
Assessment of the reduced allergenic properties of the Santana apple

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Background: Approximately 2% of the Northern and Central European population is allergic to apples. Because of symptoms of oral allergy syndrome, many individuals avoid eating fresh apples. A strategy to allow apple allergic individuals to eat apples is the development of hypoallergenic apple varieties.

Objective: To investigate the allergenicity of a putatively hypoallergenic apple cultivar, the Santana apple, on apple allergic individuals using a method of challenge in which loss of allergenicity is minimized.

Methods: The study population consisted of 15 apple allergic individuals, who underwent an open oral challenge with 3 different apple cultivars: Santana, Golden Delicious, and Topaz. Food challenges were performed between February 1 and May 31, 2005, during the birch tree pollen season. Sensory perception during challenges was minimized by use of a blindfold and nose clip and randomization of challenge order. Subjective symptoms were scored by a visual analog scale.

Results: After challenge with the Santana apple, 8 of 15 study participants (53%) developed no symptoms, which was a significantly greater proportion than after challenge with the Topaz apple (1 participant) and Golden Delicious apple (1 participant) ($P = .002$). The visual analog scale scores for the Santana apple (dose 1) were significantly lower than that for the Golden Delicious and Topaz apples. A total of 73% of the participants stated they would eat Santana apples in the future.

Conclusion: The Santana apple caused significantly fewer allergic symptoms in apple allergic individuals than the Golden Delicious and Topaz apples. The Santana apple may be a good choice for most apple allergic individuals who want to eat apples. This conclusion may not apply to apple allergic individuals with severe symptoms after apple consumption.

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INTRODUCTION

The apple is the most frequently eaten and cultivated fruit in the Netherlands.^{1,2} Up to 2% of the Northern and Central European population is allergic to apples.³ The apple is one of the principal foods that cause IgE-mediated symptoms associated with the oral allergy syndrome (OAS).⁴ These symptoms arise immediately after ingestion of fresh, unprocessed apple.^{5,6} Processed products of apples, such as juices and pulp, are well tolerated by most patients. Patients with OAS often avoid eating fresh apples and many other fresh fruits, which probably has an impact on their quality of life.^{7–9}

A novel approach to this problem is the development of apple varieties that are less allergenic than existing cultivars. A study by Bolhaar et al¹⁰ revealed major differences in allergenicity among various cultivars, using skin prick tests (SPTs) and double-blind, placebo-controlled food challenges in 5 patients. Golden Delicious (GD) and Elstar appeared to be the most allergenic apple cultivars. Santana, Topaz, Elize, and Breaburn were the least allergenic. However, in the preparation of apple-derived materials for clinical tests such as double-blind, placebo-controlled food challenges, loss of

allergenicity occurs.^{5,6,11} The aim of this study was to further investigate the allergenicity of the Santana apple in apple allergic patients using a method of challenge in which loss of allergenicity is minimized.

METHODS

Study participants

Apple allergic adults were included in the study if they had a positive SPT result with the GD apple, self-reported OAS symptoms after ingestion of a fresh apple with and without peel, and a positive open challenge test result with the GD apple. Patients with systemic reactions after ingestion of a fresh apple were excluded. The study participants all gave signed informed consent. The study protocol was approved by the local ethics committee of the University Medical Centre Groningen.

Food challenges

Of each apple cultivar, 3 increasing doses were given: dose 1 consisted of a bite from an intact apple, including peel (approximately 20 g); dose 2 consisted of 30 g cut from an intact apple, including peel; and dose 3 consisted of 100 g cut from an intact apple, including peel. The interval between doses was 15 minutes. The challenge was stopped at the lowest dose that induced symptoms of OAS. Participants were blindfolded and wore a nose clip during testing to minimize sensory perception. The order of the apples used for challenge was randomized by computer.

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Skin prick tests

SPTs were performed on the volar side of the forearm with the help of a 1-mm-tip lancet (ALK, Horsholm, Denmark) according to Dreborg.¹¹ The GD apple (prick to prick), Topaz apple (prick to prick), and Santana apple (prick to prick) were tested. The SPT result was regarded as positive when the diameter of the wheal was at least 3 mm. Histamine-equivalent prick (HEP) values (the ratio of the wheal size of the prick test to apple cultivars were given. These challenges consisted of a challenge with the Santana apple, a challenge with the GD apple as a positive control, and a challenge with the Topaz apple as a negative control. Apples used for challenge were as ripe as possible. The order of the challenges was randomly determined by computer. Symptoms were registered by the participants themselves on a visual analog scale (VAS) after 1, 5, 10, and 15 minutes after every dose.^{13,14} Patients were asked for each of the apple cultivars whether they would consider eating this apple variety after the study.

