

18.2 PRINCIPLE OF PROTEIN AND PROTEOLYSIS

MEASUREMENT (35,36,37)

Protein measurements are made by mixing weighed samples with a known volume of reagent dye solution containing acid orange-12 dye. Proteins in the sample react with the dye to form a precipitate. The remaining unreacted dye concentration is inversely proportional to the protein content of the sample. A Udy colorimeter is used to measure the dye concentration can be set to display the percent protein directly. The Udy colorimeter is described in more detail in the measurement section. The following paragraphs give additional information about the chemistry of the reaction.

The U.S.A. dye, acid orange-12, is used because it, 1) binds strongly with proteins, 2) has an excellent color/protein sensitivity ratio, 3) gives clear, stable solutions, and 4) is easily purified for use as its own primary standard. It is essentially non-hydroponics in pure form. Dyes which have been tried by others do not have these properties and so have limited use. Acid orange-12 reacts with the three basic amino acid (BBA) functional groups on proteins. The strong electrostatic bond forms an insoluble protein dye complex. This precipitate is easily filtered or centrifuged so that the remaining dye concentration can be measured color metrically. Solutions of the dye have broad absorption peak at 482nm and follow Beer's Law of optical absorption.

Proteins from a natural source have an essentially constant ratio of basic amino acids to total amino acids or protein. This is genetically controlled by the organism. Each basic site originating from lysine (amino group), arginine (guanidino group) or histidine (imidazole ring) binds one molecule of dye. Measuring both Kjeldahl total nitrogen and equipment dye concentration on a large number of samples of a given commodity, permits the calculation of a regression equation. From this, a dye binding capacity (DBC) value for that specific protein system is established. This is a genetic constant for the given protein system, and does not change.

The DBC constant incorporates an average non-protein nitrogen (NPN) value since it is derived from the correlation with numerous Kjeldahl total nitrogen measurements. Individual samples may have appreciably different NPN levels. A total nitrogen measurement for such a sample would be less indicative of the true protein value. Once the DBC is established for a given protein system, the dye-binding method of estimating protein is independent and absolute. It is not responsive to variations in NPN.

When the ratio of BBA to protein is not genetically controlled, such as in random mixtures of commodities, or when the percentage of NPN is changes, as in the ultra filtration concentration of whey proteins, protein measured by dye binding may not correlate as well with Kjeldahl total nitrogen measurements. Denaturation of lysine by overheating or Maillard type reactions will also lower the correlation between protein measured by dye-binding and Kjeldahl total nitrogen.

The protein dye reaction requires some controls over certain factors that influence the equilibrium dye concentration. Temperature of the sample dye mixture has a small but significant effect on the degree of protein dye association. Different ions and buffer systems have potentially large effects on the DBC value. A mass action effect is significant at low dye concentrations. Mass action and ionic effects are negligible or absent entirely in the normal concentration range used for the buffer system now used in Udy Reagent Dye Solution. Starch from some commodities binds a small, but significant, amount of dye. This does not cause loss of accuracy because the starch content and its dye binding are essentially constant. Correlations coefficients over 99% are normally obtained between equilibrium dye concentration and total nitrogen measurements.

The Udy dye binding method is being used on a large number of protein systems. These include, but are not limited to, all grains, oilseeds, forages, legumes, and meat and dairy products. Proteolysis in dairy products (cheese, yogurt, starter media etc.) can also be studied using the Udy dye binding method due to the fact that as the larger protein complexes breakdown from the inferent enzyme degradation, heat denaturations etc. they will show decreases of true

protein levels using the Udy dye binding method (over a given time or due to experimentally treated conditions). An explanation of this is the Udy Reagent dye binds with the larger protein complexes (larger than 3 amino acids units). So, as the protein complexes breakdown to smaller polypeptide chain lengths, the ratio of large readable complex proteins to the unreadable amino acid units' change. The result is a decrease in protein read on the Udy protein colorimeter for the given product (detected proteolysis).

Apparatus and Reagents

Udy model MKP protein analyzer including an Udy dairy tester (colorimeter) along with standard Reagents and reference dye solutions.

Babcock centrifuge for spinning down the culture

Initial set-up

1. Drain bottle and tubing attach the end of the drain tube (feed tubing up through the bottom of the unit) to the covet tip. It may be necessary to moisten the tip of the covet with water to make the attachment easier. Wipe off any fingerprints on the covet with a soft tissue or a soft cloth.
2. After the tubing is attached, seat the covet stop making sure that the scribe mark on the top of the covet faces the front.
3. Connect the power supply to the unit and plug the power supply transformer into the appropriate outlet. Make sure the unit switch is in the off position before plugging in the unit.
4. Turn on and warm up the unit for 30 minutes before operating.
5. Follow the operating instructions below.

Protein test set-up

1. Fill the covet with de-mineralized water. Start the flow through process by gently squeezing the drain bottle. Place your finger over the vent hole of the drain bottle cap and release.
2. Place colorimeter switch to **CAL** position.
3. Adjust **ZERO** potentiometer to read .000 on meter.
4. Fill the covet with ".660" standard reference dye solution.
5. Adjust **GAIN** potentiometer to read .660 on the meter.

6. Place the colorimeter switch to percentage protein position.
7. Place **MENU** switch on appropriate channel No. 1, 2, 3, or 4 for raw or homogenized milk, starter etc.
8. Filter sample (25—30 drops or until stable) into cuvet and read percentage protein or use (in the Cal position) calibration chart supplied by Udy Corp. *(See sample prep).
9. For doing proteolysis studies compare protein differences between treated and untreated samples (can also compare as product ages).

Sample prep. Milk, starter media, cheese, supernatant etc.

1. Warm sample to 37C and mix.
2. Weigh out 2.06 grams (2.0ml) of milk, starter media etc. sample into a tared 60ml polyethylene sample bottle.
3. Dispense 40.37grams (40ml) Reagent dye solution into the sample bottle. Cap the sample bottle and shake at least 15-20 seconds.
4. Adjust the temperature of the solution to 25C.
5. Add the filter Cap assembly to the sample bottle. Filter 24-30 drops into the cuvet. Read in the Cal mode use percentage protein=6.10 (1.257 reading).

18.3 Food challenge test, for checking allergic response in humans, using cheese made with starter media which has non enzyme treated soy solids vs. cheese made with starter media which has enzyme treated soy solids vs. cheese made with starter media which has no soy solids as an ingredient:

