Frozen fruit skin prick test for the diagnosis of fruit allergy

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Summary

Background: **Diagnosis of fruit sensitisation by skin prick test (SPT) is fast and easy to perform. Nevertheless, some fruit is not available throughout the year. Freezing aliquots of these fresh fruits to be defrosted would be a good solution to perform SPT at any time.**

Objective: **To compare the reproducibility of SPT with *Rosaceae* and *Cucurbitaceae* frozen fruit with fresh and commercial fruit extracts.**

***M*ethods: SPT with the following fruit were performed: apricot, cherry, strawberry, nectarine, *Japanese* medlar, peach, (peel and pulp), yellow and red plum, melon and watermelon. We compared fresh fruit, commercial extract and fruit which had been frozen at -18°C. Results were read by planimetry (Inmunotek prick-filmTM) after 15 minutes.**

Results: **The study group comprised 48 patients (9 males, 39 females) with a mean age of 31, 6 ± 2,0 years. Concordance of positive and negative results was extremely high and significant in all cases. Correlation between frozen fruit and commercial extract, frozen fruit and fresh and commercial extract and fresh fruit was statistically significant in all cases except for strawberry.**

Conclusions: **The use of frozen fruit is a valid method, as the performance of the SPT is similar to that of fresh fruit. This enables diagnostic procedures with seasonal fruit at any time of the year. *(Asian Pac J Allergy Immunol 2010;28:275-8)***

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Introduction

Fruit is the most common cause of food allergy in the adult population in Europe.[1] Nowadays, the diagnosis of food allergy is based on a thorough case history supported by skin prick tests (SPT), specific serum IgE or recombinant allergens and includes its confirmation by labial and food challenges.[2] Of all these methods, for diagnosis of fruit sensitisation, SPT is the fastest and easiest to perform.[3] On the other hand, SPT performed with fresh fruit has higher sensitivity and specificity than commercial extracts.[4,5] Nevertheless, some seasonal fruits are not available throughout the year, as is the case of some fruit of the *Rosaceae* family such as apricot, cherry, strawberry, nectarine, *Japanese* medlar, peach, yellow plum and red plum and *Cucurbitaceae* family such as melon and watermelon. Freezing fruit aliquots might be a solution to overcome the problem of availability of some fruit at certain periods of the year. Therefore, we designed the study to evaluate the performance of frozen fruit in SPT to diagnose sensitisation. So, the objective of our study was to compare the accuracy of SPT with *Rosaceae* and *Cucurbitaceae* frozen fruit with fresh fruit and commercial fruit extracts.

Methods

Subjects

A total of 58 subjects who accepted to participate in the study were recruited in the Allergy Section from Vall d'Hebron Hospital in Barcelona (Spain). Of the 58 subjects, 48 were patients allergic to fruit (*Rosaceae* and/or *Cucurbitaceae* families). We also recruited 3 male and 7 female healthy control subjects. They were

all subjects with no history of allergy. An informed consent was obtained from all the study participants. The study was performed from June 2008 to July 2010. The diagnosis of fruit allergy to *Rosaceae* and *Cucurbitaceae* families was based on a thorough case history supported by SPT, specific serum IgE and reactions were confirmed by labial, and in select cases, open food challenges.The study was conducted with the approval of the Vall d'Hebron ethics committee.

Skin prick tests

SPT were performed with the following fruits: melon, watermelon, apricot, cherry, strawberry, nectarine, *Japanese* medlar, peach (peel and pulp), yellow plum and red plum. Fresh fruit was puréed separately, divided into aliquots and frozen at -18°C until the day of SPT. SPT with all fruits were performed simultaneously with commercial extracts (kindly supplied by Laboratorios LETI S.L., Spain), frozen and fresh fruit. Histamine hydrogen chloride 10 mg/ml was used as the positive control and physiologic saline as the negative one. To avoid puncture technical bias all tests were performed by the same researcher.[6] All SPTs were conducted using injections in the volar surface of each forearm in inverse order with respect to the other. SPT were performed following EAACI recommendations.[7] At 15 minutes papules were measured by planimetry (Inmunotek prick-filmTM), scanned and processed by a specific software program. Papules were considered positive if they were greater than 7.1 square millimetres (mm^2) which would equal a papule of 3 millimetres in diameter. All patients were instructed not to take medications during the 2 weeks before the test.

