® Postepy Hig Med Dosw (online), 2020; 74: 20-27
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 01.03.2019
Accepted: 29.01.2020
Published: 19.02.2020

An assessment of the risk of allergenicity associated with selected strawberry cultivars on a guinea pig model*

Ocena działania uczulającego wybranych odmian truskawki na zwierzęcym modelu świnki morskiej

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Magdalena Jasińska-Stroschein[1, A, B, C, D, E, F], Piotr Szcześniak[1, B, D], Jacek Owczarek[3, B, C], Krzysztof P. Rutkowski[2, A, B, D, E, F], Jarosław Markowski[2, B, D], Artur Miszczak[2, B], Daria Orszulak-Michalak[1, A, E]

[1] Department of Biopharmacy, Medical University of Łódź, Poland
[2] Research Institute of Horticulture, Skierniewice, Poland
[3] Department of Hospital Pharmacy, Medical University of Łódź, Poland

Summary

Aim of the study was to assess the risk of any allergic reaction or food hypersensitivity resulting from topical application and chronic oral administration of the fruit of selected strawberry cultivars ('Elsanta' and 'Honeoye') from farms managed organically. Materials and methods. The plantations were managed according to organic (OFP) as compared to integrated production (IFP) systems. The experiments were performed on outbred young, adult, white albinotic guinea pigs (Dunkin Hartley). Fruit characteristics included total soluble solid content, titratable acidity, sugar, polyphenol content and macro- and micronutrients. Results. The most pronounced changes in guinea pig skin followed topical exposure to 'Elsanta' strawberries from plantations managed organically showed discrete, moderate or intense erythema and swelling. Chronic oral administration of selected fruit extracts did not cause any skin reactions in groups receiving 'Elsanta' or 'Honeoye' from organic or integrated productions. The skin prick test did not show any immediate skin reactions compared to exposure to 1% histamine hydrochloride solution. Conclusion. Organic method of strawberry production cannot be concerned as more allergenic one as compared to integrated system. Any strict relationship between type of cultivar and selected macro-, micronutrients contents or fruit characteristics on the possible increase in allergenicity risk, was not found, either.

Keywords: strawberry • allergy • guinea-pig • organic farming • integrated fruit production

GICID 01.3001.0013.8550
DOI: 10.5604/01.3001.0013.8550
Word count: 4502
Tables: 4
Figures: 1
References: 25

*This research is supported by a grant from the EU Regional Development Fund through the Polish Innovation Economy Operational Program, contract N. UDA-POIG.01.03.01-10-109/08-00.

Adres autorki: Author's address: Magdalena Jasińska-Stroschein, Department of Biopharmacy, Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź, Poland; e-mail: magdalena.jasinska-troschein@umed.lodz.pl

INTRODUCTION

Allergy has been described as the epidemic of the 21st century, affecting up to 40% of the general population of developed countries. The most common allergy incidences caused by fruits are linked to those of the Rosaceae. They are widely consumed and have been increasingly reported as causes of allergic reactions. The Rosaceae includes fruits such as peach, almond, cherry (*Prunoideae* subfamily), apple, pear (*Pomoideae* subfamily), as well as blackberry and strawberry (*Rosoideae* subfamily). Several allergens that have been identified in the fruit of the Rosaceae may be responsible, especially for the cross-reactivity phenomenon linked to birch pollinosis. Among the Rosaceae, strawberries are currently one of the most popular berries grown at estimated 7.8 million metric tonnes per year [7]. However, strawberries have an unjustified reputation among the general population of being an allergenic fruit. The incidences of such hypersensitivity are commonly reported but poorly investigated [25]. An example trial with Portuguese schoolchildren revealed that the most frequently implicated food groups were fresh fruits, including strawberries, and both IgE-associated reactions and negative fruit-specific IgE levels were observed [10].

