Appendix 3

BACKGROUND PAPER FOR

GRAS AFFIRMATION OF OATRIM

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1. INTRODUCTION AND DESCRIPTION OF OATRIM

This background paper sets forth Quaker's rationale why Oatrim is safe and generally recognized as safe (GRAS). Quaker's rationale is based on published scientific literature and information generally known to qualified scientific experts, and is corroborated by unpublished information.

Oatrim is an enzymatically processed fraction of oat flour that contains amylodextrins and gluco-oligosaccharides, which are not found in oat flour. It also contains somewhat higher levels of water-soluble beta-glucans than the oat flour from which it was derived, and from which the water, insoluble fat, protein and insoluble fiber components have been largely removed. Oatrim represents about 60% of the starting material which is whole oat flour.

The intended function of Oatrim is as a fat replacer in a variety of products, such as fresh ground meat and poultry, processed meats and poultry products, salad dressings, mayonnaise, baked goods, baking mixes, cheese (processed), yogurt, ice cream and frozen desserts, snack foods, margarines and spreads, icings and frostings, frozen entrees, and confections. It may function as a total or partial fat replacer.

Appendix 1 is a summary table of Oatrim's characteristics.

For all uses of Oatrim, it is expected that Quaker's first year sales of Oatrim will be about 1.5 mm lbs., increasing in 1994 to 4 mm lbs., and to 30 to 75 mm lbs. by 2002.

2. OATRIM MANUFACTURING PROCESS

A. Processing Patent

U.S. patent 4,996,063 was issued on February 26, 1991 to the USDA for the Oatrim process (Appendix 2). Quaker is licensed to manufacture Oatrim under that patent.

2. OATRIM MANUFACTURING PROCESS - Continued

B. Oatrim Process Formula

Water	87.325170%
Oat flour	12.428770%
Sodium hydroxide	0.004700%
Phosphoric acid	0.030600%
Calcium chloride, 100%	0.016290%
Alpha amylase	0.194470%
	100.000000%

Water is used for processing only, and will not be declared as an ingredient of Oatrim.

C. Process Description for Oatrim

Step 1: Water containing at least 50 ppm of calcium chloride is slurried with an appropriate amount of oat flour. The pH is appropriately adjusted.

Step 2: The slurry is pumped through a jet cooker at a temperature of 285°F with a residence time of 10 to 15 seconds for the purpose of gelatinizing the starch fraction of the oat flour.

Step 3: Suitable alpha-amylase, at a dosage of 3 units per gram of oat flour substrate, is added to the gelatized slurry. Enzymatic hydrolysis is conducted at 190°F for six minutes.

Step 4: After hydrolysis, the pH of the slurry is reduced to 4.0 by addition of phosphoric acid and the temperature is increased to 200°F for 10 minutes to inactivate the enzyme.

Step 5: The enzyme-hydrolyzed slurry is centrifuged through a decanter and the supernatant from the decanter is further centrifuged through a disc bowl centrifuge.

Step 6: Supernatant from the disc bowl centrifuge is adjusted to pH 6.0 with sodium hydroxide.

Step 7: The supernatant is either spray dried or drum dried at a drum surface temperature of 285°F and a residence time of 25 to 30 seconds.

Step 8: The resultant product is Oatrim in the form of a creamy white powder.

Appendix 3 is a schematic diagram of the Oatrim process.

3. REGULATORY STATUS OF OATRIM INGREDIENTS AND INTERMEDIATE PRODUCTS

A. Alpha Amylase (Carbohydrase)

While any suitable alpha-amylase may be used in the production of Oatrim, the current enzyme of choice is the thermo-stable alpha-amylase produced by *Bacillus stearothermophilus*. The enzyme from *Bacillus stearothermophilus* currently being used is supplied by Enzyme Bio-Systems, Ltd., and is marketed under the trade name "G-Zyme G995 Alpha-Amylase". Appendix 4 is the technical data sheet for G-Zyme G995.

