labeling requests.

1. The label for the product containing glutamine will be given the name Stabilized Transglutaminase, abbreviated STG, to distinguish it from the product that does not contain glutamine (i.e. Transglutaminase, abbreviated TG)

2. The ingredient section of the label for the product containing glutamine (STG) will state that the product contains '...transglutaminase (contains hydrolyzed wheat protein)...' whereas the label for the non-stabilized product will state 'contains...transglutaminase...'.

In addition, the manufacturer will advise its clients that the use of STG in foods, requires that the ingredient labeling of those foods must include a statement that this food contains glutamine peptide (hydrolyzed wheat protein).

Sincerely,

(b)(6)

Bruce K. Bernard, President
Staff Officer, GRAS Panel

(b)(6)

Jay I. Goodman, Ph.D.
GRAS Panel Member

000192

SRA International, Inc.

29 March 2002

Philip S. Portoghese, Ph.D.
Distinguished Professor
Dept. Of Medicinal Chemistry
College of Pharmacy
University of Minnesota
308 Harvard Street, SE
Minneapolis, MN 55455

Re: Addition to GRAS certificate

Dear Dr. Portoghese:

You were a member of the expert panel, which on 15 February 2001 determined the above-identified enzyme to be GRAS under specified conditions of intended use.

At the request of the U.S. Food and Drug Administration, you recently approved a modification to the original GRAS certificate. The FDA requires the signatures of each panel member on this change to the GRAS certificate. Therefore, below please find the statement you approved and a space for your signature. We appreciate your assistance in this matter.

The following will be added to the GRAS certificate:

CONDITION OF SAFE USE

While some experts believe that an allergic reaction to wheat protein is a 'threshold phenomenon', the GRAS Panel agrees that conclusive scientific information quantifying this threshold level is not currently available. In the absence of these data, the Panel is unable to determine if a level of ≤ 3.0 ppb, which is the maximum level of hydrolyzed wheat protein that would result from the use of transglutaminase as stated, is below that threshold.

Under these conditions, the Panel recommends two labeling requirements as a condition of safe use.

1. The manufacturer has been requested to employ a different name for this product in order to distinguish it from the TG product that does not contain glutamine peptide.

2. The manufacturer has been requested to identify, in the ingredient section of the label, that the product contains "...hydrolyzed wheat protein...".

RESPONSE OF THE MANUFACTURER

The manufacturer agrees to comply with both of the Panel's labeling requests.

1. The label for the product containing glutamine will be given the name Stabilized Transglutaminase, abbreviated STG, to distinguish it from the product that does not contain glutamine (i.e. Transglutaminase, abbreviated TG)

2. The ingredient section of the label for the product containing glutamine (STG) will state that the product contains '...transglutaminase (contains hydrolyzed wheat protein)...' whereas the label for the non-stabilized product will state 'contains...transglutaminase...'.

In addition, the manufacturer will advise its clients that the use of STG in foods, requires that the ingredient labeling of those foods must include a statement that this food contains glutamine peptide (hydrolyzed wheat protein).

Sincerely,

(b)(6)

Bruce K. Bernard, President
Staff Officer, GRAS Panel

(b)(6)

Philip S. Portoghese, Ph.D.
GRAS Panel Member

SRA International, Inc.

29 March 2002

William J. Waddell, M.D.
14300 Rose Wycombe Lane
Prospect, KY 40058

Re: Addition to GRAS certificate

Dear Dr. Waddell:

You were a member of the expert panel, which on 15 February 2001 determined the above-identified enzyme to be GRAS under specified conditions of intended use.

At the request of the U.S. Food and Drug Administration, you recently approved a modification to the original GRAS certificate. The FDA requires the signatures of each panel member on this change to the GRAS certificate. Therefore, below please find the statement you approved and a space for your signature. We appreciate your assistance in this matter.

The following will be added to the GRAS certificate:

CONDITION OF SAFE USE

While some experts believe that an allergic reaction to wheat protein is a 'threshold phenomenon', the GRAS Panel agrees that conclusive scientific information quantifying this threshold level is not currently available. In the absence of these data, the Panel is unable to determine if a level of $\leq$ 3.0 ppb, which is the maximum level of hydrolyzed wheat protein that would result from the use of transglutaminase as stated, is below that threshold.

Under these conditions, the Panel recommends two labeling requirements as a condition of safe use.

