DETECTION AND CHARACTERIZATION OF SESAME SEED ALLERGENS

By

Gülsen Söylemez

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Food Science and Technology

Under the Supervision of Professor Sue L. Hefle

Lincoln, Nebraska

May, 2003

UMI Number: 3092599

ProQuest Information and Learning Company
300 North Zeeb Road
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DISSERTATION TITLE

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University of Nebraska, 2003

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Identification and characterization of both black and white sesame seed proteins were investigated using biochemical and IgE-binding techniques. Extracts were made from both non-defatted and defatted seeds using both phosphate-buffered saline (PBS; pH 7.4) and deionized water (D-H_2O). Extracts were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by IgE-immunoblotting, and radioallergosorbent assay (RAST) with sera from sesame seed-allergic subjects. The SDS-PAGE results revealed differences in the extractable proteins obtained from each extract. Sesame seed extracts contained several proteins (13-26) with molecular weights in the range of 6.5-151 kD. Immunoblotting studies demonstrated the presence of several IgE-binding proteins with estimated molecular weights in the range of 6.5 to 87 kD, and IgE-binding patterns and RAST results differed between extracts for each sesame seed-allergic subject.

Polyclonal antibodies against the mixture of one part black and three parts of white sesame seeds were produced in goats, sheep, rabbits, and chickens. Antibodies were purified from the animal antisera and egg yolk. The immune response of each

animal was monitored using an indirect enzyme-linked immunosorbent assay (ELISA). The sheep, goats, and chickens generated good immune responses to the sesame seed immunogen, while rabbits gave a weaker immune response to the immunogen.

A sandwich ELISA was developed using the goat serum and chicken egg yolk antibodies for quantification of sesame seed residues in foods with a detection limit of 2 ppm sesame seed flour. Goat IgG and chicken egg yolk IgY antibodies were used as capture and detector antibodies, respectively. Binding was visualized by addition of commercial goat anti-chicken IgY-alkaline phosphatase conjugate and subsequent substrate addition. Validation of the assay included testing pasta containing different known amounts of sesame seed flour (0-100 ppm), positive and negative samples, and cross-reactivity assessment. Minimal cross-reactivity was observed with allspice and kidney beans. A limited retail survey and positive samples demonstrated that the assay produced statistically consistent results ($P > 0.05$).

ACKNOWLEDGEMENTS

This dissertation is dedicated to my whole family, whose love and encouragement have enabled me to pursue my academic goals. My parents, Ali & Kaymak Söylemez, my sisters, Gülseren Gülener, Derya Öder, and Hülya Utku, my brother A. Mutlu Söylemez, my brother-in-laws Altan Gülener, Serdar Utku and Özgur Öder, and my sister-in-law Selda Söylemez. I would like to thank them all for their encouragement, loving support and their patience. I would also thank to my second family, Chad & Jill Walrod and Glenn & Char Cedeberg. This dissertation would not be completed without their ability to understand the challenges I have faced and the love they have provided. I would like to give a special acknowledgment to my nieces, Sena Gülener, and Buse Utku and my nephew Ali Kemal Gülener. Their love gave me the strength to continue during the most difficult times.

I would like to express my gratitude to the Türkiye Ministry of Agriculture, Dr. Sue L. Hefle and Dr. Steve L. Taylor for giving me the opportunity to continue my Ph.D. program.

I would like to express my thanks and appreciation to Dr. Sue L. Hefle for her guidance, encouragement, and support during the course of this research project. I would also like to express my appreciation to the other members of my committee, Dr. Steve L. Taylor, Dr. Michael Zeece, Dr. Susan Cuppett, Dr. Judy Driskell, and Dr. Durward Smith for their suggestions and serving on my committee. I would also like to thank Dr. David Marx for providing his assistance with statistical design and analysis.

A special thanks to Julie Nordlee for her technical assistance, her guidance, her patience, her kindness and her friendship. To my friends, Lynn Newman, Debra Lambrect, Tina Boilesen, Jamie Kabourek, Carolina Estrada, Ashley Lardizabal, Rebekah Lowe, and Pat Gergen; thanks for your encouragement, support and friendship.

I would also like to express my gratitude to my friends, Dr. Asiye Kumru, Dr. Sevket and Yuksel Cetin, Dr. Nedim and Songül Mutlu, Dr. Hurol and Asli Aslan, Erol and Ozgul Durukan who have offered encouragement, support and stress relief throughout my graduate studies.