A group of 11 people were selected as human subjects to consume the cheeses made with both IMAC starter medium which has enzyme treated and non enzyme treated soy solids as an ingredient, separately. Out of eleven people, 3 people are allergic to soy products (prior clinical history and also confirmed by skin prick test), but not to milk or wheat. The skin prick test procedure used is as follows: 10 grams of soy protein concentrate was dissolved in 90ml of water. Ph of the solution was adjusted to 7.0 (neutral). The solution was autoclaved at 120C for 15 minutes and cooled to room temperature. A drop of the sterilized preparation was used as antigen to determine the soy sensitivity. After placing a drop of the sterile soy protein solution on the skin, a sterile needle was inserted through the external surface of the skin, but not deep enough to draw the blood. The individual was watched for 15 minutes for the positive wheal and flare reaction. If there is no redness and wheal and flare reaction, the individual according to skin prick test is not sensitive to a particular allergen. Their age groups are 11, 33 year old males and a 57 year old female. One person is allergic to milk (lactose intolerance) but not to fermented cheese, soy, and wheat. Five people are not allergic to milk, wheat, and soy. Their age groups range from 25 to 59, which includes two females and three males. Two people are not allergic to milk or wheat, but they do not know their exact status on allergy to soy, because they do not consume any soy products. Their age groups are 35 year old male and 45 year old female. One of the panel members is a registered nurse, who can monitor the allergic symptoms better than others. All eleven of these members were told ahead of time they will be eating cheese on salad and pizza. However, they were not told which cheese is made with which starter culture. This test is a double blind placebo controlled food challenge or double blind challenge, where none of the panel members knew what cheese they were eating on pizza and salad. The cheese made with IMAC starter medium with non enzyme hydrolyzed but fermented soy solids are treated as a positive control. The cheese made with IMAC starter with enzyme hydrolyzed and fermented soy solids, are treated as a test sample. Whereas, the cheese made with no soy

solids in the starter, is treated as a negative control or placebo. All the panel members were asked not to eat any soy products, and also not to take any antihistamine drugs or other medications, two weeks prior to taking the test and also during the test period. If they had any abnormal symptoms, prior to taking the food challenge test, they were asked to report promptly. Each member was given a minimum of 75 grams of cheese either on salad or on pizza. Each person was fed 1 ounce of shredded cheese on salad. Afterwards they were given two slices of pizza with 50 grams of cheese. All of them were asked to watch for symptoms of allergy such as watery eyes, itching, sneezing, upset stomach and loose motions etc. First day, they were fed cheese made with IMAC starter with non enzyme treated soy solids. Two days later they were fed cheese made with IMAC starter with enzyme treated soy solids. At the end of the trial they were fed cheese made with starter cultures with no soy in it. None of the eleven members complained about any allergy symptoms indicating that soy has been rendered non allergenic. It was surprising to note that enzyme immunoassay detected some allergens in the cheese made with starter with non enzyme treated soy solids. However, even soy allergic people did not exhibit any symptoms. It goes to prove that the high heat treatments and fermentation employed in starter making, and rennet enzyme used in cheese making along with the starter bacterial growth must have made soy Non allergenic in human body. These results were reproduced several times with the same panel. We have been conducting such tests several times in 2003, 2004 and 2005. This test was conducted under the guidance of Ms. Marlene Hickman.

Although, enzyme immunoassay did detect some soy allergens in the cheese, none of the soy sensitive people exhibited any allergy symptoms. However, the cheese made with IMAC starter with enzyme treated (protease) soy solids, was negative for soy allergens with enzyme immunoassay. As expected, it did not induce any allergy symptoms in the soy allergic group of people. Since IMAC system utilizes enzymatic treatment of soy solids in the starter medium, it maybe safe to assume that it will not cause any allergic reactions of soy. It further proves why over 500,000,000 people in the U.S., who have consumed over 650,000,000lbs. of cheese made with enzyme treated soy solids in IMAC medium, did not exhibit any allergy symptoms, as evidence by no complaints from the health departments anywhere in the United States. Although it cannot

be called as a controlled test, this is more like "an open challenge testing" in medical terms. If the results are coded properly, it is the best form of proof or test. In our case, we did monitor it carefully. We could not make such comment without the wide range of statistics we have collected. This control test only proves that even people, who are allergic to soy, can consume cheese made with IMAC starter with no problems, provided it is enzyme treated and fermented, and made into cheese using coagulating enzymes etc. Of course it is the only way cheese can be manufactured. For our own verification and for the benefit of public health, we have communicated on a bi-monthly basis with all the Q.A. personnel of the manufacturing companies using IMAC system, for any field or health department or customer complaints on cheeses.

In a separate test, the same panel members were fed blended cheeses, individually made with enzyme hydrolyzed and fermented IMAC media with soy solids. The cheese blend was prepared by blending 20% of each cheese (cheddar, provolone, asiago, pamesan and romano). Two ounces of such blend was spread on salad was fed to each panel member. This was an open challenge food test, where placebo is not included, and the food being tested is known to the panel. Each panel member was asked to report any abnormal symptoms promptly. Our intention here was to segregate and feed individual cheeses, if any panel member exhibited allergy symptoms for the mixed blend. None of the panel members reported any allergy symptoms indicating the cheese blend made using IMAC medium with enzyme hydrolyzed and fermented soy solids, did not have any allergic soy antigens.

To start out, allergy is caused by protein molecules which have longer chain peptides. When the protein is proteolyzed using protease enzyme, and/or microbial protease enzyme and/or, protease enzymes used in cheese making, additional bacterial fermentation in the cheese vat along with the reduced oxygen conditions encountered in the cheese, the soy allergenic protein is rendered non allergenic. If the protein is broken down significantly, the human body cannot detect the antigen because the antigen is further metabolized in the human body very easily. If the soy solids are used through any other route other than cheese starters, apparently they will induce allergic reactions in human beings sensitive to such proteins. To date there is no prior literature in the world stating that

cheese made with starter with enzyme treated and fermented soy proteins, caused allergies in human beings. The reason we have selected three different age groups who are sensitive to soy solids, is to check the affect of age on induction of symptoms of allergy. All three age groups did not exhibit any signs of allergy indicating fermented starter is safe and will not induce soy allergy even in sensitive individuals. Also a person who is allergic to milk, but not cheese was included, to check if soy solids alter his allergic reactions to milk solids in cheese. The subject did not exhibit any allergic reactions indicating that the cheese made with IMAC starter medium with either enzyme treated or non enzyme treated soy solids did not induce any allergic reactions.

IMAC took total liability to pay all the bills and compensation if a person got severely sick during their participation in our study. One of the panel members is a registered nurse.

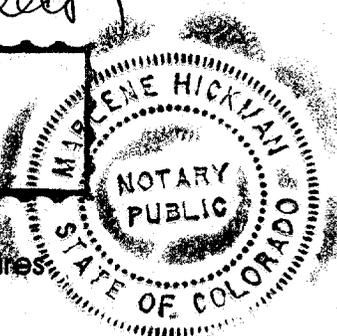
List and names of the members who participated in our allergy studies						
Number	Name	Age	Gender	Prior known Allergies		
				Milk	Wheat	Soy
1	Marlene Hickman	41	Female	-	-	-
2	Mali Reddy	58	Male	-	-	-
3	Venkat Mantha	45	Male	-	-	-
4	Renee W.	37	Female	-	-	-
5	Kalvin Hickman	11	Male	-	-	+ (a)
6	Michael Hickman	33	Male	-	-	+ (a)
7	Adam Rose	27	Male	-	-	Not sure (b)
8	Chuck Groves	32	Male	-	-	Not sure (b)
9	Laura Mounce	44	Female	-	-	Not sure (b)
10	Robert Williams	44	Male	+ (c)	-	
11	Juanita Gonzales	57	Female	-	-	+ (a)

- (a) This group cannot stand soy and do not feel well after eating and proven allergic to soy using skin prick test.
- (b) This group does not particularly care for it. If it does not bother them, they are ok.
- (c) Robert who is lactose intolerant. However, the fermented dairy products such as cheese, yogurt etc. does not bother him.

I, Marlene Hickman, Supervised the above food challenge tests. I certify the data is true and accurate, to the best of my knowledge.

