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Review

Investigation of undeclared food allergens in commercial Thai food products

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ABSTRACT

This study investigates the presence of undeclared allergens – namely milk, egg, wheat and peanut – in commercial processed food products in Thailand. Out of 142 commercial products, 55 positive cases were found (by quantitative ELISA method) to contain an undeclared allergen greater than 10 ppm. Among all positive products, undeclared milk appeared the most frequently (21 cases), followed by egg and wheat with similar frequency (17 cases), while peanut was rarely found. Milk- or egg-positive products were further confirmed by the presence of the milk protein, casein, or the egg protein, ovalbumin, by Western blot test. Our results should help to increase the awareness among Thai food manufacturers of the need for more careful management of allergenic food ingredients, and to encourage the labeling of allergen information for allergic consumers, thus reducing the health hazard from food allergy.

Contents

1. Introduction

It is well recognized that food allergy has become a major health concern worldwide, especially in industrialized countries. It has been estimated that around 1–2% of the population, and up to 8% of children, suffer from food allergy (Bousquet et al., 1998; Helm & Burks, 2000; Jansen et al., 1994), with symptoms ranging from relatively mild to severe, or sometimes even fatal consequences (Sampson, 2005). Many foods are known to elicit food allergy; among those, eight types – i.e. peanuts, nuts, wheat, soy, milk, egg, fish and shellfish – are reported to be responsible for causing the majority (>90%) of allergic reactions (Bush & Hefle, 1996). In Thailand, food allergy is still unfamiliar, and only limited information has been reported. One emergency department in a Thai university hospital reported the most common causes of anaphylaxis to be foods, mostly seafood, followed by milk and wheat

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(Poachanukoon & Paopairochanakorn, 2006). Another study of adverse food reactions including food allergy in 656 Thai children revealed that milk and egg were the two foods most commonly reported to cause adverse reactions in young children; whereas seafood, particularly shrimp, was the most common allergen for older children (Santadusit, Atthapaisalsarudee, & Vichyanond, 2005). These studies suggested that the existence of food allergy in Thailand is similar to other countries, although the majority of Thai people are still not aware of food allergy.

Currently, the most effective way of preventing allergic reactions is the strict avoidance of the offending allergic food, due to the fact that there is no known medication for food allergy (Vierk, Koehler, Fein, & Street, 2007). In order to ensure avoidance of the offending food allergen, a labeling system to provide information regarding food allergens used in food products has been adopted globally (Mills et al., 2004; Yamakawa et al., 2007). Thailand, however, has not participated in this program. Regardless of labeling, complete avoidance is still difficult, because food products may not be properly labeled or may contain a food allergen by mistake – e.g. an ingredient derived from a food allergen may be overlooked (De Luis, Lavilla, Sanchez, Calvo, & Perez, 2008). Also, unintentional inclusion of food allergens might arise from cross-contamination in the food production process (Holzhauser & Vieths, 1999). Undeclared food allergens in food products pose a major risk for allergic individuals; hospital emergency departments have reported that severe allergic reactions have been caused by the accidental intake of food containing allergenic materials (Wüthrich & Ballmer-Weber, 2001). At present, the scientific study of undeclared allergens in commercial Thai food products is lacking. Surveys are urgently required in order to assess the health hazards from food allergy, as well as to ensure the safety of commercial food products.

Our present work is the first investigation to report undeclared food allergens in commercial food products in Thailand, using a quantitative immunoassay (ELISA) suitable for examining processed foods. Furthermore, any products testing positive for milk or egg allergens by ELISA assay were further confirmed for the presence of specific milk or egg protein by Western blot (WB) test.

2. Materials and methods

2.1. Materials

Quantitative ELISA test kits for determining egg, milk, wheat and peanut – as well as qualitative WB test kits for determining specific egg and milk proteins (ovalbumin and casein, respectively) – were provided by Morinaga Institute of Biological Science, Inc., Yokohama, Japan. The limit of quantification (LOQ) of the ELISA kit was 0.3 μg allergen protein/g food (0.3 ppm), and the limit of detection of WB kit was 0.5 ppm.