The study followed rigorously the international ethical recommendations for the investigation and clinical tests in humans (Declaration of Helsinki on clinical tests, Edinburgh 2000), following the recommendations of Spanish Ministry of Health and the ethical Deontological code of the General Council of Physicians of Spain.

Statistical analysis

The statistical evaluation was performed using a software program (SPSS; SPSS Inc, Chicago, IL). The normality of the distribution of the data was determined using the Kolmogorov-Smirnov test. The non-parametric test Kruskal-Wallis One Way Analysis of Variance was used to compare the differences in the wheal sizes. A Bland Altman analysis was used to determine the agreement between frozen and fresh fruit SPT, frozen and commercial fruit SPT and fresh and commercial fruit SPT. Lineal correlations were used to evaluate the correlation between commercial extract and frozen fruit, fresh and frozen fruit and fresh and commercial fruit SPT. A p value = 0.01 was set to determine level of significant correlation.

Results

48 patients with fruit allergy were included (9 males, 39 females) with a mean age of 31, 6 ± 2,0 years. Commercial extracts of apricot, nectarine and watermelon were not available at the time of study, so only frozen and fresh fruit were compared in these cases. When analysing the correlation between frozen fruit and commercial extract, frozen fruit and fresh and commercial extract and fresh fruit statistically significant correlation was found in all cases except for strawberry (Table 1). A Bland Altman statistical

Table 1. Pearson's Correlation Coefficient between frozen fruit and commercial extracts, frozen and fresh fruit and fresh fruit and commercial extracts for each fruit

	Frozen fruit / Commercial extract	Frozen fruit / Fresh fruit	Fresh fruit/ Commercial extract
Apricot (n = 44)	ND	0,817 (p < 0,001)	ND
Cherry (n = 43)	0,794 (p < 0,001)	0,863 (p = 0,002)	0,730 (p < 0,001)
Strawberry (n = 35)	0,394 (p = 0,394)	0,748 (p < 0,001)	0,338 (p = 0,059)
Nectarine (n = 45)	ND	0,839 (p < 0,001)	ND
Peach (complete) (n = 46)	0,751 (p < 0,001)	0,787 (p < 0,001)	0,705 (p < 0,001)
Peach (peeled) (n = 46)	0,835 (p < 0,001)	0,882 (p < 0,001)	0,693 (p < 0,001)
Japanese Medlar (n = 43)	0,792 (p = 0,009)	0,837 (p < 0,001)	0,647 (p = 0,003)
Yellow plum (n = 44)	0,808 (p < 0,001)	0,802 (p < 0,001)	0,717 (p < 0,001)
Red plum (n = 44)	0,751 (p < 0,001)	0,703 (p < 0,001)	0,698 (p = 0,007)
Melon (n = 44)	0,908 (p < 0,001)	0,842 (p < 0,001)	0,847 (p < 0,001)
Watermelon (n = 44)	ND	0,993 (p < 0,001)	ND
All	0,897 (p < 0,001)	0,718 (p < 0,001)	0,795 (p < 0,001)

(ND Not done; statistically significant correlation p=0.01)

test was used in order to compare different methods (frozen fruit, fresh fruit and commercial extract SPT) that measure the same parameter (diameter of the papule). Hence, we compared two clinical measurements with a new measurement technique. As you can see in Table 2, this analysis revealed an average discrepancy between commercial extract and frozen fruit SPT of 8.4 mm² (papules were considered positive if they were greater than 7.1 mm²). However, when comparing fresh and frozen fruit and commercial extract and fresh fruit the average of discrepancy was lower (5.6 mm² and 6.0 mm² respectively). Moreover, when analysing each fruit, positives and negatives values of the papules were well correlated in all Bland Altman analysis fruits except for strawberry (Table 2). Therefore, this Bland Altman analysis makes the point that any two methods that are designed to measure the same parameter will have a good correlation. On the other hand, when analysing negative and positive results for each fruit (Table 3) the number of positive SPT using frozen fruit was higher than using commercial extracts in all cases but for peeled peach and yellow plum. In conclusion, the concordance of the results of the tests performed with frozen fruit was even higher and significant and frozen fruit papules tended to be larger.