Another issue is the growing interest of organic cultivation. By definition, organic fruit production (OFP) relies on natural mechanisms to control the growth, yield and health status of the plants, without the use of soluble mineral fertilisers, herbicides and synthetic chemical pesticides [16]. In contrast, integrated fruit production (IFP) is defined as the economical production of high quality fruits, which minimizes the undesirable side effects and use of agrichemicals to enhance safeguards to the environment and human health [4].

Although food products of organic origin are believed to be healthier than the corresponding conventional foods [2], clear experimental evidence supporting this assumption remains incomplete and further research is required to determine the nutritional potential of these products. Another question is whether such technologies could cause any changes in the expression of fruit allergens, resulting in alterations of allergenic potential.

Our purpose was to assess if the risk of any allergic reaction or food hypersensitivity to selected strawberry cultivars increases when they are managed organically. Such potential reactions were examined after topical application and chronic oral administration of strawberry fruit in accordance with cultivar and production system. Two cultivars ('Elsanta' and 'Honeoye') were chosen; each was cultivated according to the organic *vs* integrated method. We suggested that safety would possibly decrease due to the method of cultivation in both cultivars. In turn, the increase in allergenicity potential due to cultivar would occur in both production systems.

The potential risk of any allergic reaction or food hypersensitivity was thought to result from topical application (Guinea Pig Maximization test-GPMT, prick test) and chronic oral administration of selected strawberry cultivar. Sensitization methods (GPMT) address delayed-type hypersensitization (Type IV, (Gell and Coombs), while for the recognition of type I IgE mediated reactions, prick/intradermal skin testing, oral provocation and total IgE measurement were used. Another aim of the study was to reveal if the observed reactions on strawberry cultivars are IgE-dependent or IgE-independent.

METHODS

Biological material: The plant material were the fruits of two strawberry cultivars, 'Elsanta' and '*Honeoye', cultivated according to IFP or OFP methods in two orchards located in the central parts of Poland:* plantations in Pustkowa Góra run according to rules of integrated production with systematic monitoring of nitrates, heavy metals or herbicides (*Elsanta* – EIP and *Honeoye* – HIP), and plantations in Mokra Lewa run according to the rules of organic production (Elsanta – EOR and *Honeoye* – HOR). During production, no synthetic herbicides, fertilizers, concentrates, genetically modified organisms (GMO), industrial fodders or ionizing radiation were applied. After harvest, fully ripe fruits were frozen at –25 C, disintegrated in the frozen state, finely ground in solid CO_2 with Blixer 3 (Model 712033, Robot Coupe, France) and then packed into 120g portions, called thereafter fruit preparations. Such portions were kept frozen until used. The characteristics of the fresh fruit are presented in table 1.

Reagents and substances: Freund Adjuvant Complete -CFA (batch no.: 029K8708, Sigma-Aldrich), Histamine hydrochloride (batch no.: 100896320, Sigma-Aldrich), Sodium lauryl sulphate-Ph.Eur (batch no.: 1052, POCH S.A.), Vaseline (batch no.: 110495. Pharma Cosmetic), Ascorbic acid (batch no.: 110158, Pharma Cosmetic), Benzocaine (batch no.: 110030, Pharma Cosmetic) and Aqua pro injection (Polpharma).

Animals: The experiments were performed on eighty-four outbred young, adult, white albinotic guinea pigs (*Dnkin Hartley*) of both sexes, with an average weigh of 403.7 g (± 100,2), fed on granulated fruits, with free access to water. The temperature of the experimental animal room was 20 C (±3 C) and the relative humidity 30-70 percent with a 12-hour light, 12-hour dark photoperiod. The animals were housed in standard cages. During the experiments, all guinea pigs received an