Marlene Hickman

MARLENE HICKMAN
Notary Public
State of Colorado



My Commission Expires
May 15, 2007

Table A: Symptoms monitored for allergic reactions after feeding mozzarella (a) cheese made with enzyme treated soy solids used as an ingredient in the fermented starter culture.

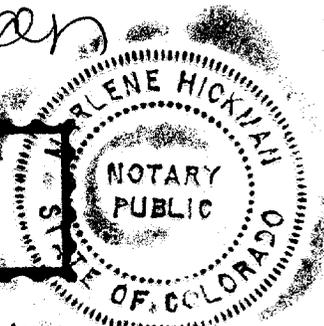
Number	Person	Allergy symptoms monitored						
		Watery eyes	Nasal itching	Uneasiness in breathing	Stomach irritation	Diarrhea	Dizziness	Any other abnormal systems
1	Marlene Hickman	-	-	-	-	-	-	-
2	Mali Reddy	-	-	-	-	-	-	-
3	Venkat Mantha	-	-	-	-	-	-	-
4	Renee W.	-	-	-	-	-	-	-
5	Kalvin Hickman	-	-	-	-	-	-	-
6	Michael Hickman	-	-	-	-	-	-	-
7	Adam Rose	-	-	-	-	-	-	-
8	Chuck Groves	-	-	-	-	-	-	-
9	Laura Mounce	-	-	-	-	-	-	-
10	Robert Williams	-	-	-	-	-	-	-
11	Juanita Gonzales	-	-	-	-	-	-	-

(a) One ounce of shredded mozzarella cheese spread on salad was given to each individual. In addition 50 grams of cheese was baked on pizza and fed to each person/per serving.

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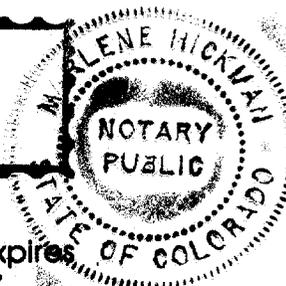
Table C: Symptoms monitored for allergic reactions after feeding mozzarella cheese made with fermented starter culture with no soy solids used as an ingredient.

Number	Person	Allergy symptoms monitored						
		Watery eyes	Nasal itching	Uneasiness in breathing	Stomach irritation	Diarrhea	Dizziness	Any other abnormal systems
1	Marlene Hickman	-	-	-	-	-	-	-
2	Mali Reddy	-	-	-	-	-	-	-
3	Venkat Mantha	-	-	-	-	-	-	-
4	Renee W.	-	-	-	-	-	-	-
5	Kalvin Hickman	-	-	-	-	-	-	-
6	Michael Hickman	-	-	-	-	-	-	-
7	Adam Rose	-	-	-	-	-	-	-
8	Chuck Groves	-	-	-	-	-	-	-
9	Laura Mounce	-	-	-	-	-	-	-
10	Robert Williams	-	-	-	-	-	-	-
11	Juanita Gonzales	-	-	-	-	-	-	-

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State of Colorado**



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Table D: Symptoms monitored for allergic reactions after feeding a blend of parmesan, romano, asiago, provolone, and cheddar cheeses made with enzyme treated soy solids used as an ingredient in the fermented starter culture (1, 2).

Number	Person	Allergy symptoms monitored						
		Watery eyes	Nasal itching	Uneasiness in breathing	Stomach irritation	Diarrhea	Dizziness	Any other abnormal systems
1	Marlene Hickman	-	-	-	-	-	-	-
2	Mali Reddy	-	-	-	-	-	-	-
3	Venkat Mantha	-	-	-	-	-	-	-
4	Renee W.	-	-	-	-	-	-	-
5	Kalvin Hickman	-	-	-	-	-	-	-
6	Michael Hickman	-	-	-	-	-	-	-
7	Adam Rose	-	-	-	-	-	-	-
8	Chuck Groves	-	-	-	-	-	-	-
9	Laura Mounce	-	-	-	-	-	-	-
10	Robert Williams	-	-	-	-	-	-	-
11	Juanita Gonzales	-	-	-	-	-	-	-

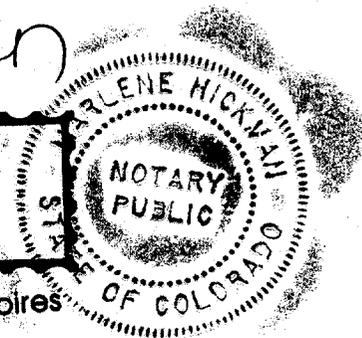
(1) If any member showed any allergic reaction to the blend, our test protocol was to run tests on each cheese with that particular individual.

(2) For every 100 grams twenty grams of each cheese is blended and two ounces of such blend is spread on salad and fed to each panel member.

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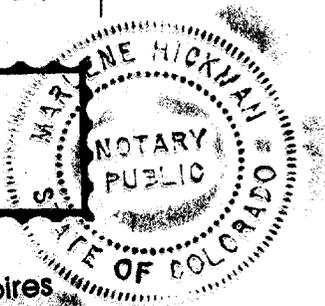
Table B: Symptoms monitored for allergic reactions after feeding mozzarella cheese made with non enzyme treated soy solids used as an ingredient in the fermented starter culture.

Number	Person	Allergy symptoms monitored						
		Watery eyes	Nasal itching	Uneasiness in breathing	Stomach irritation	Diarrhea	Dizziness	Any other abnormal systems
1	Marlene Hickman	-	-	-	-	-	-	-
2	Mali Reddy	-	-	-	-	-	-	-
3	Venkat Mantha	-	-	-	-	-	-	-
4	Renee W.	-	-	-	-	-	-	-
5	Kalvin Hickman	-	-	-	-	-	-	-
6	Michael Hickman	-	-	-	-	-	-	-
7	Adam Rose	-	-	-	-	-	-	-
8	Chuck Groves	-	-	-	-	-	-	-
9	Laura Mounce	-	-	-	-	-	-	-
10	Robert Williams	-	-	-	-	-	-	-
11	Juanita Gonzales	-	-	-	-	-	-	-

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The food allergy in human being to the intact soy allergen in the food (cheese) can be detected with the following procedures: 1. laboratory methods 2. experimental clinical test laboratory methods. The laboratory methods include: 1. radio allerge sorbent test (rast); 2. radio allegro sorbent procedure (RASP); 3. food immune complex assay (FICA); 4. fluorescent allegro sorbent test (FAST); 5. cytotoxic test; 6. skin testing; 7. provocation neutralization and serial dilution titration technique; 8. acupuncture technique; 9. kinesiology (muscle testing). Whereas, the experimental clinical tests or experimental challenge methods include: 1. oligoantigenic dye; and 2. dietary food with food challenge.

The details of the above tests with their relative advantages and disadvantages are clearly written in the article, titled Food Allergy testing by Stephen Barrie (41). This article clearly states that according to many physicians, orthodox practitioners, clinical ecologists and naturopathic physicians, the food challenge is an accurate and useful procedure and is the definite method ("gold standard") for diagnosing food sensitivities. This article summaries with a conclusion that since every procedure has its advantage and disadvantage, each practitioner will have to develop their own operative plan. Article also states that no matter which methods are used for food allergy testing, there is a wonderful cross check for results if the offending food eliminated, the patient feels better, if it isn't he or she doesn't!

