Dust mite infestation in cooking flour: experimental observations and practical recommendations

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Summary

Background: **The first documented case of oral mite anaphylaxis has recently been reported in Thailand, with mites possibly originating from cooking flour.**

Objective: **Our study was designed to assess the effects of cooking flours enhancement and storage conditions on mite proliferation and to provide practical recommendations to prevent mite anaphylaxis. .**

Methods: **In a factorial experiment, six commercial brands of cooking flours were selected and either inoculated or set free of mites and stored in one of the four containers chosen for the study: original package, plastic bag, plastic box and glass bottle. The resulting experimental units where then stored at either room temperature or in a refrigerator (+4C). In order to determine levels of Der f 1 allergen, 0.1 gram of flour was sampled from each experimental unit and tested by ELISA. Sampling was carried out immediately after inoculation and subsequently at week 2, 4, 6, 8, 10, 12, 16 and 20.**

Results: **Levels of Der f 1 allergen in the inoculated samples increased significantly in all conditions 6 weeks after inoculation (p <0.001) and reached the highest levels at week 8. While experimental units left at room temperature showed higher levels of mite growth (p <0.001), no statistical differences were found among types of containers. The highest amount of Der f 1 was observed for Gogi, followed by Gold Label, tempura flour, corn flour, wheat flour and tapioca starch, respectively (p <0.01).**

Conclusions: **In the context of our experiment, mites preferably grew in cooking flours containing high amounts of wheat at room temperature, particularly after 8 week of storage. According to our results, we thus advise to keep household cooking flour refrigerated and while the type of container does not matter, storage should not exceed 20 weeks. *(Asian Pac J Allergy Immunol 2015;33:123-8)***

Keywords: *mite growth, cooking flour, allergen contamination, mite allergen, oral mite anaphylaxis*

Introduction

House dust mites (HDM) allergens have been shown to be one of the most important and ubiquitous allergens involved in asthma and allergic rhinitis pathogenesis. The most abundant species found in house worldwide are belong to the species *Dermatophagoides pteronyssinus and D. farinae.*[1] HDM allergens most often enter patients' body via inhalation, ingestion or skin contact.[2] Anaphylaxis caused by the ingestion of mite-contaminated foods was first documented in 1993 by Erben et al.[3] Since then, a number of cases who developed anaphylactic reactions after ingestion of dust or food infested by mites, a reaction commonly named the "Pancake Syndrome", have been reported from all over the world, including Asia.[4] Recently, reported cases of Pancake Anaphylaxis have been reviewed by Sanchez-Borges et al.[5] and designated as "Oral Mite Anaphylaxis (OMA)" in order to describe and diagnose this life threatening allergic reactions. While the occurrence of OMA is rare, its potentially fatal outcome underline the importance to make precise diagnosis especially in cases of recurrent anaphylaxis of unknown origin. Mite species most often associated with OMA include the domestic

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species (*D. pteronyssinus, D. farinae*), as well as the storage mites (*Blomia tropicalis, Sudasiamedanensis, Aleuroglyphusovatus, Lepidoglyphus destructor, Tyrophagus putrescientiae, Thyreophagus entomophagus and B. freemani*).[5] Most cases were reported in the tropical and sub-tropical countries where high temperature and humidity can promote mite proliferation.[6]

In Thailand, the first OMA case was recently reported at King Chulalongkorn Memorial Hospital, Bangkok. In previous reports, all patients were atopic and sensitive to mite allergens, and anaphylaxis occurred shortly after the ingestion of mite-contaminated food. These findings suggest that the allergenicity of mite allergens persists despite cooking and the thermal challenge associated to it.[7] Indeed, skin prick test involving the heated contaminated flour extract yielded lower response but persistently positive wheal-and-flare,[5,8] indicating that group 2 mite allergens may be involved in OMA due to its heat-stable characteristics.[9]

Additionally, mite contamination in food items may be facilitated during product initial manufacturing or while at home if the product holding container remains open.[8] Recent evidence suggest that using waterproof grip bags, insuring cold storage at 0-7°C and maintaining low humidity (i.e. < 12 % relative humidity) could prevent mite infestation,[10] and be effective measures to reduce OMA risk. In order to further assess the validity of these preventive procedures, the objective of this study was to evaluate the competency of several common cooking flours for dust mite growth, assess how storage conditions can affect mites proliferation and determine if the type of container in which flour is stored as well as the duration of storage can affect mite presence and proliferation.