Table 1. Fruit characteristics

Cultivar	'Elsanta'		'Honeoye'	
Type of production	IFP	OFP	IFP	OFP
Dry matter (%)	10.3 (± 0.1)	11.4 (± 0.1)	9.5 ± (0.1)	12.0 (± 0.1)
Total soluble solids (%)	8.7 (± 0.01)	9.6 (± 0.01)	7.6 (± 0.01)	10.2 (± 0.1)
Titratable acidity(%)	0.79 (± 0.01)	0.63 (± 0.01)	1.02 (± 0.01)	0.95 (± 0.01)
Anthocyanins (mg/100ml)	33.7 (± 0.1)^{d}	32.5 (± 0.4)^{c}	65.6 (± 1.1)	68.0 (± 3.9)
Polyphenols (mg/100ml)	277.0 (± 2.0)	307.0 (± 4.0)	301.0 (± 4.0)	296.0 (± 3.0)
Malic acid (mg/100g)	191.2 (± 12.0)^{d}	191.4 (± 5.2)^{c}	251.9 (± 9.8)	251.7 (± 7.6)
Ascorbic acid (mg/100g)	20.5 (±3.9)^{d}	12.4 (±7.2)	31.1 (±2.0)^{c}	18.3 (±1.9)
Citric acid (mg/100g)	841.2 (±54.9)$^{a, d}$	592.0 (±6.0)^{c}	970.4 (±29.8)	877.9 (±20.8)
Saccharose (g/kg)	< 0.01	2.3 (±0.8)	3.1 (±0.8)	5.5 (±2.1)
Glucose (g/kg)	32.3 (± 1.2)$^{a(p=0.053), d}$	34.6 (± 0.7)	21.0 (± 0.6)^{c}	35.2 (± 1.5)
Fructose (g/kg)	37.6 (± 1.5)^{d}	39.7 (± 0.6)	24.9 (± 0.5)^{c}	39.5 (± 1.8)
Ferrum (mg/kg)	1.8 (± 0.1)^{a}	3.2 (± 0.2)	2.4 (± 0.1)	2.5 (± 0.1)
P (mg/kg fw)	276.4 (± 1.6)^{a}	244.9 (± 7.3)^{c}	271.6 ± (8.4)	285.0 (± 4.7)
K (mg/kg fw)	1824.0 (± 11.3)$^{a, d}$	1380.0 (± 11.3)^{c}	1909.5 (± 7.8)^{c}	1773.0 (± 1.4)
Mg (mg/kg fw)	144.2 (± 1.4)$^{a, d}$	129.3 (± 3.2)	153.1 (± 0.3)^{c}	128.8 (± 0.3)
Ca (mg/kg fw)	179.6 (± 2.1)^{a}	248.7 (± 12.0)^{c}	194.2 (± 5.5)^{c}	229.1 (± 3.7)
B (mg/kg fw)	1.5 (± 0.01)^{d}	1.6 (± 0.01)^{c}	0.9 (± 0.01)^{c}	1.4 (± 0.02)
Cu (mg/kg fw)	0.3 (± 0.03)	0.4 (± 0.01)	0.4 (± 0.01)	0.3 (± 0.01)
Fe (mg/kg fw)	3.2 (± 0.02)$^{a, d}$	1.8 (± 0.04)^{c}	2.4 (± 0.01)	2.5 (± 0.01)
Mn (mg/kg fw)	3.6 (± 0.04)$^{a, d}$	2.1 (± 0.04)$^{b, c}$	5.2 (± 0.2)^{c}	3.8 (± 0.1)
Zn (mg/kg fw)	1.3 (± 0.03)$^{a, d}$	0.6 (±0.01)^{c}	1.5 (± 0.1)	1.6 (± 0.1)
S (mg/kg fw)	77.6 (± 0.1)$^{a, d}$	87.3 (± 1.8)	66.6 (± 0.8)^{c}	88.0 (± 2.6)

a – as compared to organic Elsanta; b – as compared to Elsanta cultivated by integrated production; c – as compared to organic Honeoye; d – as compared to Honeoye cultivated by integrated production; $p<0.05$, fw – fresh weight

adequate amount of ascorbic acid. The animals were weighed before the test commenced and at the end.