Using IMAC starter media (enzyme treated and fermented hydrolyzed soy solids as one of the ingredient), 650,000,000lbs. of cheese was manufactured in the Unites States and it was consumed by over 500,000,000 people, without a single registered complaint anywhere in the United States. It goes to prove that cheese made with the enzyme treated and then fermented with appropriate starter culture with soy solids, cannot be allergenic to human beings. It is better than gold standard. You cannot have better experimental clinical test (food challenge test) than this. However, we also ran laboratory food challenge tests in limited human subjects using the cheese made with enzyme treated and fermented

starter with soy solids vs. non enzyme treated but fermented starter soy solids vs. control starter with no soy solids. These studies confirmed that soy allergens are absent in cheese made using enzyme hydrolyzed and fermented starter with soy solids. The comments made by FDA i.e. Dr. Gottlieb (21) have excellent validity. Notification did not determine the lack of antigenicity in the starter medium. However, notification proved that cheese made with starter with soy solids i.e. enzyme hydrolyzed and then fermented with appropriate lactic culture, did not have any antigen as per the invitro allergen test. As per the "guidelines of the radioallergosorbent test (rast) methods for allergen specific immunoglobulin E (IgE) 510 (k) S; final guidance for the industry and FDA," the rast test (EIA) must include positive and negative controls (40). Infact in our test, we have included cheese which is positive for soy allergen (positive control) and also cheese which did not have any soy (negative control). Whereas the test sample was the cheese made with starter (with soy solids) which was enzyme hydrolyzed and then fermented with appropriate starter culture. Also, according to FDA guidelines (40) the rast test results should not be based on vitro test only, they must be backed up by challenge tests and clinical history. In our case, we have proved them with human subjects using double blinded placebo studies. We had a wide statistic of 600,000,000lbs. of cheese made with IMAC starter and such cheese fed to 500,000,000 people in U.S. with not even single complaint in the last 5 years. The geographical distribution of such cheese manufacture and distribution to consumers in the U.S. was known to the concerned U.S.D.A., and state level inspections and health authorities, as evidence by their communications and dialoging with IMAC. Invitro allergen tests have been reproduced in duplicates and the results were highly reproducible. Four different cheese samples (100 grams) from each batch were blended to arrive at 400 grams total cheese. Like that, similarly the cheese samples were collected from 4 different batches. The entire mass was blended to arrive at 1600 grams of cheese. Out of this 1600 grams 10 grams was used for each test samples. Duplicate samples were prepared by selecting another 10 grams of cheese from the four batch blend of 1600 grams. This gave us an excellent sample for the

test. Ten grams of the cheese was blended with 90ml of 2% sodium citrate to arrive at 10% solution, which is used for the invitro allergen test. Similar sampling and preparation of samples were followed with the positive control cheese, (where non enzyme hydrolyzed but fermented media with soy solids used), test sample cheese, (where enzyme hydrolyzed and fermented media with soy solids used), and the negative control cheese, (where media used did not have any soy solids). Origin of sera was human and was obtained by the National Jewish Hospital, Immunology lab, Denver, Colorado (34).

If you transpose the proteolysis data with fluoro enzyme immunoassay test results, it is quite evident that starter media with soy solids, if they are not treated with protease enzyme but fermented with only cheese cultures, and then made cheese, tested positive for soy allergens. Whereas, media which was enzyme treated and then fermented with cheese starter culture exhibited >99.5% proteolysis in the liquid starter (IMAC procedure). When such starter was used to make cheese, the finished cheese when checked using fluoro enzyme immunoassay, tested negative for soy allergens.

Surprisingly both the types of cheese did not excite any allergic reactions with oral food challenge test, even in the soy allergic people. This is the reason why FDA insists that invitro allergic test must be interpreted in conjunction with clinical trials and other tests. It is also cited in the literature that rast test in some studies have shown poor correlation with individual food challenge, while in other studies an excellent positive correlation was obtained. The most logical explanation we can offer using our proteolysis data is that culturing alone proteolyzed 48% of the protein. When such a culture was inoculated into cheese milk to make cheese, the protein will be broken down further. Even though a small amount of allergens are detected using fluoro enzyme immunoassay in cheese, the food challenge test did not excite allergic symptoms. This would mean that the already subdued soy allergen could have been further broken down upon ingestion. It goes to explain that starter cultures have inherent ability to alter the anitgenicity of the soy

proteins in conjunction with rennet enzyme used in the cheese making. However, IMAC system of treating media with proteolytic enzyme followed by, fermentation with starter culture with extensive proteolysis, followed by the use of rennet enzyme during cheese making along with such starter culture, and finally due to liberation of other enzymes during cheese making from inactivated or dead starter cultures, alters the allergenic soy antigens to non allergenic state in the cheese. Thus, such cheese does not excite allergic reaction and will not harm even to the allergen sensitive human beings.

18.4 The effect of growing lactic cultures and coccus and rod cultures using enzyme treated soy protein as sole source of protein, followed by making laboratory controlled simulated cheese products, cheddar and mozzarella, and feeding such products to soy sensitive human subjects, to see if allergen exists in the cheese product:

Table 1: Composition of the medium:	
Ingredient	Percentage
Enzyme treated soy protein concentrate	50
Mono sodium phosphate	1.97
Di-sodium phosphate	2.0
Lactose	24
Glucose	17
Magnesium sulfate	0.01
Manganese chloride	0.01
Ferrous sulphate	0.01
Calcium chloride	2.0
Autolyzed yeast extract	3.0

The medium was reconstituted at 10% solids and held at 37C for one hour. Then it was heat treated to 185F and held at that temperature for 45 minutes. The liquid medium was divided into two parts, 1 part (100 ml) was inoculated with a mixed lactic culture (3 strains) and incubated at 90F for 16 hours. The other 100 ml was inoculated with a mixed culture having two strains of streptococcus thermophilus and 3 stains of each of lactobacillus bulgaricus, lactobacillus lactis and lactobacillus helveticus. The inoculated medium was incubated at 108F for 8 hours. The microscopy and other quality control tests were conducted to check the suitability of the finished starter to use it in the cheese making.

Cheddar (American) cheese simulation:

1000ml of pasteurized whole milk was inoculated with lactic culture (grown with enzyme hydrolyzed soy solids as a source of protein) at the rate of 2.0%t i.e., 20ml/1 liter of cheese milk. The milk was incubated at 90F for 45 minutes (cheese ripening period). The coagulant, rennet (chymosin), was added to the ripened milk at the rate of 1½ ounce/1000lbs. of milk i.e. 1.1ml/liter of cheese milk. The milk was allowed to coagulate for 30 minutes at 90F (setting). The milk was heated to 102F with in a span of 45 minutes (cooking). Then the milk was held at that temperature for an additional 20 minutes (comparable to dipping

and matting). Then, the temperature of the contents were lowered to 98F and held at temperature until pH of the coagulum came down to 5.15 to 5.2 (comparable to cheddaring and milling). At this stage, 30 grams of salt was added to the coagulum and blended (salting). The salted coagulated mass was allowed to sit at room temperature for 6 hours (which is comparable to pressing). Then the entire contents including whey were placed in a refrigerator for 3 days. For the sake of the test, we kept it only for 3 days (generally cheddar cheese is ripened for 3 to 9 months). At this stage, the entire one liter of the salted coagulated mass with whey was blended with 1 liter of orange juice, and 150ml of the blend was given to each panel member (non allergic to milk) to consume, to check for any allergic reactions to soy. The panel members were asked not to eat any soy products or take any antihistamine drugs or medications two weeks prior to taking the test. This is an "open challenge food testing" where panel member is aware of what he or she is eating or drinking. The panel members were asked to report any abnormal allergy related symptoms promptly. In this test we have included whey also, to mass balance the migration of any intact allergen. The results are presented in table 1. None of the panel members including three soy allergic individuals showed any signs of allergy, indicating that the cheese and whey did not have any intact soy antigens to excite allergy symptoms. Since one panel member was "intolerant to lactose", he was not included in this study.