Thank you all. I could not have done it without any of you. Above all, I thank God for everything he has given me.

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CHAPTER I: INTRODUCTION

Food allergy is an important food safety issue and has had a significant impact on the food industry. As awareness grows, food allergens have become a general concern to consumers, food manufacturers, and regulatory agencies. The presence of undeclared allergens in food products is a serious health hazard to consumers with food allergy, as there are estimated 29,000 emergency room visits per year and 200 deaths caused by allergic reactions to foods in the U.S. The prevalence of food allergy is increasing in the U.S. as a whole, as are reports of severe reactions and deaths, for unknown reasons. The majority of food-allergic individuals are children, and food allergy can significantly affect their quality of life, both socially and medically. Research on food allergy can help to improve diagnostic and treatment methods, and new detection methods for undeclared allergens in foods will help to improve the quality of life for those living with food allergies. Food allergy is a major cause of medical care in susceptible individuals, as they report usually 2-3 reactions per year.

In addition to food-allergic individuals, farmers and food companies are highly affected by food allergen concerns, from a legal and financial standpoint. There is an increase in the number of food recalls because of the undeclared allergenic food residues. Smaller companies have more at stake as a claim against them could put them out of business. Therefore, food allergen issues can have a major economic impact on food companies. The availability of detection methods for allergenic foods is desirable for the food industry. Because of the significance of allergic disease on every part of the food manufacturing process, from farm to table, research on food allergy is needed.

Sesame seed is probably one of the oldest foods in the human diet. Sesame seed is a member of the *Pedaliaceae* family, which is likely the most ancient seed known and grown for edible oil, called 'the queen of the oil seed crops'. Archeological evidence shows that sesame seed was a highly-prized oil crop in Babylon and Assyria (Iraq and Syria) at least 4,000 years ago. In ancient Babylon, sesame seeds were used for making cakes, candy bars, wine, and brandy, and sesame seed oil was used for cooking. Also, Greek and Roman soldiers carried sesame seed for food on long marches. A 4,000-year-old archeological finding in an Egyptian tomb shows that sesame seed was used as an ingredient in baking. Also, "Open Sesame" was the magical password that opened the entrance of the cave in the story of "Ali Baba and the Forty Thieves" in reference to the shattering properties of ripe sesame seed pods.

The origin of sesame seed is in doubt (either Africa or India), but the latest evidence suggests India. Sesame seeds spread through West Asia, to China and Japan. Sesame seeds were introduced to the United States late in the 17th century by slaves, but interest in it as a food started in the early 1940s due to its high oil and protein content. They have been grown in some parts of United States: Texas, South Carolina, Arizona, California and Kansas. However, domestic production has been limited due to lack of cultivators to mechanically harvest the seeds. Therefore, most of the seeds have been imported from South America and China.

Sesame seeds are small, flattened, and oval shaped seeds that have a nut-like flavor, and range in color from yellowish white to red, brown, and black. Most of the seeds that are used in the United States are pearly white with a glossy finish. Sesame

seeds can be found as a constituent of a wide variety of foods. White sesame seeds have been commonly used in foods in many forms, such as hulled, unhulled, or ground; the oil is used in the cosmetic industry, and in pharmaceutical products as a solvent. Black sesame seeds have primarily been used as toppings in bakery products. Sesame seeds are consumed raw, roasted or dried. An increasing worldwide utilization of sesame seeds for food suggests a possible future for sesame seeds as an increasing source of food protein.

Sesame seeds can cause severe, life-threatening allergic reactions in sensitive individuals. The prevalence of sesame seed allergy is not known. However, the increasing consumption of sesame seeds is likely to cause an increase in the incidence of allergy to sesame seeds. Allergic reactions to sesame seeds experienced by sesame seed-allergic individuals vary from mild to severe life-threatening reactions. The only treatment for a food allergy is elimination of the offending food from the diet, and avoidance is easy if seeds are visible on the food or if sesame seed-allergic individuals are conscious about the food groups that contain sesame seeds, but can sometimes be quite difficult since trace quantities of sesame seeds can trigger reactions. Accidental ingestion of sesame seed proteins could result from failure to declare the presence of or mislabeling of food products, lack of knowledge of the composition of food, cross-contamination with sesame seeds during food production or preparation, or insufficient cleanup procedures after production of sesame seed-containing foods before manufacturing other products on shared equipment. Because of the potential hazards of undeclared sesame seed in processed foods, sensitive and specific methods are needed to

detect residues, which can provide the food industry with a useful tool for the control of sesame seed contamination for the protection of sesame seed-allergic consumers.