The experimental procedures were carried out in accordance with the international guidelines for care and use of laboratory animals. All efforts were made to minimize animal suffering and reduce the number of animals used in the experiments. All the procedures in these experiments were approved by the Ethics Committee of the Medical University of Lodz, Poland.

The animals were randomly allocated into groups, as follows: I. Strawberry 'Elsanta' IFP, (n = 12) and the control group (n=5); II. Strawberry 'Elsanta' OFP, (n = 12) and control group (n=5); III. Strawberry '*Honeoye*' IFP, (n = 12) and control group (n=5); IV. Strawberry '*Honeoye*' OFP, (n = 12) and control group (n=5); V. Animals exposed to benzocaine during the validation process, (n = 11) and control group (n = 5).

EXPERIMENTAL: GUINEA-PIG MAXIMIZATION TEST (GPMT)

The sensitivity and reliability of the experimental technique was confirmed by the use of benzocaine: a substance with mild-to-moderate skin sensitization properties. All procedures were performed according to OECD guidelines for skin sensitization tests (406) [17, 18]. In brief, the test animals were initially exposed to the fruit preparations by intradermal injection. Following a rest period of 10-14 days (induction period), during which an immune response may develop, the animals were exposed to a test. The extent and degree of skin reaction to the challenge in the test animals was compared with that demonstrated by control animals, which underwent the same treatment during induction and were exposed to the tested substance. Approximately 24, 48 and 72 h after this procedure, skin reactions were observed and recorded according to the OECD guideline scaling by two independent investigators.

Induction: Day 0 (treated groups): Three pairs of intradermal injections of 0.1 ml volume were administered in the shoulder region, which was cleared of hair so that one of each pair lies on either side of the midline. Injection 1: a 1:1 mixture (v/v) FCA/Aqua pro injectione; Injection 2: Fruit preparation, according to animal group (i.e. 'Elsanta' IFP; 'Elsanta' OFP; '*Honeoye'* IFP; '*Honeoye' OFP*); Injection 3: Fruit preparation formulated in a 1:1 mixture (v/v) FCA/Aqua pro injectione.

Day 0 (control group): Three pairs of intradermal injections of 0.1 ml volume were administered in the same sites as in the treated animals. Injection 1: a 1:1 mixture (v/v) FCA/Aqua pro injection; Injection 2: the undiluted vehicle; Injection 3: a 50% w/v formulation of the vehicle in a 1:1 mixture (v/v) FCA/Aqua pro injectione. Day 5–7(treated and control groups): Approximately twenty-four hours before the topical induction application and after shaving, the skin area was painted with 0.5 ml of 10% sodium lauryl sulphate in vaseline, to create a local irritation. Day 6–8(treated groups): The selected fruit preparations, according to animal group (i.e. 'Elsanta' IFP; 'Elsanta' OFP; 'Honeoye' IFP; or 'Honeoye' OFP) were applied on the test areas, after renewed shaving.

Day 6–8 (control group) Only the vehicle was applied, in a similar manner, to the test area.

Challenge: Day 20–22 (treated and control groups): The selected fruit preparations, according to animal group (i.e. 'Elsanta' IFP; 'Elsanta' OFP; 'Honeoye' IFP; or 'Honeoye' OFP) or vehicle were applied to the test areas. Approximately 48 and 72 hours after this procedure, the skin reactions were observed and recorded according to the OECD guideline scaling (0 = no visible change; 1 = discrete or patchy erythema; 2 = moderate and confluent erythema; 3 = intense erythema and swelling).

Chronic administration of selected fruit extracts

The fruit preparations were administrated *per os* (10g/day/animal) according to each animal group for a 30-day period. This was performed to assess whether chronic feeding with such fruit preparations can result in any skin reaction.