Table 2: Symptoms monitored for allergic reactions after feeding simulated cheddar cheese along with whey, made with enzyme treated soy solids used as an ingredient in the fermented starter culture.

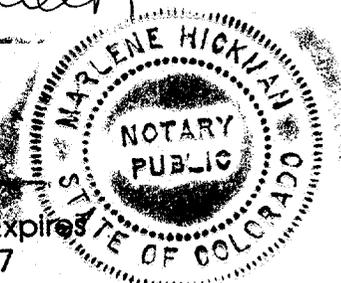
Number	Person	Allergy symptoms monitored						
		Watery eyes	Nasal itching	Uneasiness in breathing	Stomach irritation	Diarrhea	Dizziness	Any other abnormal systems
1	Marlene Hickman	-	-	-	-	-	-	-
2	Mali Reddy	-	-	-	-	-	-	-
3	Venkat Mantha	-	-	-	-	-	-	-
4	Renee W.	-	-	-	-	-	-	-
5	Kalvin Hickman	-	-	-	-	-	-	-
6	Michael Hickman	-	-	-	-	-	-	-
7	Adam Rose	-	-	-	-	-	-	-
8	Chuck Groves	-	-	-	-	-	-	-
9	Laura Mounce	-	-	-	-	-	-	-
10	Robert Williams- DP	ND	ND	ND	ND	ND	ND	ND
11	Juanita Gonzales	-	-	-	-	-	-	-

DP- Did not participate in the test because of his lactose intolerance.
 ND- Not determined.

I, Marlene Hickman, Supervised the above food challenge tests. I certify the data is true and accurate, to the best of my knowledge.

Marlene Hickman

MARLENE HICKMAN
 Notary Public
 State of Colorado



My Commission Expires
 May 15, 2007

Mozzarella (Italian) cheese simulation:

1000ml of the pasteurized whole milk was inoculated with 30ml of the mixed streptococcus thermophilus and lactobacillus culture (prepared according to the procedure using enzyme treated soy protein as sole source of protein). The inoculated milk was incubated at 95F (ripening). The chymosin (rennet) was added at the rate of 1½ ounces/1000lbs. of milk i.e. 1.1ml/1000ml milk. The milk was allowed to coagulate for 30 minutes (setting). The temperature was raised from 95F to 113F, in a span of 45 minutes (cooking). The entire contents were allowed to sit at this temperature until pH came down to 5.2. At this stage, the contents were salted at 0.5% i.e., 5 grams/1000ml. Then the curd and whey were heated to attain 140F to 145F and held at that temperature for 45 minutes (mixing and molding). Then additional 1.25% salt i.e. 12.5 grams/1000ml was added to the contents and blended. This blend was held at room temperature for 2 hours. Later, the entire contents including whey were placed in the refrigerator for 3 days. The entire 1000ml of the contents were mixed with 1000ml orange juice and blended uniformly. Each panel member (non allergic to milk) was asked to consume 150ml of the blend, to check for any signs of soy allergies. The similar precautions and instructions were given to panel members as in the case of simulated cheddar cheese trial. The results are presented in table 3. None of the panel members including 3 soy allergic individuals exhibited symptoms of allergy upon consumption of cheese and whey, indicating that no detectable amount of soy allergen was present. This observation is in accordance with the latest FDA guidance for the industry, released by CFSAN in April 2006 (51). According to the FDA communication, even the unaltered soy protein has the lowest observed adverse effect level or LOAEL compared to other major allergens. In our case, the soy protein has been enzyme treated, fermented, and hydrolyzed during starter making, cheese making and during storage (due to the liberated proteolytic enzymes), rendering itself non allergenic. The details of this data are presented in the petition.

Table 3: Symptoms monitored for allergic reactions after feeding simulated mozzarella cheese along with whey, made with enzyme treated soy solids used as an ingredient in the fermented starter culture.

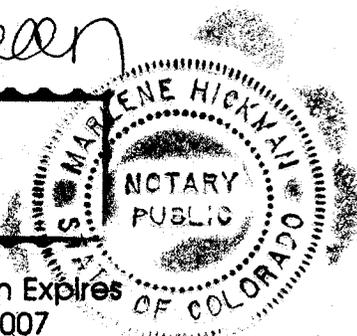
Number	Person	Allergy symptoms monitored						
		Watery eyes	Nasal itching	Uneasiness in breathing	Stomach irritation	Diarrhea	Dizziness	Any other abnormal systems
1	Marlene Hickman	-	-	-	-	-	-	-
2	Mali Reddy	-	-	-	-	-	-	-
3	Venkat Mantha	-	-	-	-	-	-	-
4	Renee W.	-	-	-	-	-	-	-
5	Kalvin Hickman	-	-	-	-	-	-	-
6	Michael Hickman	-	-	-	-	-	-	-
7	Adam Rose	-	-	-	-	-	-	-
8	Chuck Groves	-	-	-	-	-	-	-
9	Laura Mounce	-	-	-	-	-	-	-
10	Robert Williams-DP	ND	ND	ND	ND	ND	ND	ND
11	Juanita Gonzales	-	-	-	-	-	-	-

DP- Did not participate in the test because of his lactose intolerance.
 ND- Not determined.

I, Marlene Hickman, Supervised the above food challenge tests. I certify the data is true and accurate, to the best of my knowledge.

Marlene Hickman

MARLENE HICKMAN
 Notary Public
 State of Colorado



My Commission Expires
 May 15, 2007

18.5 Statistical data on the amount of cheese made using IMAC starter media with enzyme hydrolyzed and fermented soy as an ingredient and consumption of such cheese without any complaints in U.S. for the past five years (wide range practical food challenge test):

The following cheese producers in different geographic locations of U.S. are using IMAC starter medium that has enzyme hydrolyzed and fermented soy as an ingredient.

I) F & A Dairy (Newman, CA), U.S.D.A. Inspected plant

- a) Pounds of milk processed per day-**1,500,000**
- b) Number of days of production per week-**6**
- c) Cheese yields → 10% → **150,000lbs./day**
- d) **16 inch** pizza will approximately have **6 ounces (oz)** of cheese
- e) According to US standards, 16 inch pizza is consumed by **4 people**
- f) Approximately one pound of cheese is consumed by **11 people**
- g) Annual cheese production at F & A dairy is $150,000 \times 6 \times 52$ → **46,800,000lbs.**
- h) Total number of people eating F & A cheese per year $46,800,000 \times 11$ → **514,800,000**
- i) Assuming that each person repeatedly eats pizza once a month
- j) Hence total number of people eating pizza per year that has F& A cheese is → $514,800,000 / 12$ → **42,900,000**
- k) For five years → $5 \times 42,900,000$ → **214,500,000 people.**
- l) Hence in five years **234,000,000lbs** of cheese have been consumed by **214,500,000 people** in U.S.