Table 2. Bland Altman analysis

	Frozen fruit / Commercial extract	Frozen fruit / Fresh fruit	Fresh fruit/ Commercial extract
Apricot (*n* = 44)	ND	1,5 mm²	ND
Cherry (*n* = 43)	9,5 mm²	14,8 mm²	10,5 mm²
Strawberry (*n* = 35)	8,0 mm²	6,4 mm²	6,5 mm²
Nectarine (*n* = 45)	ND	1,9 mm²	ND
Peach (complete) (*n* = 46)	3,3 mm²	3,2 mm²	2,9 mm²
Peach (peeled) (*n* = 46)	4,9 mm²	2,6 mm²	5,2 mm²
Japanese Medlar (*n* = 43)	49,7 mm²	30,4 mm²	40,0 mm²
Yellow plum (*n* = 44)	2,4 mm²	5,0 mm²	5,2 mm²
Red plum (*n* = 44)	2,6 mm²	5,2 mm²	3,8 mm²
Melon (*n* = 44)	4,3 mm²	4,4 mm²	4,9 mm²
Watermelon (*n* = 44)	ND	2,0 mm²	ND
All	8.4 mm²	5,6 mm ²	6,0 mm²

(ND Not done)

About our control group, of the ten control subjects included in the study (3 males and 7 females, mean age 35,8 ± 4,2 years), all of them had negative SPT to frozen fruit, fresh fruit and commercial fruit extracts which indicate that SPT extracts used in this study did not produce irritation and therefore did not induce false positive tests in healthy controls.

Finally, we take into account that all SPTs were performed on both arms and no differences were observed.

Discussion

Because some seasonal fruit is not available throughout the year as is the case of some fruit of the *Rosaceae* and *Cucurbitaceae* families, the high correlation and concordance found between commercial extract and frozen fruit SPT and between fresh and frozen fruit suggests that, at least in our population, the use of frozen fruit can be considered a useful tool for detection of sensitization to *Rosaceae* and *Cucurbitaceae* families. In an extensive review of the literature

Table 3. Number of negative and positive results for frozen, fresh and commercial SPT extracts for each fruit

	Frozen fruit Positive/Negative	**Fresh fruit Positive/Negative**	**Commercial extracts Positive/Negative**
Apricot (*n* = 44)	20/24	20/24	ND
Cherry (*n* = 43)	29/14	27/16	25/18
Strawberry (*n* = 35)	5/30	1/34	3/32
Nectarine (*n* = 45)	35/10	36/9	ND
Peach (complete) (*n* = 46)	39/7	37/9	38/8
Peach (peeled) (*n* = 46)	16/30	13/33	18/28
Japanese Medlar (*n* = 43)	10/33	8/35	2/41
Yellow plum (*n* = 44)	21/23	26/18	24/20
Red plum (*n* = 44)	24/20	24/20	24/20
Melon (*n* = 44)	13/31	14/30	8/36
Watermelon (*n* = 44)	7/37	6/38	ND

(ND Not done)

we found that few authors have addressed this methodology. Ziegert and co-workers[8] reported a study which included thirty-two children with atopic dermatitis in which the results of SPTs with fresh and frozen cow's milk and eggs were compared. The results of the procedures were similar and the authors concluded that both methods were equivalent. Our results cannot be transferred automatically to other common foods. Therefore, studies of a similar design are warranted if further foods are intended to be used for SPT after freezing. Our results suggest that the use of frozen fruit is a valid method as the performance of SPT is similar to that of fresh fruit and commercial extracts. Therefore, the methods may be used interchangeably. Moreover, in both comparisons frozen fruit papules tended to be larger. One hypothetical explanation for these results could be that frozen fruit extracts used for SPT were puréed before freezing at -18°C and this processing may produce a high number of multiple allergenic proteins exposure when doing SPTs with frozen fruit.

On the other hand, not only do frozen fruit seem to be similar to fresh fruit and commercial extracts for SPT but, as mentioned above, they also offer the added advantage that the same material can be used for SPT and oral food challenges. However, another study comparing frozen and fresh fruit in oral challenges should be carried out. Hence, from all these results we can conclude that the use of frozen fruit for SPT is a valid method as the performance of SPT is similar to that of fresh fruit. This makes it possible to carry out diagnostic SPTs with seasonal fruit at any time of the year.

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