Skin prick test

For all tests, 0.9% of physiologic saline solution was used as negative control and aqueous 1% (10 mg/ml) of histamine hydrochloride solution was used as positive control. A drop of saline solution and histamine solution were pipetted on the intact animal skin and pricked with lancets (M Mediware, Blutlanzette, steril, Premium Quality, REF B2 01). Asymmetrical wheals were measured as follows: wheal size perpendiculars to each other were measured, divided by two and the average wheal diameter showed in millimeter (mm) [5]. Testing was performed by two investigators blinded to the group to which the test subject belonged. Skin prick tests were read after 10 and 20 minutes and quantified on the basis of wheal diameter, as compared to negative and positive control.

Total IgE measurement

At the end of the experiment, for further serum analysis, 0.5ml of blood samples were taken from the left atrium under general anesthesia. Sera were obtained by centrifugation and stored at -20 C until used. The total IgE antibody content was determined using commercial Guinea Pig Immunoglobulin E, IgE ELISA Kit (Catalog No: CSB-E06768Gu, Cusabio Biotech Co., Ltd).

STATISTICS

The statistical analysis was carried out using Statistica version 12.0 software (Statsoft). The statistical evaluation of quantitative variables among more than two groups was performed using the analysis of variance (ANOVA), and *post hoc* comparisons by the Tukey test. Normal distribution of a parameter was checked by means of the Shapiro-Wilk test and the homogeneity of variance by the Brown-Forsythe test. If the data was not normally distributed, the values of variance were different, and so ANOVA with the Kruskal-Wallis was used. The Mann-Whitney U-test was used for one-factor comparisons of ordered variables between two groups, and ANOVA with the Kruskal-Wallis test for comparisons among more than two groups. All parameters were considered statistically significantly different if $p<0.05$.

RESULTS

Guinea-Pig Maximization Test (GPMT) – Magnusson-Kligmann test

During the validation of the Magnusson-Kligmann test, skin reactions to benzocaine were manifested in particular with discrete or patchy erythema, and were observed in 63.6 % of examined animals, moderate or confluent erythema in 18.2% and intense erythema or swelling in 9.1%. In general, 90% of the animals revealed some changed skin 48h following the test. Mean ranks for points being classified according to Magnusson-Kligmann scale at 24 and 48 hours after the test were different between animals exposed to benzocaine and the control group (Table 2).

The Magnusson-Kligmann test results revealed no significant changes in skin reactions with regard to controls in guinea pigs exposed to extracts from Honeoye strawberries derived from organic production (OFP) as compared to those exposed to Honeoye strawberries derived from integrated production (IFP). Non-significant changes involving discrete, moderate or intense erythema with swelling were observed in less than 20% of the animals belonging to the Honeoye IFP group and less than 10% of the animals belonging to HIP group. Skin reactions to Honeoye OFP were only manifested

Table 2. The severity of skin reactions observed during validation of GPMT according to the percentage (%) of animals.

Group	Benzocaine n = 11		Control group n = 5	
Time after exposure	24 h	48 h	72 h	24-72 h
Point average (±SD)	0.82* (±0.6)	1.27* (±0.8)	0.0 (±0.0)	0.0 (±0.0)
% of animals with skin reactions	72%	90%	0%	0%

* – as compared to control group at the same time point; p<0.05. * express statistically significant differences of mean ranks for points being classified according to Magnusson-Kligmann scale (0-3) between animals exposed to benzocaine and the control group at 24, 48 and 72 hours after challenge. Mean ranks for points being classified according to Magnusson-Kligmann scale at 24 and 48 hours after challenge are different between animals exposed to benzocaine and the control group.

with discrete or patchy erythema and were observed in 8.3% of the examined animals. Skin reactions to Honeoye IFP involved discrete or patchy erythema (16.7%); moderate erythema (8.3%) or intense erythema or swelling in 8.3% of the animals. Skin reactions to Elsanta OFP were manifested with discrete or patchy erythema (41.7%), moderate or confluent erythema (33.3%) and intense erythema or swelling in 25% of the examined animals. However, no skin changes were observed in guinea pigs receiving Elsanta cultivar derived from integrated production (Figure 1).