Methods

Six common brands of cooking flours including Gogi, Gold Label, McGarett tempura flour, Knorr corn flour, whole wheat (bread flour) and Happy Baht tapioca starch were selected for this experiment. The main ingredient of each cooking flours is reported in Table1. From each individual flour brand, a sample of 50 grams was inoculated with 1 gram of *D. farinae* mites (approximately 924 ± 45 mites per gram of flour), whereas another 50 grams was left un-inoculated. Both groups were then divided according to the storage conditions, which included four different containers (original package tied with elastic band, plastic bag tied with elastic band, plastic box with lid and glass bottle), and two different storage temperatures (home refrigerator and kitchen cupboard). Flour samples (0.5 grams) were taken every second week until week 12 (7 samples) and every four weeks thereafter until week 20 (2 samples) and sent to laboratory for Der f 1 allergen examination. Temperatures and relative humidity were recorded daily during the whole experiment.

Mite allergen measurement

In each flour sample, levels of Der f 1, the major allergen of *D. farinae* mites, was determined using the commercial sandwich ELISA kit (Indoor Biotechnology, U.K.). The assay was performed according to the manufacturer instructions.

Data analysis

The statistical differences in levels of Der f 1 allergen between samples from different treatment categories were assessed using both one-way and repeated measures ANOVA followed by Bonferroni posthoc tests used to evaluate differences between levels of factors. Statistical significance was considered be met when P value < 0.05.

Results

This study was carried out from August to December 2012. The mean temperature and relative humidity in the refrigerator were 8.2 °C (range = 2.0 - 12.2 °C) and 36.7% RH (range = 22.0 - 51.4% RH), while those in the kitchen cupboard were 26.6 °C (range = 25.0 - 28.9 °C) and 69.6 %RH (range = 60.0 - 77.3 %RH), respectively. We observed that the levels of Der f 1 allergen in all mite-inoculated flour samples stored at room temperature had significantly increased after 6 weeks (p <0.05; Figure 1). There was no statistical difference of Der f 1 levels among storage containers at room temperature (Figure 2) but the allergen levels in these samples were significantly higher than those in all flour samples stored in the refrigerator (Figure 3). Meanwhile, none of the non-inoculated flour samples showed presence of Der f 1 allergens.

At room temperature, the average levels of Der f 1 allergen had significantly increased in Gogi, Gold label, wheat flour, Knorr corn flour and Happy Baht tapioca starch when compared to initial content (p <0.05), while levels in McGarrett tempura flour had significantly increased only after 8 weeks as shown

in Figure 1. Overall, all flour taken into account, the highest levels of Der f 1 allergen were observed after 8 weeks. Finally, all time period accounted, the mean highest levels of Der f 1 were found in Gogi and Gold Label followed by McGarrett tempura flour, Knorr corn flour, wheat flour and Happy Baht tapioca starch ($p < 0.01$) as shown in Figure 4.

Discussion

The incidence of respiratory allergy such as allergic rhinitis and asthma is now increasing over the global population. Over 80% of these respiratory allergic cases acquire allergy to domestic HDM via inhalation and this group of patients might be at risk of OMA. Similar to what is found in other countries, the prevalence of HDM allergy in Thailand is also about 80% of atopic patients. Although *D. farinae* is one of the common species causing OMA, the prevalence of *D. pteronyssinus* and *D. farinae* can be as high as 97% in Thailand, while *E. maynei* and *B. tropicalis* are found less frequently. [11] Moreover, the high season of mite proliferation is known to be August to November with average room temperatures found to be ranging between 25-28 °C and with a RH of approximately 75 %. The case reported from the King Chulalongkorn Memorial hospital, OMA was induced by *D. farinae* contaminated cooking flour, which was an incentive for us to investigate further the patterns of proliferation of this mite in households food items. In 1996, two Japanese children developped anaphylaxis soon after the ingestion of mite contaminated flour, namely okonomi-yaki powder. The first patient ate the products that were stored for 5 months in a cupboard. The second case consumed the flour which was kept in the same condition for 7 months. The SPT reactions to individual ingredients of the homemade "okonomi-yaki" and the original commercial mixtures were negative in both patients, and no adverse reaction was observed in the oral provocation tests with the original mixture. Approximately, 487 *T. putrescentiae* mites were found in 100 mg of the mixture eaten by patient 1 whereas lower number of *D. farinae* mites (198 mites) were detected in 100 mg of ingested product in the case of the second patient. The result of CAP-RAST analysis of *T. putrescentiae* in the serum of patient 1 was 2.09 Ua/ml, and that of *D. farinae* of the patient 2 was 159.00 Ua/ml.[12]