1. The manufacturer has been requested to employ a different name for this product in order to distinguish it from the TG product that does not contain glutamine peptide.

000195

2. The manufacturer has been requested to identify, in the ingredient section of the label, that the product contains "...hydrolyzed wheat protein...".

RESPONSE OF THE MANUFACTURER

The manufacturer agrees to comply with both of the Panel's labeling requests.

1. The label for the product containing glutamine will be given the name Stabilized Transglutaminase, abbreviated STG, to distinguish it from the product that does not contain glutamine (i.e. Transglutaminase, abbreviated TG)

2. The ingredient section of the label for the product containing glutamine (STG) will state that the product contains '...transglutaminase (contains hydrolyzed wheat protein)...' whereas the label for the non-stabilized product will state 'contains...transglutaminase...'.

In addition, the manufacturer will advise its clients that the use of STG in foods, requires that the ingredient labeling of those foods must include a statement that this food contains glutamine peptide (hydrolyzed wheat protein).

Sincerely,

(b)(6)

Bruce K. Bernard, President
Staff Officer, GRAS Panel

(b)(6)

William J. Waddell, M.D.
GRAS Panel Member

000196

SRA International, Inc.

29 March 2002

Bernard Wagner, M.D.
38 Mohawk Road
Short Hills, NJ 07078

Re: Addition to GRAS certificate

Dear Dr. Wagner:

You were a member of the expert panel, which on 15 February 2001 determined the above-identified enzyme to be GRAS under specified conditions of intended use.

At the request of the U.S. Food and Drug Administration, you recently approved a modification to the original GRAS certificate. The FDA requires the signatures of each panel member on this change to the GRAS certificate. Therefore, below please find the statement you approved and a space for your signature. We appreciate your assistance in this matter.

The following will be added to the GRAS certificate:

CONDITION OF SAFE USE

While some experts believe that an allergic reaction to wheat protein is a 'threshold phenomenon', the GRAS Panel agrees that conclusive scientific information quantifying this threshold level is not currently available. In the absence of these data, the Panel is unable to determine if a level of ≤ 3.0 ppb, which is the maximum level of hydrolyzed wheat protein that would result from the use of transglutaminase as stated, is below that threshold.

Under these conditions, the Panel recommends two labeling requirements as a condition of safe use.

1. The manufacturer has been requested to employ a different name for this product in order to distinguish it from the TG product that does not contain glutamine peptide.

000197

2. The manufacturer has been requested to identify, in the ingredient section of the label, that the product contains "...hydrolyzed wheat protein...".

RESPONSE OF THE MANUFACTURER

The manufacturer agrees to comply with both of the Panel's labeling requests.

1. The label for the product containing glutamine will be given the name Stabilized Transglutaminase, abbreviated STG, to distinguish it from the product that does not contain glutamine (i.e. Transglutaminase, abbreviated TG)

2. The ingredient section of the label for the product containing glutamine (STG) will state that the product contains '...transglutaminase (contains hydrolyzed wheat protein)...' whereas the label for the non-stabilized product will state 'contains...transglutaminase...'.

In addition, the manufacturer will advise its clients that the use of STG in foods, requires that the ingredient labeling of those foods must include a statement that this food contains glutamine peptide (hydrolyzed wheat protein).

Sincerely,

(b)(6)

Bruce K. Bernard, President
Staff Officer, GRAS Panel

(b)(6)

Bernard Wagner, M.D.
GRAS Panel Member

000198

SRA International, Inc.

29 March 2002

Dr. Vernon Young, Ph.D.
Nutrition Department
Massachusetts Institute of Technology
50 Ames St., Bldg. 17-434, Room E
Cambridge, MA 02139

Re: Addition to GRAS certificate

Dear Dr. Young:

You were a member of the expert panel, which on 15 February 2001 determined the above-identified enzyme to be GRAS under specified conditions of intended use.

At the request of the U.S. Food and Drug Administration, you recently approved a modification to the original GRAS certificate. The FDA requires the signatures of each panel member on this change to the GRAS certificate. Therefore, below please find the statement you approved and a space for your signature. We appreciate your assistance in this matter.