Chronic administration of selected fruit extracts

Chronic oral administration of selected fruit extracts did not cause any skin reactions in groups receiving Elsanta or Honeoye from organic or integrated productions.

Skin prick test (Dreborg test)

Non-significant skin reactions resulting from topical exposure during the skin prick test were observed for extracts from Elsanta or Honeoye strawberries derived from integrated or organic production. No differences were observed between the examined groups, compared to control groups (Table 3).

Total IgE measurement

Non-significant (p >0.05) differences in IgE serum concentration were observed in animals exposed to organically grown Elsanta, Elsanta derived from integrated production and placebo. Average IgE serum concentration did not differ significantly (p >0.05) between animals exposed to organic *Honeoye,* conventional *Honeoye or placebo* (Table 4).

DISCUSSION

Although hypersensitivity to strawberry is commonly reported, it is a poorly investigated phenomenon, as only a few cases of patients with adverse reactions to strawberries are listed in the literature [25]. Strawberries can cause IgE- mediated sensitivity reactions; however, other factors that cause non-IgE mediated reactions cannot be excluded [10]. At the first stage of the current survey we performed GPMT that addresses delayed or type IV (Gell and Coombs) hypersensitivity reactions. Accord-

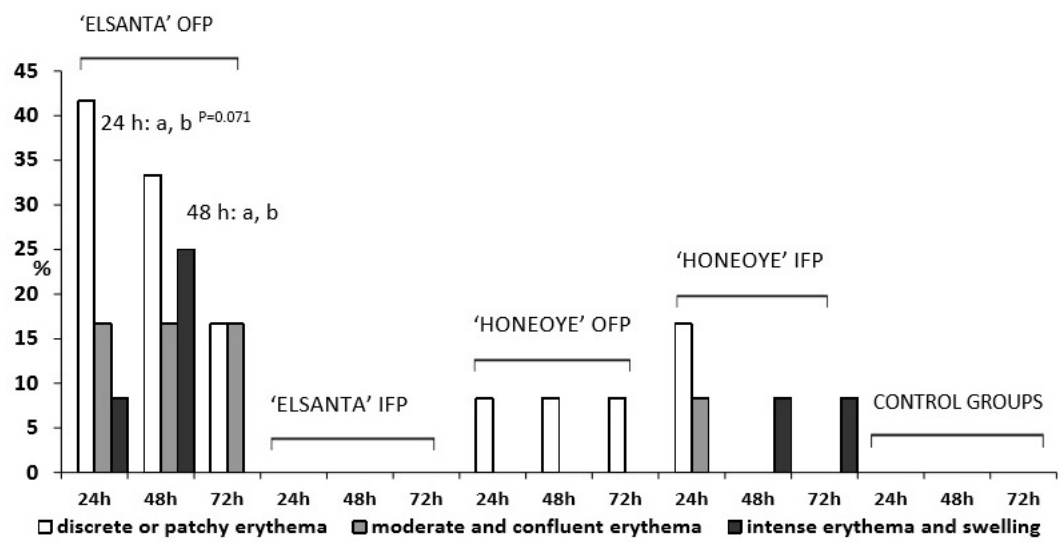

Fig. 1. The severity of skin reactions on fruits in GPMT as percentage (%) of animals

Table 3. The severity of skin reactions in the histamine Dreborgs test, expressed as wheal diameter (mm), average ± SD

Cultivar	'Elsanta'		'Honeoye'	
Type of production	IFP n= 12	OFP n = 11	IFP n = 10	OFP n = 10
Histamine	5.6 (±4.7)*	8.2 (±4.7)	7.6 (±4.3)*, p=0.058	7.6 (±3.8)*, p=0.058
Fruit cv	(±0.0)	2.3 (±4.0)	1.4 (±3.0)	1.0 (±1.7)
Negative control (0.9% NaCl)	(±0.0)	(±0.0)	(±0.0)	(±0.0)