Alpha amylase is also known as carbohydrase. The carbohydrase enzyme from *Bacillus stearothermophilus* is the subject of a GRAS affirmation petition for use as a direct human food ingredient, filed by CPC International Inc. in September 1983 (Appendix 5 *Federal Register* notice announcing petition filing).

It is expected that other suitable alpha-amylase enzymes, such as those produced by *Bacillus subtilis* and *Bacillus lichenformis*, may be used in the production of Oatrim. In separate communications (Appendix 6), FDA has indicated that the following carbohydrase enzymes may be used in food, subject only to a good manufacturing practice (GMP) limit:

Aspergillus flavus-oryzae
Bacillus subtilis strains
Aspergillus niger
Bacillus lichenformis (mixed carbohydrase and protease enzyme)

Consistent with FDA's correspondence, various amylases from plant, animal, fungal and bacterial sources have been in use for many years and have wide commercial food application, as shown in the following table:

	Bacterial	Fungal	Plant
Syrup manufacture	X	X	X
Dextrose manufacture	X	X	
Baking	X	X	X
Saccharification of fermentation mashes			
Distillery	X	X	X
Brewing	X	X	X
Food dextrin and sugar products	X	X	X
Dry breakfast foods	X	X	X
Chocolate and licorice syrups	X	X	
Starch removal from fruit extracts and juices and from pectin		X	
Scrap candy recovery	X	X	

Source: "Starch Modification in Vegetables", *Handbook of Food Additives*, 1972, p.46.

3. REGULATORY STATUS OF OATRIM INGREDIENTS AND INTERMEDIATE PRODUCTS - Continued

B. Oat Flour

Oat flour is a common ingredient of longstanding food use, and is GRAS on the basis of that usage. There are no known questions about the safety of oat flour.

C. Sodium Hydroxide

Sodium hydroxide is used to adjust the final pH of the oat flour/enzyme/water slurry. Sodium hydroxide has been affirmed as GRAS as a pH control agent, subject only to a GMP limit. 21 CFR 184.1763.

D. Phosphoric Acid

Phosphoric acid is used to adjust the pH of the slurry for optimal enzyme activity. Phosphoric acid is GRAS as a multiple purpose ingredient, subject only to a GMP limit. 21 CFR 182.1073.

E. Calcium Chloride, 100%

Calcium chloride (100%) is used as a synergist to enhance the activity of the enzyme during enzymolysis. Calcium chloride has been affirmed as GRAS for use as a synergist, subject to a GMP limit. 21 CFR 184.1193.

F. Sodium Acid Phosphate

To neutralize the supernatant which has been acidified with phosphoric acid to a pH of 4.0, sodium hydroxide is added to obtain a pH of 6.0. The reaction products are sodium acid phosphate (NaH_2PO_4) and water:

$$H_2PO_4 + NaOH \longrightarrow NaH_2PO_4 + H_2O$$

Sodium acid phosphate is GRAS, subject only to a GMP limit. 21 CFR 182.6085.

G. Ingredient Specifications

Appendix 7 contains ingredient specifications.

4. QUAKER'S RATIONALE WHY OATRIM IS SAFE AND GRAS

In Quaker's view, several independent factors support the conclusion that Oatrim is safe and GRAS. First, Oatrim is similar to oat starch and maltodextrin, two food ingredients of acknowledged safety. Second, the enzymatic manufacturing process for Oatrim is analogous to the biological process for the digestion of starch in humans. Third, safety evaluations of Oatrim's constituents do not reveal any areas of concern. The conclusion that Oatrim is safe and GRAS is corroborated by a short-term toxicity study with Oatrim.

4. QUAKER'S RATIONALE WHY OATRIM IS SAFE AND GRAS - Continued

A. Oatrim is Similar to Oat Starch and Maltodextrin

Starch, a mixture of amylose and amylopectin, can be found in all organs of most higher plants, including the endosperm of seeds. The endosperm of the oat kernel is the starch source for the production of Oatrim.

The amylose fraction of starch is essentially a linear polymer of glucose, with a Degree of Polymerization (DP) ranging from 100-10,000 glucose residues. Amylopectin is a branched polymer of glucose with a DP ranging from 10,000-100,000 glucose residues. The amylose content of oats comprises about 27% of the starch fraction, the balance being amylopectin.