Although sesame seed allergy is defined as an IgE-mediated food allergy based on biological and clinical characteristics such as specific IgE measurement and provocation tests with sesame seed, and the observation of anaphylactic reactions in individuals, there is some debate about whether sesame seed allergy is IgE-mediated or non-IgE-mediated. Some studies report reactions in individuals with very convincing histories of sesame seed allergy but possessing low or negative sesame seed-specific serum IgE. These results could be due to low quality of diagnostic extracts. Low quality extracts could result in misdiagnosis and could be due to extraction methods or varieties of food, which could result in differences in protein profiles for the food of interest. Therefore, one of the major objectives of this study was to compare black and white sesame seeds for differences in protein profiles and IgE-binding using different extraction buffers, which could be important considerations in preparation of diagnostic sesame seed extracts. To investigate the IgE-binding qualities of sesame seed allergens, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE), IgE-immunoblotting, and radioallergosorbent assay analysis were performed. Details and results of the studies on characterization of sesame seed proteins are presented in Chapter III.

In addition, identification and characterization of sesame seed allergens is necessary to gain knowledge in what causes certain proteins to become allergens. Better knowledge of allergen identity and structure will result in improved diagnostic tools and

therapeutic methods (such as immunotherapy) for sesame seed and other food-allergy sufferers. Information on allergenic structure also will contribute information to be used in safety assessment of the potential allergenicity of genetically-modified crops.

Because of the significance of sesame seed allergy, there is a need for an analytical method to detect sesame seed residues in foods for the food industry for protection of consumers suffering from sesame seed allergy. For detection/identification and quantification of allergenic sesame seed residues in sesame seed-free foods, radioimmunoassays were the historical method. However, these methods require radioactive reagents, expensive equipment, trained personnel and large amounts of sera from individuals who are allergic to sesame seeds. However, human sera and radioisotopes are not appropriate for food processing environments. Enzyme-linked immunosorbent assays (ELISAs), in which radiolabels are replaced by enzymes and human sera are replaced by animal sera, are the most versatile, specific, and sensitive methods to detect undeclared allergenic residues such as sesame seed. Because of the lack of human sera and radioisotopes, ELISAs can be used to detect inadvertent contamination and assess cleaning processes in the food-manufacturing environment. Therefore, another major objective of this present study was the development of a specific and sensitive ELISA method for detecting potentially hazardous undeclared sesame seed residues in food products, and to survey retail food samples for sesame seed contamination.

The first step in development of an ELISA for allergenic foods is the production of polyclonal antibodies against the food of interest. The ability to produce specific

polyclonal antibodies using a crude extract instead of a specific allergen, and other benefits of polyclonal antibodies including high specificity and high titer, make this approach very attractive. Approaches used in the production of polyclonal antibodies against sesame seed proteins, and results, are presented in Chapter IV. The development of a sandwich type ELISA for sesame seed residues is described in Chapter V.

CHAPTER II: LITERATURE REVIEW

INTRODUCTION

Food is an essential substance to maintain life. On the other hand, food can cause fatal or near-fatal reactions in sensitive individuals due to being recognized by the immune system as a harmful substance. Adverse reactions to food are individualistic and affect a small percentage of population. Some are mild, but some can be life-threatening or fatal.

ADVERSE REACTIONS TO FOOD

An individualistic adverse reaction to food or food sensitivity is defined as any abnormal response due to an ingested food or food additive that occurs in some individuals in the population but not others (*192, 193, 197*) (Figure 1). Food sensitivity is categorized into two groups; primary food sensitivity and secondary food sensitivity. Secondary food sensitivity is an adverse reaction to food or food additives as a result of combination of certain conditions with ingestion of food (*192, 197*). Primary food sensitivities are further categorized into food intolerance and food hypersensitivity. Food intolerance is defined as an abnormal physiologic response attributed to the ingestion of food or food additive that is not immunologically mediated, including idiosyncratic, pharmacologic, anaphylactoid, or metabolic reactions (*198*). Food hypersensitivity is an immunologic hypersensitivity reaction resulting from the ingestion of a food or food additive, and is categorized into immunoglobulin E (IgE)-mediated and non-IgE-