* - as compared to fruit *cv*; $p < 0.05$

Table 4. The total IgE in guinea pig serum. Average ± SD; $p > 0.05$

Animal group	Total IgE (ng/ml) ± SD
Elsanta OFP	621.1 ± 66.4
Elsanta IFP	686.7 ± 82.3
Honeoye OFP	487.4 ± 77.5
Honeoye IFP	805.8 ± 88.1
Control groups	631.5 ± 78.1

Average IgE serum concentration does not differ significantly ($p > 0.05$) between animals exposed to organic or conventional cultivars.

ing to OECD guideline (406, for testing skin sensitization) adjuvant test in which sensitization is potentiated by the injection of Freunds Complete Adjuvant (FCA) is given preference over other methods. It is recognized as the best and the most precise tool available for the non-clinical study of sensitization [17]. The validation of the Magnusson-Kligmann test, which was performed with the use of benzocaine, a substance with mild-to-moderate skin sensitization properties, confirms the sensitivity and reliability of the experimental technique. In a properly conducted test, a response of at least 30% of subjects should be expected [18]. In our present study, skin reactions including discrete or moderate erythema were observed in 90% of the examined animals.

Our findings demonstrate that topical exposure to organically-produced Elsanta strawberries caused skin reactions, including swelling or discrete, moderate or intense erythema. Such changes in skin image were observed in more than 30% of the animals, which influences the positive result of the Magnusson-Kligmann test and the potential allergenicity risk of such organically-produced cultivars. Interestingly, the Magnusson-Kligmann test did not indicate that the Elsanta cultivar derived by integrated production or *Honeoye* cultivars demonstrated similar properties.

Further tests involved the assessment of type I hypersensitivity from topical exposure (prick-test) and oral test. They did not confirm similar worrying results attributed to organically-grown Elsanta. No significant or immediate skin reactions were noted in the skin prick test as a result of exposure to selected fruit extracts either from integrated or organic production, in contrast to 1% histamine hydrochloride solution. Also, no differences were observed resulting from chronic oral exposure to selected strawberry cvs obtained from IFP or OFP production. Similarly, our previous study did not demonstrate any effect of the selected cultivars of cherries ('Sabina' and 'Debreceni Bötermö) nor the type of fruits production on the guinea pig skin as a result of topical exposure or chronic feeding with fruits [9].

According to our knowledge this is the first study that compares different strawberry cultivars and methods of their production in the aspect of allergenic potential. Previous reports only refer to oral allergy syndrome (OAS) and urticaria as major symptoms. In contrast to other Rosaceae fruit, type I allergies for fruit belonging to the Rosoideae subfamily (strawberries and blackberries) are poorly documented [25]. The non-specific lipid transfer protein Fra a 3, profilin Fra a 4 (Mediterranean area) and protein Fra a 1 (North and Central Europe) are mainly responsible for strawberry allergies. Many incidences result from cross-reacting sensitizations. Bet v 1, the major birch pollen allergen, was described to be homologous with Fra a 1 [6, 11], and this can explain the phenomenon that about 30% of patients sensitized to Bet v 1 report allergenic reactions after consumption of strawberry fruits [3]. Also, Fra a 1-17.5 kDa proteins belong to pathogenesis-related proteins (PR-10), and allergy to rosaceous fruits was also described to be caused by 31 kDa thaumatin-like proteins (TLPs, PR-5) with anti-fungal activity [8, 14] or 9 kDa non-specific lipid transfer proteins (nsLTPs, PR-14) and a 14 kDa proline-binding protein known as profiling [22]. While previous studies have ranked the allergenicity risk of several apple cultivars [1], similar rankings have not been observed for strawberry cultivars.