The following will be added to the GRAS certificate:

CONDITION OF SAFE USE

While some experts believe that an allergic reaction to wheat protein is a 'threshold phenomenon', the GRAS Panel agrees that conclusive scientific information quantifying this threshold level is not currently available. In the absence of these data, the Panel is unable to determine if a level of ≤ 3.0 ppb, which is the maximum level of hydrolyzed wheat protein that would result from the use of transglutaminase as stated, is below that threshold.

Under these conditions, the Panel recommends two labeling requirements as a condition of safe use.

1. The manufacturer has been requested to employ a different name for this product in order to distinguish it from the TG product that does not contain glutamine peptide.

000199

2. The manufacturer has been requested to identify, in the ingredient section of the label, that the product contains "...hydrolyzed wheat protein...".

RESPONSE OF THE MANUFACTURER

The manufacturer agrees to comply with both of the Panel's labeling requests.

1. The label for the product containing glutamine will be given the name Stabilized Transglutaminase, abbreviated STG, to distinguish it from the product that does not contain glutamine (i.e. Transglutaminase, abbreviated TG)

2. The ingredient section of the label for the product containing glutamine (STG) will state that the product contains '...transglutaminase (contains hydrolyzed wheat protein)...' whereas the label for the non-stabilized product will state 'contains...transglutaminase...'.

In addition, the manufacturer will advise its clients that the use of STG in foods, requires that the ingredient labeling of those foods must include a statement that this food contains glutamine peptide (hydrolyzed wheat protein).

Sincerely,

(b)(6)

Bruce K. Bernard, President
Staff Officer, GRAS Panel

(b)(6)

Vernon Young, Ph.D.
GRAS Panel Member

000200

AM

SRA International, Inc.

FAX

To:	Lane Highbarger, Ph.D	**From:**	Bruce K. Bernard, Ph.D.
Company:	CFSAN, FDA		
Fax:	202.418.3131	**Date:**	12 February 2002
Phone:	202.418.3032	**Pages:**	2
Re:	FDA GRAS Notification SRA for Ajinomoto USA Transglutaminase	**CC:**	

☐ **Urgent** X **For Review** ☐ **Please Comment** ☐ **Please Reply**

Comments

Dear Dr. Highbarger,

Thank you for pointing out the three (3) places on page 23 of the original submission in which grams (g) was typed instead of milligrams (mg).

Attached please find a corrected page 23 for your substitution in this notification.

Sincerely,

(b)(6)

Bruce K. Bernard, Ph.D.

000163

1920 L Street, NW, Suite 420, Washington, DC 20036
Telephone: (202) 728 1400 Telefax: (202) 331-3393

Blank Page Inserted for
Pagination

Computer Technology Services, Inc.

000163.001

2) Use of Protein Consumption Data

A second and distinct method for estimating consumption of transglutaminase, as included in all food groups, is possible based upon the known distribution of protein consumption.

According to a number of USDA surveys (i.e. Economic Research Service [1970 – 1991, 1988 – 1991], Household Food Consumption Survey [1994 – 1996]), the intake of protein in the various U.S. subpopulations ranges from 25 – to 111 g/p/day. The estimate of overall mean protein intake is 75 g/p/day. Estimates of the 90th percentile of intake based upon published surveys (USDA Household Food Consumption Survey [1994 – 1996], HHS-NHANES III) range from 84 – 154.5 g/p/d, with an overall estimate of 140 g/p/day. If transglutaminase were added to all food containing protein (100% of the food consumed), an obvious worst case and clearly unachievable situation, total consumption would be 28.5 mg/p/day with a 90th percentile consumption of 53.2 mg/p/day.

Estimates of exposure levels of transglutaminase, like most such intake assessments, tend to substantially over estimate the amount of TG ingested for a host of reasons, like that noted above. These overestimates are especially seen when use level is based on the protein content of a food. For example, there are a large number of protein containing foods consumed in which the enzyme would likely never be used, e.g. nuts consumed as snacks, whole cuts of fish or meat, etc. Hence, taking the unattainable worst case scenario, the addition of transglutaminase to all protein-containing food would result in a consumption of approximately 50 mg/p/day, a level that is clearly consistent (and in many cases lower) than consumption of other enzymes currently permitted in US foods.

3) Application of Transglutaminase at Good Manufacturing Practice (GMP) Levels of Use

Previous requests for determination of the GRAS status of TG have involved food applications in which use levels of the enzyme in commercial practice had been somewhat established by prior extensive laboratory testing, pilot scale implementation and full production experience in other