In most reported cases, the major source of mite allergens was wheat flour but the anaphylaxis may also be induced by other kinds of flour. In the case of a 18-year-old-woman who developed anaphylaxis a few minutes after eating polenta; a dish prepared with cooked corn flour, allergic sensitization was assessed *in vivo* by SPT test using food and common inhalant allergens as well as *in vitro* tests for the

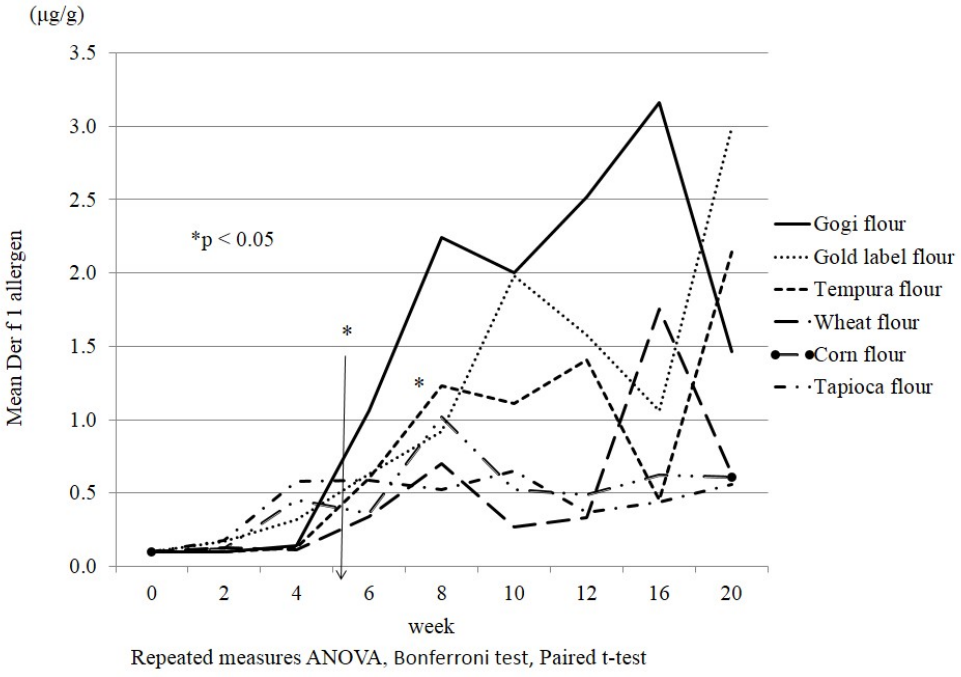

Figure 1. Mean Der f 1 allergen level of different type of mite-inoculated cooking flours kept in room temperature

Table 1. Components of 6 common brands of cooking flours tested

Component	Gogi flour	Gold label	Tempura	Bread flour	Corn flour	Tapioca
Wheat	90%	81%	50%	100%	-	-
Tapioca	6%	-	-	-	-	100%
Corn	-	6%	41%	-	100%	-
Baking powder	3%	1%	-	-	-	-
Other	1%	12%	9%	-	-	-

levels of specific IgE against *D. pteronyssinus* and *B. tropicalis* in patient's serum. Skin tests returned negative results to common foods, including corn flour, but the patient showed strong reactions to mite allergens. A sample of the corn flour in her home found a total number of 36 mites/gm of corn flour.[13]