F & A Dairy has been making cheese with IMAC systems for the past five years. F & A cheese is distributed throughout the U.S. by national distributors. Hence it is understood that all age groups, race, and gender consume F&A cheese. For further information please contact: Ms. Debbie Barry (Q.A. Director), F & A Dairy, Phone: 1-800-626-6580.

II) Cedar Valley Cheese (Belgium, WI), U.S.D.A. Inspected plant

- a) Pounds of milk processed per day-**1, 000,000**
- b) Number of days of production per week-**5**
- c) Cheese yields → 10% → **100,000lbs./day**
- d) **16 inch** pizza will approximately have **6 ounces** of cheese.
- e) According to US standards 16 inch pizza is consumed by **4 people.**

- f) Approximately **11 people** consume **one pound** of cheese.
- g) The annual cheese production at Cedar Valley cheese is $100,000 \times 5 \times 52$
→ **26,000,000 lbs.**
- h) Total number of people eating Cedar Valley cheese → $26,000,000 \times 11$ →
286,000,000
- i) Assuming that each person repeatedly eats pizza once a month
- j) Hence total number of people eating pizza per year that has Cedar
Valley cheese is $286,000,000 / 12$ → **23,833,333**
- k) For 2 years → $23,833,333 \times 2$ → **47,666,666** people
- l) Hence in 2 years → **52,000,000 lbs.** of cheese is consumed by
47,666,666 people

Cedar Valley cheese has been making cheese with IMAC starter systems for the past two years. Cedar Valley cheese is distributed throughout the U.S. Hence it is understood that all age group, race and gender consume Cedar Valley Cheese. For further information please contact: Mr. Jeff Hiller, Cedar Valley cheese, Belgium, WI, Phone: 920-994-4415.

III) **Tropical Cheese (Perth Amboy, NJ)**, (Spanish cheese), U.S.D.A. Inspected plant

- a) Pounds of milk processed per day-**600, 000**
- b) Number of days of production per week-**5**
- c) Cheese yields → 15% → **90,000 lbs./day**
- d) Total cheese production per year → $90,000 \times 5 \times 52$ → **23,400,000 lbs.**
- e) It is assumed that 6 ounce of cheese is consumed per person per month.
- f) It is assumed that one pound of cheese is consumed by **11 people**
- g) Total number of people eating cheese made by Tropical cheese is-
 $23,400,000 \times 11$ → **257,400,000**
- h) Hence total number of people consuming food made with cheese made by
Tropical cheese per year is → $257,400,000 / 12$ → **21,450,000**
- i) For five years → $21,450,000 \times 5$ → **107,250,000**
- j) Hence in five years → **117,000,000 lbs.** of cheese is consumed by
107,250,000 people

Tropical cheese is using IMAC systems for the past five years. The cheese is distributed nationally and is consumed by all segments of the population. For further information please contact: Ralph Mendez, Tropical cheese, Perth Amboy, NJ, Phone: 1-800-332-4791.

IV) Biazzo Dairy (Ridgefield, NJ), U.S.D.A. Inspected plant

- a) Pounds of milk processed per day-**200, 000**
- b) Number of days of production per week-**5**
- c) Cheese yields → 10% → **20,000lbs./day**
- d) Total annual cheese production- $20,000 \times 5 \times 52$ → **5,200,000lbs.**
- e) **16 inch pizza** has **6 ounces** of cheese.
- f) According to US standards 16 inch pizza is consumed by **4 people**.
- g) It is understood that one pound of cheese is consumed by **11 people**
- h) Total number of people eating Biazzo dairy cheese per year $5,200,000 \times 11$
→ **57,200,000**
- i) Assuming that each person repeatedly eat pizza once a month.
- j) So total number of people eating pizza with Biazzo dairy cheese per year-
 $57,200,000 / 12$ → **4,766,666**
- k) For five years → $5 \times 4,766,666$ → **23,833,330**
- l) For 5 years → **26,000,000 lbs.** of cheese is consumed by **23,833,330**
people

Biazzo dairy has been making cheese with IMAC starter systems for the past 5 years. The cheese is distributed nationally and is being consumed by all segments of the population. For further information please contact: Alberto Plumacher, Biazzo Dairy, Ridgefield, NJ, Phone: 201-941-6800.

V) Schreiber Foods (Green Bay, WI), (Cream cheese), U.S.D.A. Inspected plant, and also State of Wisconsin

- a) Pounds of cream plus milk processed per day-**500, 000**
- b) Number of days of production per week-**5**
- c) Cheese yields → 25% → **125,000**
- d) Total annual cheese production— $125,000 \times 5 \times 52$ → **32,500,000 lbs.**
- e) It is assumed that **6 ounces** of cream cheese is consumed per person per month.
- f) One pound of cheese is consumed by **11 people**
- g) Total number of people consuming Schrieber foods cream cheese per year- $32,500,000 \times 11$ → **357,500,000**
- h) So total number of people consuming food that has cream cheese per year is- $357,500,000 / 12$ → **29,791,666.**
- i) For five years → $5 \times 29,791,666$ → **148,958,330** people.

- j) For 5 years → **162,500,000 lbs.** of cheese is consumed by **148,958,330** people

Schrieber foods have been using IMAC systems for the past five years. The cheese is distributed and consumed all over U.S. For further information please contact: Kay Wallsten, Schrieber Foods, West Bend, WI, Phone: 262-675-6533.

VI) **Tucumcari Cheese** (Tucumcari, NM), (American style cheese), Inspected by State of New Mexico, Health department.

- a) Pounds of milk processed per day-**250, 000 lbs.**
- b) Number of days of production per week-**4**
- c) Cheese yields → 10% → **25,000 lbs.**
- d) Annual cheese production-**25, 000x4x52 → 5,200,000 lbs.**
- e) It is assumed **6 ounces** of cheese is consumed per person per month.
- f) One pound of cheese is consumed by **11 people**
- g) Total number of people eating Tucumcari cheese product per year is-**5, 200,000x11 → 57,200,000**
- h) Number of people eating food that has Tucumcari product per year is-**57, 200,000/12 → 4,766,666**
- i) For five years → **4,766,666x5 → 23,833,330 people.**
- j) For five years → **26,000,000 lbs.** of cheese is consumed by **23,833,330 people**

IMAC has been supplying its starter systems to Tucumcari cheese for the past five years. The cheese is consumed all over the United States. For further information please contact: Charles Krause, Tucumcari Cheese, Tucumcari, NM, Phone: 505-461-4045.

VII) **Lucille Farms** (Swanton, VT), U.S.D.A. Inspected plant

- a) Pounds of milk processed per day-**1, 000,000**
- b) Number of days of production per week-**5**
- c) Cheese yields → 10% → **100,000 lbs.**
- d) Total annual cheese production → **100,000x5x52 → 26,000,000**
- e) **16 inch** pizza will have **6 ounces** of cheese.
- f) According to US standards **16 inch** pizza is consumed by **4 people.**
- g) One pound of cheese is consumed by **11 people**
- h) Total number of people consuming Lucille farms cheese per year-**26, 000,000x11 → 286,000,000**
- i) Assuming that each person repeatedly consume pizza once a month.