The principal difference between oat starch and Oatrim is that about 35% of the starch in Oatrim has been hydrolyzed by alpha-amylase to form lower DP glucose polymers. Normally, the lower DP limit for amylose is about 100, and for amylopectin, about 10,000. The DP resulting from enzymatic hydrolysis ranges from 2-100. The glucose polymers of DP2-DP10 are designated as gluco-oligosaccharides, the higher DP polymers are designated as amylodextrins.

Oatrim is the water soluble, partially enzymatically hydrolyzed starch fraction of oats, but, because the separation of the treated starch fraction from the protein/fat/insoluble fiber fraction is not complete, some protein, fat, and insoluble fiber are found in Oatrim. A comparison of these fractions is shown below:

	Oat Flour*	Oatrim*
Protein	15%	4.0%
Fat	7.5%	2.1%
Total Dietary Fiber (TDF)	14%	6.9%
Beta-glucan	3.8%	4-6%

*Values will vary as a result of growing conditions and different cultivars.

Maltodextrin, a product similar in several respects to Oatrim, is produced by the partial enzymatic hydrolysis of corn starch. Maltodextrin is GRAS, 21 CFR 184.1444. The principal differences between Oatrim and maltodextrin produced from corn starch are as follows:

- Differing degrees of polymerization, but still within the DP2-DP100 range.

- Presence of some protein, fat and TDF, including beta-glucan, in Oatrim, whereas there is virtually none of these constituents in maltodextrin.

Therefore, Oatrim is similar to oat starch and maltodextrin, two food ingredients of acknowledged safety. The differences between Oatrim and oat starch or maltodextrin are unrelated to the safety of the ingredients.

4. QUAKER'S RATIONALE WHY OATRIM IS SAFE AND GRAS - Continued

B. The Enzymatic Manufacturing Process for Oatrim is Analogous to The Biological Process for the Digestion of Starch in Humans

1. Key Reaction Products of the Enzymatic Hydrolysis of Oat Starch by Alpha-Amylase

Partial, random, "endo" cleavage of alpha 1-4 linkages of the starch polymers by alpha-amylase, results in amylodextrins, gluco-oligosaccharides and unchanged starch (Figure 1). The alpha-amylase has no effect on the oat constituents: fiber, beta-glucan, protein, fat and minerals.