In the currently reported Thai case, we found a number of living *D. farinae* mites but not storage mite in the cooking flour that is believed to have contributed to induce anaphylaxis. The presence of *D. farinae* against other storage mite that are usually responsible for OMA, may be explained by the highest abundance and distribution of domestic mites which proliferation is certainly accentuated by the tropical location of our country. Most of the studies examining mite presence in households report densities ranging from 5 to 5,000 mites/g.[3-5,8,12-22] Our results are consistent with previous reports. Mean Der f 1 allergen levels detected in Gogi and Gold label flours (Figure 1 & 4) were also significantly higher than any other types of cooking flours. This suggests that mites likely proliferate in cooking flour that contained high wheat such as Gogi flour, Gold label (Table 1 & Figure 1). A hundred percent wheat or corn flour and tapioca starch was associated with a lower abundance of mites. We postulate that baking powder may change the texture of the flour, increasing aeration and facilitating mite proliferation as opposed to what could be observed in 100% flour. In Thailand, Gogi and gold label brands are the most popular flours used for cooking in households and restaurants, hence, these products may increase risk of OMA in Thai mite-sensitive patients.

As already reported, optimal conditions for mite growth include temperature ranging between 25 and 28 °C with approximately 75 %RH.[11] Our observation that the level of Der f 1 allergen

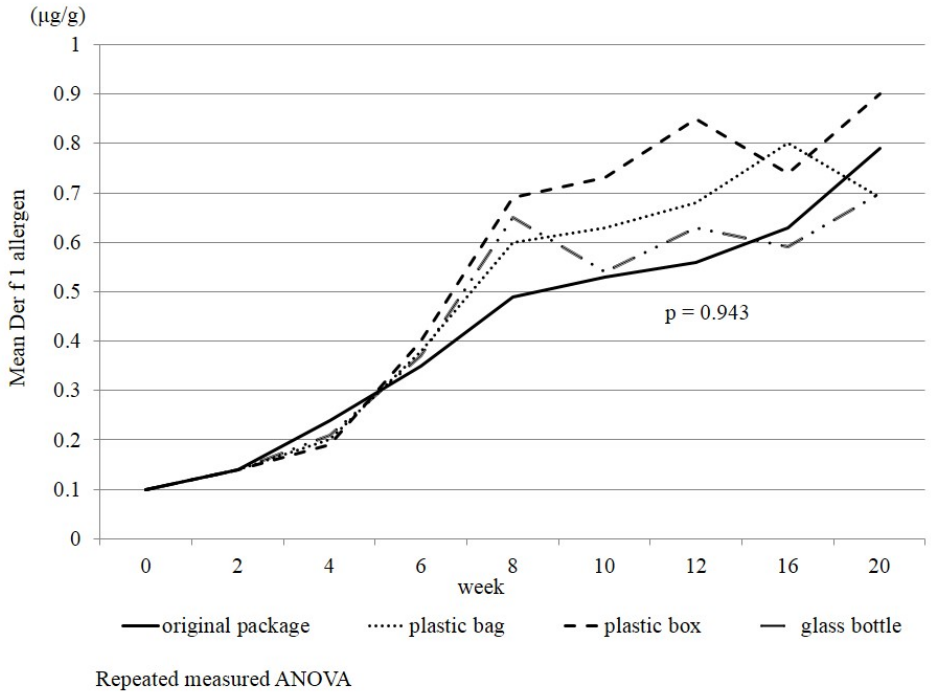

Figure 2. Mean Der f 1 allergen level in different type of mite-inoculated cooking flours kept in various storage wares