One hundred and forty-two commercial Thai food products were collected during June to September 2008 from local food stores, supermarkets, and the exhibition of Thai foods for export ("THAIFEX 2008") held in Bangkok, Thailand. The product name and its ingredients declared on the label were recorded.

2.2. Methods

2.2.1. Quantitative ELISA test

2.2.1.1. Sample preparation. The forms of the surveyed food products were diversified, from liquids to solids, including powders, cakes and pastes. These food products were sometimes not homogenous in the serving package; in that case the test sample for the assay was prepared from whole one serving, or by collecting samples from different parts of the package to represent the product of interest. Each product was mixed thoroughly, and then ground and homogenized to a fine powder or homogenous mixture. Sample preparation was performed with clean instruments, and the prepared samples were kept in clean containers to avoid cross-contamination.

2.2.1.2. Allergen determination by ELISA. Each homogenous sample was treated and the allergen content determined according to the ELISA kit instructions. An observed ELISA result with an allergen content equal to or greater than 10 ppm (10 μg per 1 g food product) was classified as "positive," since this low level of food allergen is considered to be the threshold value for eliciting allergic reactions (Bindslev-Jensen, Briggs, & Osterballe, 2002). Additionally, the Japanese government has established 10 ppm as the level for mandatory labeling of food allergens.

2.2.2. Confirmatory examination of the specific allergen protein by WB test

WB examination for specific milk (casein) or egg (ovalbumin) proteins was performed on all food products having a positive ELISA result for milk or egg allergen. WB procedure was carried out according to the manufacturers' instructions. In brief, the sample extract (a standard allergen protein, casein or ovalbumin), Kaleidoscope Prestained Standards, and a molecular weight marker (Bio-Rad Laboratories, Inc., USA) were applied to SDS-gel electrophoresis (Laemmli, 1970), after which the SDS gel was blotted onto a PVDF membrane (Amersham Hybond-P, GE Healthcare, UK) by Trans-Blot semi-dry electrophoretic transfer cell (Bio-Rad Laboratories). During immunostaining, rabbit antibodies against casein or ovalbumin protein supplied in the WB kit reacted with the blotted membrane; the membrane was then treated using a VECTASTAIN ABC-AP Rabbit IgG kit (Vector Laboratories, USA), and finally a BCIP/NBT Alkaline Phosphatase Substrate Kit IV (Vector Laboratories). The immunostained band appearing at the respective molecular mass location (casein 33–35 kDa, ovalbumin 50 kDa) was interpreted as a "positive" result.

3. Results

One hundred and forty-two commercial pre-packaged Thai food products, collected locally, were categorized into 25 product types based on their characteristics and use. The most abundant group was instant noodles, either from wheat or rice (14.8%), followed by sauces (10.6%), snacks (9.9%), rice flour products such as dry rice noodles/rice paper/rice flakes (8.5%), meat balls (fish, shrimp, cuttlefish and pork) (7.7%), salad dressing (7.7%), curry powder/spices (7.0%), coconut milk/coconut cream/cereal cream (6.3%), imitation crab stick/imitation salmon stick (4.2%), instant rice porridge (3.5%), and others (Table 1).

Quantitative ELISA results for undeclared food allergens in commercial food products are presented in Fig. 1. Foods with an allergen declared on the label were omitted from ELISA testing, and were classified as "not tested" (NT). Most of the food products examined by ELISA were determined to have an allergen content less than the LOQ (0.3 ppm), shown as "ND" (not detected). The egg ND group was observed in 106 cases in 129 food products; milk in 103 cases in 129 products; wheat in 81 cases in 124 products; and peanut in 139 cases in 141 products. This meant that the labeling was properly done in most of the examined products. On the other hand, there were a significant number of cases containing undeclared food allergen greater than 1%, namely wheat (5 cases), egg (3 cases) and milk (3 cases).

Out of 142 commercial food products, a total of 55 positive cases of undeclared food allergens were observed. The frequency of undeclared milk-positive cases was 21, followed by wheat- and