Action of Amylases on Starch

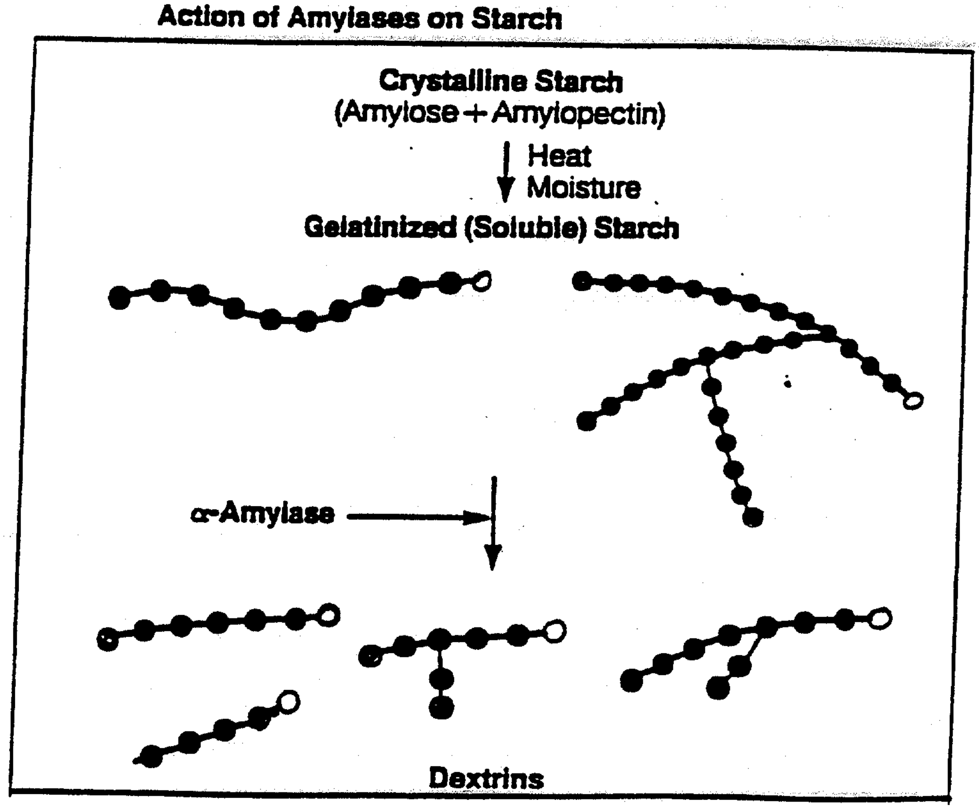

Figure 1

○ Reducing Unit

● Glucose Unit

4. QUAKER'S RATIONALE WHY OATRIM IS SAFE AND GRAS

B. The Enzymatic Manufacturing Process for Oatrim is Analgous to the Biological Process for the Digestion of Starch in Humans - Continued

2. Starch Digestion in Humans

The action of alpha-amylase on oat starch to produce shorter chain glucose polymers is very similar to the action of alpha-amylase in the human body to digest starch. In humans, alpha-amylases are produced by the salivary and pancreatic glands. However, the action of salivary amylase is limited to the hydrolysis of starch to lower DP polymers.

The principal hydrolysis of starch by pancreatic alpha-amylase occurs within the lumen of the small intestine. This action, as with starch in oats, produces shorter chain polymers of lower average DP than the intact starch molecule. The lower DP polymers are subsequently acted on by other enzymes, including glucoamylase, which cleaves the polymers into individual glucose residues that are absorbed by the body.

It can be readily seen that the external action of alpha-amylase on oat starch is equivalent to the internal action by salivary and pancreatic alpha-amylase. In both cases, shorter chain polymers are produced, which are, in either case, after ingestion, subjected to the action of glucoamylase in the small intestine to produce glucose monomers.

C. Safety Evaluations Do Not Reveal Any Areas of Concern

1. Joint FAO/WHO Expert Committee on Food Additives

The Joint FAO/WHO expert Committee on Food Additives (Expert Committee) has prepared monographs on enzymes derived from *Aspergillus oryzae* and *Aspergillus niger*, and carbohydrase enzymes derived from *B. lichenformis*. WHO Food Additive Series 20, 22 "Toxicological Evaluation of Certain Food Additives and Contaminants" (Appendix 8). No comparable monograph has yet been prepared for alpha-amylase from *B. stearothermophilus*, but the enzyme is currently being reviewed by the Expert Committee (see Appendix 9 newsletter article).

The relevant findings of the Joint Expert Committee were as follows:

a. *Aspergillus oryzae*

The level causing no toxicological effects in the rat, at a level of 10% in the diet, is equivalent to 7 g/kg b.w./day. The Expert Committee concluded that the enzyme is "acceptable for use in food when used according to good manufacturing practices".

4. QUAKER'S RATIONALE WHY OATRIM IS SAFE AND GRAS

C. Safety Evaluations Do Not Reveal Any Areas of Concern

1. Joint FAO/WHO Expert Committee on Food Additives - Continued

b. Carbohydrase from Aspergillus niger

All enzyme preparations tested showed no-observed-effect-levels greater than 100 mg TOS/kg b.w./day in 90 day studies in rats. The estimate of acceptable daily intake was 0-1 mg TOS/kg b.w. for each tested enzyme preparation.

c. Carbohydrase from B. lichenformis

The no-observed-effect level in a short-term study in dogs was 2% of the diet, equal to 450 mg/kg b.w. An acceptable daily intake for man was "not specified".