Study design

Food challenges were performed in the outpatients' clinic between February 1 and May 31, 2005, during the birch tree pollen season. Participants underwent an open challenge and a SPT with the GD apple. Each study participant underwent 3 single-blind challenges, with participants blinded to the order that apple cultivars were given. These challenges consisted of a challenge with the Santana apple, a challenge with the GD apple as a positive control, and a challenge with the Topaz apple as a negative control. Apples used for challenge were as ripe as possible. The order of the challenges was randomly determined by computer. Symptoms were registered by the participants themselves on a visual analog scale (VAS) after 1, 5, 10, and 15 minutes after every dose.^{13,14} Patients were asked for each of the apple cultivars whether they would consider eating this apple variety after the study.

Statistical analysis

Differences between SPT results for the apples tested expressed as diameter in millimeters and in HEP values were analyzed for all study participants with the Wilcoxon signed rank test. Differences in maximum VAS score of dose 1 among the 3 apple cultivars and differences in maximum VAS score within an apple cultivar among the various doses were analyzed for all study participants with the Wilcoxon signed rank test. Differences in observed frequencies were tested with the Pearson χ^2 test for the number of participants who ingested dose 1 and doses 2 and 3, the number of participants who remained asymptomatic after ingesting dose 3, and the number of participants who indicated that they would continue to eat the Santana apple after the study. Correlation between the size of the SPTs and the corresponding maximum VAS score of the respective apple cultivar was tested with the Spearman correlation test. Differences in SPTs between the patients who reacted after ingesting apple and the patients who had no reaction after ingesting apple were analyzed with the Mann-Whitney test. For all tests, differences with a $P < .05$ were considered statistically significant.

RESULTS

Study participants

In total, 18 adults were recruited. Three of them were excluded from participation in the study because of a negative SPT result with the GD apple. In total, 15 individuals were

challenged. Of the 15 study participants, 4 were men and 11 were women. The mean age of the participants was 29 years.

Skin prick tests

The SPT result for birch pollen was positive for all 15 participants (range, 5–16 mm). A positive SPT result was present for the GD and Topaz apples; for the Santana apple, this was the case for 13 of 15 participants. The mean HEP value of the SPT for the Santana apple (0.67) was significantly lower than for the Topaz (0.95; $P = .004$) and GD (1.00; $P = .002$) apples.

Number of challenged doses

Table 1 indicates the number of participants who received different doses. The number of participants who received dose 3 of the Santana apple differed significantly from the number of patients who had eaten dose 3 of the GD and Topaz apples ($P = .001$).

Asymptomatic consumption of apples

Eight patients were free of symptoms after the final dose with the Santana apple, whereas this was true for only 1 patient each for the GD and Topaz apples ($P = .002$). After the challenge, the number of participants who stated they would eat the apple in the future was 5 of 15 for the GD apple, 3 of 15 for the Topaz apple, and 11 of 15 for the Santana apple (73%). In this respect, the Santana apple differs significantly from the GD and Topaz apples ($P = .009$). For the Santana apple, no difference could be found in the size of the SPT results between the patients who reacted after ingesting the Santana apple and the patients who had no reaction after ingesting the Santana apple.

VAS scores

Figure 1 shows that the maximum VAS score for the Santana apple for all persons is significantly lower than the maximum VAS score for the Topaz and GD apples. The maximum VAS score did not differ between the Topaz and GD apples. Because too few participants had dose 2 or 3 of the GD, Santana, and Topaz apples, no comparison could be made. Within the various cultivars, no difference could be found among the maximum VAS scores of the different doses. No correlation was found between the results of the SPTs and the VAS scores.

Table 1. Number of Study Participants by Dose and Apple Type

Dose ^a	GD	Topaz	Santana
1	15	15	15
1 and 2	5	5	12
1, 2, and 3	3	2	11 ^b

Abbreviation: GD, Golden Delicious.

^a Dose 1 consisted of a bite from an intact apple, including peel (approximately 20 g); dose 2 consisted of 30 g cut from an intact apple, including peel; and dose 3 consisted of 100 g cut from an intact apple, including peel.