- j) Total number of people consuming pizza that has Lucille farms cheese per year- $286,000,000/12 \rightarrow 23,833,333$

Lucille farms uses IMAC starter systems in their cheese making process. The cheese is enjoyed all over U.S. For further information please contact: Carl Millerick, Lucille Farms, Swanton, VT, Phone: 802-868-7301.

VIII) Suprema Specialties (Manteca, CA), (Ogdensburg, New York), and (Blackfoot, Idaho), U.S.D.A. Inspected plants

- a) Pounds of milk processed per day-**1,000,000**
- b) Number of production days per week-**5**
- c) Cheese yields $\rightarrow 10\% \rightarrow 100,000$ lbs.
- d) Annual cheese production $\rightarrow 100,000 \times 5 \times 52 \rightarrow 26,000,000$ lbs.
- e) **16 inch** pizza will have **6 ounces** of cheese.
- f) According to US standards 16 inch pizza is consumed by **4 people**.
- g) One pound of cheese is consumed by **11 people**
- h) Total number of people consuming Suprema cheese per year- $26,000,000 \times 11 \rightarrow 286,000,000$
- i) Total number of people consuming pizza with Suprema cheese per year- $286,000,000/12 \rightarrow 23,833,333$

Suprema Specialties used IMAC starter systems in their cheese making process. The cheese was distributed all over U.S., and enjoyed by millions of people all over the country. Suprema is no longer in business hence we could not give any contact person. Their R & D Director was Dr. Reyad Mahmood, who was previously employed by the United States Department of Agriculture.

With the above mentioned statistical data from different cheese manufacturers located in different geographic regions of the country, it is widely perceived that the cheese made with IMAC starter systems that has enzyme treated soy solids as an ingredient, which undergoes a myriad of treatments during starter making, cheese making and storage is non allergenic. As it always been in the past, the cheese made with IMAC starter systems can be exempted from allergen labeling in accordance with 403 (w) (6).

Strength in numbers:

We are very proud to make a note that a total of 567,000,000 people from all over U.S. have consumed 617,000,000 pounds of cheese made with IMAC products that has enzyme treated and thus hydrolyzed soy as an ingredient namely Eliminate series, Eradicate series and Enhance series. It should also be noted that the cheese producers using IMAC products hail from different geographic locations of the country.

PLEASE NOTE: There will be repetition of consumers of cheese made with IMAC products. It emboldens our understanding that the cheese made with IMAC products are non-allergenic. It is due to the fact that if the consumer misses the allergenicity the first time around, he or she cannot miss it the second time. Hence, we emphatically say that the cheese made with IMAC products are non-allergenic.

According to U.S. statistics, only 2% of the U.S. population have food (and other) allergies. Population of U.S. is approximately 325,000,000. 2% of the population is 6,500,000 people. According to the recent communication form FDA (51), it is currently estimated that 0.2% soy (which is untreated or unaltered). Based on this estimate, soy allergy appears to be less prevalent than allergies to other major food allergens, according to FDA.

A total of 617,000,000 lbs. of cheese is made with IMAC starter that has enzyme hydrolyzed and fermented soy as an ingredient and is fed to over 567,000,000 people for the past five years.

Hypothetical Situation (for allergic Reactions):

At 2 % it is 10,000,000 people

At .1% it is 500,000 people.

At .01% it is 50,000 people.

At 001% it is 5,000 people.

The total number of people at different percentage level having possible allergic reaction with IMAC starter is staggering and palpable and is easily noticed by the health officials in U.S. However, not a single complaint was filed with health department in the past five years. Hence, it goes to prove that soy solids used as an ingredient in the starter medium is rendered non-allergenic statistically as well

as test wise when such medium is thermally treated, enzyme hydrolyzed, fermented (microbial proteolysis, and acid hydrolysis), enzyme hydrolyzed with rennet enzyme, microbial proteolysis and acid hydrolysis followed by the reduction of O/R potential in cheese matrix along with aging of cheese.

18.6 Testimonials from qualified physicians and medical professors regarding safety of cheese made with IMAC media with enzyme hydrolyzed and fermented soy as one of the ingredients:

Dr. P.K. Vedantan, M.D., a practicing allergy specialist who has an allergy clinic covering State of Colorado, Wyoming, and parts of Nebraska, states that the only way to find out if allergen is present in food is if a patient responds to that food. FDA has also been stating the same thing. Upon analyzing our statistical data, he strongly feels that the allergen must not be present in the cheese. Further more, people who are allergic to soy nuts did not exhibit any signs of allergy when they ate cheese made with IMAC starter with soy as one of the ingredients. Mr. Sridhar Reddy, 30 years old, has consumed our dairy products made with our starter and did not have any signs of allergy. He has consumed various dairy products made using IMAC starter medium, over a long period of time with no allergic response. Similarly several people who have expressed that they react to soy products with gastric discomfort did not have any such signs of discomfort when they consumed the cheese made with our starter. For that matter, they did not experience any allergy symptoms. Pizza with cheese topping is very popular among young and old alike. It is a very good indicator to check the allergic responses. Since we have not received any complaints it goes to prove that the cheese made using IMAC starter media with soy as an ingredient did not exhibit any signs of allergies among all age groups (young, middle and old age) in all parts of the United States. Hence, it can be reiterated that the thermal, enzymatic and fermentation treatment of soy rendered them non-allergenic, as additionally proved by enzyme immunoassay.

Again, according to Dr. P.K. Vedantan, M.D., 600 million pounds of cheese fed to over 500,000,000 people without any allergic complaints, definitely proves, the allergen is inactivated and cannot trigger allergic response in the hypersensitive individual for soy protein. He thought it is more than adequate statistical information to draw an inference. Dr. Vedantan is also a faculty member at University of Colorado Health Science Center. He can be reached at 970-493-5290. He will be more than happy to discuss this issue.

Dr. Ron Harbeck, Ph.D., Director of Immunology Clinic at the National Jewish Hospital at Denver, Colorado, and also professor of medicine also stated that 600 million pounds of cheese fed to over 500 million people of different ages, races, gender, without any reported allergic response, definitely goes to prove that thermally treated, enzyme hydrolyzed and fermented soy in starter medium is non allergenic. If not, some body should have reacted and reported to the physicians and thus to the local health department. This is a typical protocol followed all over U.S. Dr. Harbeck did extensive research on allergy related issue and has published several articles. His work proved that denatured and/or oxidized protein loses the allergenic properties in mold related allergies. An article is being attached as a reference. Dr. Harbeck can be reached at 303-398-1337, for any discussions on this issue.

Dr. Hari M. Sharma, M.D., FRCP (Fellow of Royal College of Physicians), Professor Emeritus, College of Medicine and Public Health of the Ohio State University, has also attested that soy as one of the ingredients in starter medium, which is thermally treated (180° to 190°F for 30 to 60 minutes), enzyme hydrolyzed using protease, and fermented (enzyme plus acid) with dairy starter cultures, and then used in making cheese at the rate of 0.5 to 3.0%, with further enzymolysis due to rennet enzyme used in cheese making, and additional hydrolysis due to second bacterial fermentation during cheese making, followed by aging of cheese from 1 day to 1 year with reduced oxygen conditions will definitely make the soy non-allergenic. He also said that best proof is wide range of people (500 million people) consuming the cheese and dairy products (over 600 million pounds) made with starter containing hydrolyzed and fermented soy solids as an ingredient (stimulants), with no complaints registered, is a good enough clinical evidence to prove beyond doubt that soy allergens are inactivated when processed as starters through fermentation and then used as ingredient (<3%) in cheese. He can be reached at 614-879-7898 for further verification.