2. Alpha-amylase from B. stearothermophilus

The amount of inactivated Bacillus stearothermophilus contained in Oatrim is about 1.8 units per gram. A published study by McKenzie & Petsel, entitled "Subchronic Toxicity Studies in Dogs and In Utero Rats Fed Diets Containing Bacillus stearothermophilus Alpha-Amylase From a Natural or Recombinant DNA Host" (Appendix 10) concluded the no-observable-effect-level for this alpha-amylase fed to dogs or rats is 36 units/g food.

3. Oligosaccharides

The gluco-oligosaccharide mixture resulting from the enzymatic hydrolysis of amylose and amylopectin have degrees of polymerization (DP) from 2-10 (Appendix 11).

There has been concern about an excessive intake of oligosaccharides of the type represented by raffinose, stachyose and verbascose found in soybeans and other legumes and other non alpha-glucan polysaccharides and some sugars such as lactulose. These oligosaccharides are not enzymatically hydrolyzed in the small intestine of humans; they are subsequently fermented by the microflora in the large intestine to produce carbon dioxide and hydrogen in addition to short-chain fatty acids and lactate, which may, in turn, be absorbed and metabolized. Symptoms of high dosages and prolonged use of the non-alpha-glucan polysaccharides are flatus, enlargement of the large intestine, and may include hypercalciuria and aciduria, renal mineralization and adrenal hyperplasia.

The oligosaccharides, DP2-DP10, resulting from the enzymatic hydrolysis of starch by alpha-amylase, are not of this type and are hydrolyzed in humans to monosaccharides by glucoamylase and other carbohydrases in the lumen and brush border surface membrane of the small intestine (Appendix 12. (Crane, R. K., "Physiological Effects of Food Carbohydrates". ACS Symposium Series 15, 1975)).

4. QUAKER'S RATIONALE WHY OATRIM IS SAFE AND GRAS

C. Safety Evaluations Do Not Reveal Any Areas of Concern - Continued

4. Beta-Glucan

Beta-glucan is a mostly water-soluble fiber found in appreciable levels in oats and barley. The amount of beta-glucan varies as a function of the cultivar and growing conditions. The beta-glucan content of oats, although variable, is generally found to be about 2.8-5.5% (Appendix 13 (Oats Chemistry and Technology, F.W. Webster, Editor, American Association of Cereal Chemists, 1986. Chapter on "Oat Beta-Glucan: Structure, Location and Properties")). The specified value of beta-glucan in Oatrim is 5% and will likely vary from about 4-6%, depending on the beta-glucan content of the oats from which it was derived and by milling techniques.

As a point of comparison, the American Association of Cereal Chemists has defined oat bran as having a minimum beta-glucan content of 5.5%. The concentration of beta-glucan in Oatrim is within the range of the beta-glucan normally found in oats and oat bran, and does not raise any safety concerns (Appendix 14).

It is to be expected that, as Oatrim proliferates as a fat replacer in a wide variety of products, an increase in the intake of soluble fiber (beta-glucan) by consumers would result. An increase in fiber intake is consistent with the report on "Physiological Effects and Health Consequences of Dietary Fiber" by the Federation of American Societies for Experimental Biology (FASEB)(1987), the Surgeon General's Report on Nutrition and Health (1988) and the Public Health Service report, "Healthy People 2000", which affirms FASEB's and FDA's recommended level of intake for dietary fiber. The recommmended levels of intake for dietary fiber in the FASEB report and FDA's proposed dietary fiber "daily reference value" is in the range of 20-30 g/day for the healthy adult population of the United States, whereas the estimates of current actual intake range from 2.5-4.8 g/day to 11.1-23.3 g/day.

5. Oatrim

Hazelton Wisconsin, Inc. conducted a four week dietary toxicity study with Oatrim in rats at dietary levels of 7.5% and 15%. The researchers concluded that dietary exposure to 7.5% and 15% Oatrim for at least four weeks had no-observable-effects on the test animals. A detailed report of this study, which was submitted to FDA by ConAgra, was obtained from FDA pursuant to the Freedom of Information Act (Appendix 15). Quaker believes that this study corroborates the conclusions set forth above, namely, Oatrim is safe and GRAS.