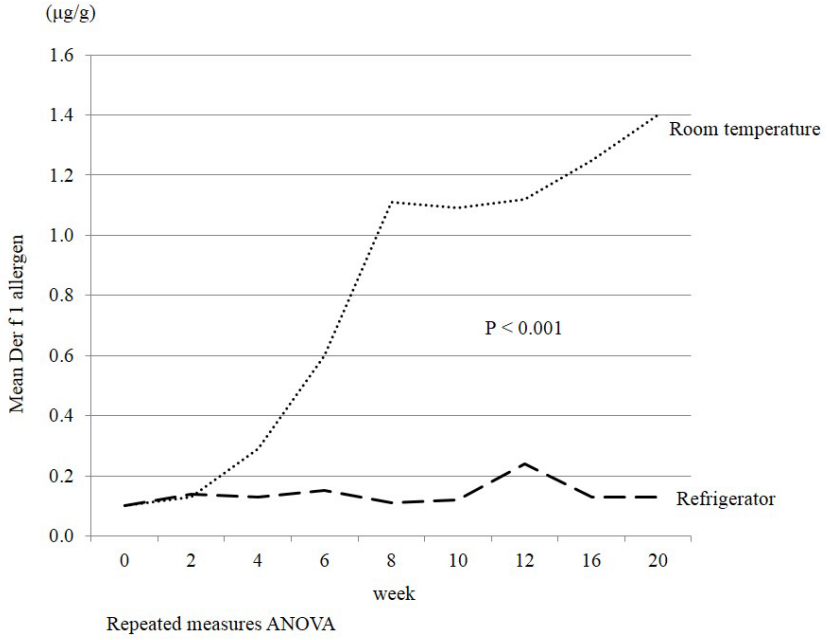

Figure 3. Mean Der f 1 allergen level in mite-inoculated cooking flours kept in room temperature and refrigerator

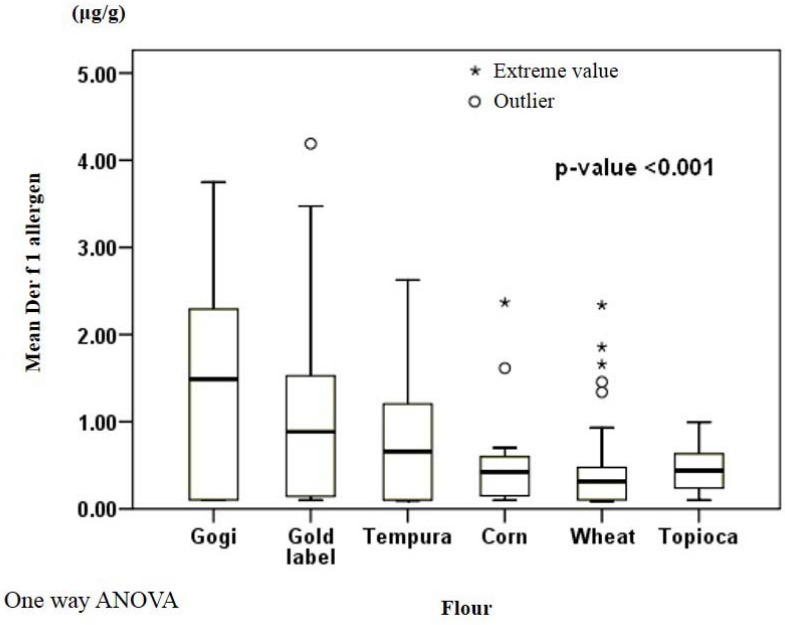

Figure 4. Mean Der f 1 allergen in all type of mite-inoculated cooking flours kept in room temperature from week 0-20

increased significantly at room temperature as compared to refrigerator storage corroborate this observation (Figure 3). Figure 2 shows trends of mite proliferation in relation to storage containers. No significant differences in mite proliferation were observed among kinds of storage wares suggesting that the type of storage containers in which flour and other items susceptible to host mites is not an important factor explaining mite proliferation. Der f 1 allergen in each non-inoculated cooking flour sample could not be detected at any time, nor were we able to detect Der f 1 allergens from kitchen dust

samples, the later observation indicating that kitchen environment can reasonably be considered free of mite or having low mite infestation.

According to our observations, we recommend flours to be kept in the refrigerator, using any kind of containers (Figure 3) but for a maximum of 20 weeks to prevent mite proliferation. High wheat mixed flours with baking powder such as Gogi flour, Gold label flour should not be kept at room temperature more than 8 weeks after opening. With such operational recommendations, mite allergic patients will minimize risks of OMA

Conclusion

Mites preferably proliferate in flour containing high wheat mixed with baking powder such as Gogi flour and Gold label flour. A hundred percent wheat or corn flour and tapioca starch are less prone to mite proliferation. Our results strongly suggest that in order to prevent mite contamination and proliferation, cooking flours should be kept in a refrigerator using any kind of container for a maximum of 20 weeks. At risk patients should avoid keeping flours containing high wheat at room temperature more than 8 weeks after opening.

Acknowledgments

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