As a Veterinarian, I, Dr. Reddy (copy of my resume is enclosed for your perusal) (31) was involved in treating canines and felines for various diseases including food allergies. Later as a cheese technologist, I have worked over 35 years with dairy starter cultures, starter medium, and various varieties of cheeses in the

United States, United Kingdom, European Economic Community, (Southern Ireland, Germany, France, Italy, Denmark etc.), Eastern Europe, and Mexico, can make an affirmative statement that to the best of my knowledge, hydrolyzed soy solids using fermentation, enzymolysis and acid hydrolysis, are rendered non-allergenic and does not emit allergic reactions in cheese. Further strengthening this statement the concentration of these solids are low in starters and thus going into cheese are small, because the amount of starter used is roughly 0.5 to 1.0 percent (47). The U.S. government restricts the usage of starter to 3.0% (47). The solids level in the liquid starter is low, ranging from 7 to 20% and most generally around 8.5 to 12.0%. The concentrations of solids vary because of the amount of buffering required in the liquid starter medium, which in turn depends on the type of fermentation. According to the latest report from FDA (51), published on April, 2006, it is currently estimated that 0.2% of children and adults in the U.S. are allergic to soy (which is untreated or unaltered). Based on this estimate, soy allergy appears to be less prevalent than allergies to other major food allergens, according to FDA. All in all, cheese made with IMAC starter medium (with soy solids) which is high heat, treated, enzyme hydrolyzed and fermented should not induce any allergenic response that poses a risk to human health.

19. SUMMARY AND CONCLUSIONS

Use of soy solids as an ingredient in the starter medium treated with high heat, enzymes, second heat treatment, fermentation (enzymolysis, acid hydrolysis), enzyme hydrolysis (rennet) in the cheese making, second fermentation in the cheese milk (enzymolysis, and acid hydrolysis) with reduced O/R potential, is safe in terms of lack of allergens in the cheese. This has been verified by testing using proteolysis, food challenge tests, and specific allergen tests in cheese. Also through field data i.e. 600 million pounds of cheese, (made using IMAC starter with enzyme hydrolyzed and fermented soy solids as one of the ingredients), fed to over 500 million people of different age groups, gender, races and geographic regions, without any complaints (zero complaints on allergy or any other complaint) for the past 5 years.

The most common symptoms of food allergies are:

1. Skin reactions-Itchy, rash, hives and eczema.
2. Stomach and intestinal reactions-Abdominal pain and bloating, diarrhea, vomiting, gas/flatulence, and cramps.
3. Nose, throat, and lung reactions-Runny nose, sneezing, watery and/or itchy eyes, coughing, wheezing, shortness of breath, and extreme cases of asthma.

In April, 2006, FDA has announced (51) that, it is currently estimated that 0.2% of children and adults in the U.S. are allergic to soy (which is untreated or unaltered). Based on this estimate, soy allergy appears to be less prevalent than allergies to other major food allergens, according to FDA.

Earlier, it has been conclusively proven, beyond doubt that soy proteins fermented with lactic acid cultures did not exhibit any toxicity or abnormal symptoms in a subculture oral toxicity study. Also, the fermented soy was demonstrated to be non mutagenic in ames test, and did not cause chromosomal damage in mammalian cells in vitro and did not induce micro-nuclei in bone marrow cells in ICR mice tested. In

humans, it did not have any adverse effects when fed as a dietary supplement over a long period of time. This is according to the work of LU, published as an invention with minute details in U.S. patent number 6,855,350 (27). Also, according to this publication, the fermented soy prevented allergy, inflammation, dermatitis and improved immunomodulation by improving the phagocytosis activity of macrophages by 71% in both vitro and vivo. It was also noticed in one of our company staff, whose immune system was greatly improved with the intake of fermented soy. The doctors were pleased with the findings. It has also been reported that beneficial bacterial cultures (probiotics) can modify allergens so that they cannot excite an immune response in sensitive human beings (U.S. pat. #6,506,980). Literature also cites the bacterial thioredoxin can reduce the allergenicity of certain proteins (26). Martyny et.al (30) proved that even the chemically denatured mold proteins are rendered non-allergenic, proving protein denaturation is a prime factor for reducing allergenicity of proteins. It was also found that fermented soy inhibited Lipoxygenases and thus act as anti-inflammatory agent, thus modulate immune response to prevent allergy. This is an excellent piece of work to demonstrate beyond doubt that fermentation of soy proteins not only eliminate their allergic response but also improve their ability to reduce allergens (27). This work ties in good with our test results. Also, 617,000,000 pounds of cheese consumed by over 567,000,000 people in U.S. (although the U.S. population is approximately 300,000,000, the entire population is exposed at least two times in the past five years) for the past five years without any complaints from either people, clinics, or health departments, across the United States, is an excellent statistical indication that soy allergens are inactivated when used as fermentation bases to make cheese. In this connection, please also refer to the article by Reddy and Reddy in terms of the effect of Probiotics on improving the immune modulation and specific mention is made that beneficial starter cultures reduce immune response in allergy. This article by, M.S. Reddy, and D.R.K. Reddy. Proyurveda (Probiotic + Ayurveda) on curing the acute and chronic disease, without any side effects was. Presented at the 23rd AAPI

Medical Convention (integrated medicine program), June 15-19, 2005, Houston, TX (32).

According to medical professionals, more than test being negative for allergens in cheese, the statistical data of no allergenicity reported in the past five years in 567 million people (practical wide angel food challenge test) is more than enough data to conclude, the procedure used to prepare IMAC starter made with enzyme hydrolysis and fermentation of soy solids and further proteolysis during cheese make procedures to inactivate the allergens in the finished cheese. However, starters have to be prepared properly using heat treatment and fermentation. It is a self- controlled prophecy because starters cannot be produced without proper heat treatment and fermentation. Furthermore, U.S.D.A., F.D.A., and State Health departments inspect this area thoroughly (47).

We conclude our communication by stating that hydrolyzed (enzyme and fermented) soy solids, which are one of the ingredients in the IMAC starter medium is rendered non-allergenic through various treatments namely thermal, enzymatic and acid hydrolysis that occurs during starter making and cheese making.

FALCPA was enacted to inform consumers, as means to protect health. Identifying on the cheese label soy as one of the minor ingredient in the IMAC fermented starter cultures, which are used to manufacture cheese, even though allergens are absent or inactivated, in the cheese is a false declaration, which contradicts the intent of FALCPA.

This petition complies with the requirements of section 403 (w) (6) of the Food Allergen Labeling and Consumer Protection Act. We sincerely request the Honorable Secretary to grant us an exemption from the labeling requirements of section 403 (w) (1), established by the Food Allergen Labeling and Consumer Protection Act (FALCPA), for IMAC media with enzyme treated soy solids as one of the ingredients.

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