^b $P < .05$ compared with GD and Topaz apples.

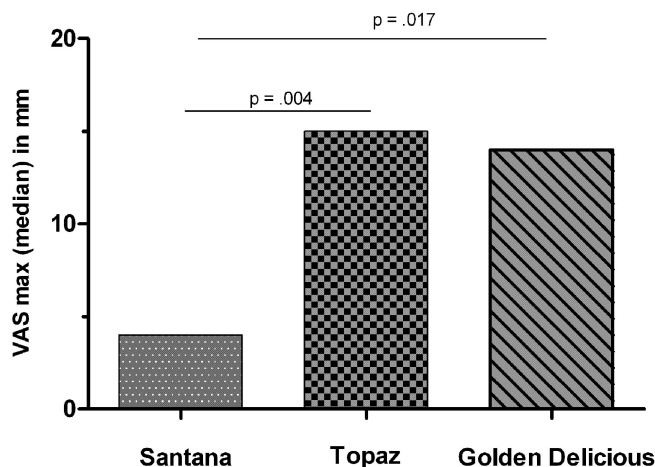


Figure 1. Maximum visual analog scale scores (VAS max) during dose 1.

DISCUSSION

We investigated the allergenicity of an apple variety with putatively reduced allergenic properties: the Santana apple. The Santana apple was selected in 1978 as a cross-breeding between the Elstar and Priscilla apples and has been on the market since 1996. Apart from its putatively reduced allergenicity, the Santana apple is of interest for 2 other reasons: the Santana apple is resistant to scab, the major apple disease, and the Santana apple is less harmful to the environment because it can be cultivated with fewer pesticides, making it suitable for ecological cultivation. Our study confirmed the data of Bolhaar et al¹⁰ that the Santana apple has reduced allergenic properties, causing fewer symptoms, even during the birch pollen season.

The study by Bolhaar et al used double-blind, placebo-controlled food challenges, which have the advantage of superior blinding of the challenge material and are generally seen as the most reliable way of diagnosing food allergy.¹⁵ The disadvantage of this method for apple and most other fruits, however, is that during preparation of challenge materials, allergenicity may be lost. This loss of allergenicity may result in overestimation of the reduced allergenicity of the apple tested. Our test procedure involved a single-blinded challenge with individuals blinded to the order that apple cultivars were given. The blinding procedure minimized sensory perception while retaining optimal allergenicity. The advantage of this method is that it approaches more closely real-life apple consumption and is thus unlikely to overestimate reduced allergenicity. Double-blind challenge methods that have been or will be developed may in the future be compared with the method used herein to ascertain whether allergenicity is preserved in the preparation of the challenge materials.

The reason for the reduced allergenicity of the Santana apple is poorly understood. The literature shows that allergenic differences among apple cultivars are mainly related to

differences in expression levels of Mal d 1¹⁶ of which 18 different isoforms are currently known.¹⁷ There are thus 2 possible explanations for the reduced allergenic properties of the Santana apple, namely, lower levels of Mal d 1 or preferential expression of Mal d 1 isoforms that are less allergenic. The relation between the occurrence of allelic diversity among cultivars and the difference in allergenicity is not clear yet, and the relation between the different isoforms of Mal d 1 and their biological function remains to be elucidated.

OAS is a unique manifestation of food allergy for a number of reasons. It is the most common form of food allergy in Northern Europe as a result of the high prevalence of birch pollinosis. It is generally benign in this context and is rarely accompanied by severe symptoms. The question arises whether OAS is a true systemic allergic reaction or whether it is a local phenomenon. Our data show that the relationship between symptoms of OAS and specific IgE as measured by SPTs is complex, with lack of correlations between severity of symptoms by VAS scores and size of the SPT results and asymptomatic sensitization seen in some participants, both of which are characteristics that pertain to systemic allergic reactions to foods.^{18,19} These observations suggest that OAS is a true systemic allergic reaction and thus an interesting model for further study.

This study showed that the Santana apple causes fewer allergic symptoms compared with the Topaz and GD apples. It is the first known example of a food developed for consumption by food allergic individuals. The Santana apple might be an alternative to other apple varieties for individuals with OAS and gives them the opportunity to consume fresh apples, which contributes to a normal, healthy, well-balanced diet. This apple variety may not be an alternative for apple allergic individuals with other symptoms such as anaphylaxis after apple consumption.

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