Topical exposure to organically-produced Elsanta strawberry caused skin reactions, including swelling or discrete, moderate or intense erythema. Such changes in the skin were observed in more than 30% of the tested animals, which influences the positive result of the Magnusson-Kligmann test and the potential allergenicity risk of such organically-produced cultivars. Interestingly, the Magnusson-Kligmann test did not indicate that *Elsanta* IFP cultivar demonstrated similar properties. Another cultivar, Honeoye, derived from organic production, did not demonstrate similar properties as Elsanta *cv*. Such phenomena can rather exclude the hypothesis that an organic production system would be more allergenic. Nevertheless, the question to answer is why 'organic' Elsanta revealed allergenicity risk as compared to other products. Further, we focused on the potential relationship between selected macro-, micronutrients contents or fruit characteristics and such indifferences in allergenicity potential.

Organic composts are known to contain micronutrients such as calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and sulphur (S), factors stimulating plant growth and seed formation, and copper (Cu), supporting root metabolism and providing utilization of proteins. The question is whether this fact could influence the results obtained in the present study. Copper is a common metal allergen. However, it is believed to be only a rare cause of allergic contact dermatitis and it has a low sensitizing potential. Similary, sulphur, being a natural element that exists in many forms (i.e. sulfites, sulfates or sulfonamides), might cause allergy. In our study, no strict correlation was found between production system and the presence of the several macro- and micronutrients. Regardless of cultivar, higher contents of sulfur were found in organic fruits than those cultivated according to IFP. In contrast K, Mg and Mn content were higher in IFP than organic fruits (Table 1). Reganold et al. (2010) also reported less potassium in organic strawberry than in conventional ones [19]. In our study 'Elsanta' from organic farming had much lower amount of iron (Fe) compare to 'Elsanta' from IFP. For 'Honeoye' fruits from both systems the amounts of Fe were similar. The possible effect of high level of Fe on the allergenicity risk requires further investigation. The other findings concerning the possible linkage between macro- and micronutrient content and type of agricultural production are unambiguous. Kelly et al. (2010) observed systematic differences in the concentrations of certain elements including manganese (Mn), calcium (Ca), zinc (Zn) and copper (Cu) [12]. Ryan et al. (2004) observed only minor variations in sulphur (S) concentrations between commercial grain cultivated on paired organic and conventional farms, however, organic grain had higher Zn and Cu but lower Mn and phosphorus concentrations than conventional grain [20]. In another study, sulphur (S) levels were significantly higher in both the roots and leaves of carrots obtained from organic productions as compared to conventional ones [21].

In general, data presented in Table 1 showed that, regardless of cultivar, fruits from organic farming had slightly ($p>0.05$) higher dry matter, total soluble solids and saccharose content and less titratable acidity, ascorbic and citric acids as compared to integrated ones. Tonutare at al. (2009) reported higher total soluble solids and dry matter content in organic fruits as compared to conventionally cultivated strawberries [21]. In contrast, other authors showed that strawberries from organic and conventional systems have the total soluble solids content at the same level [13]. The influence of farming system on polyphenols content is also not clear. More often, fruits from organic farming have a higher polyphenols content [13], but our data seem to confirm this phenomena only for 'Elsanta' *cv*. Some authors suggested the higher polyphenols quantity in organic agriculture results from two reasons. Firstly, the use of synthetic fertilizers could deliver more bioavailable sources of nitrogen, accelerating plant development and plant resources from production of secondary metabolites. Secondly, the absence of chemical protection of a plant and fruits could result in more stressful situation, leading to an enhancement of natural defense substances e.g. phenols [23, 24]. Based on the results, our conclusion is that there was no evidence of fruit characteristics, especially for 'Elsanta' *cv*, that can provoke allergic reaction.

Another point is whether switching to organic production affects the allergenicity risk as a result of changes of potential gene expression. Expression of PR-10 genes can be induced upon various abiotic and biotic factors such as pathogen attack, wounding, environmental changes and chemicals. For the most part, data on the influence of cultivation method on fruit allergens does not indicate any significant differences, however. For example, Kurze et al. (2018) established an enzyme-linked immunosorbent assay (ELISA) method for the quantification of the Fra a 1 allergen in strawberry fruits of different genotypes. The authors did not reveal any significant differences for fresh strawberries, when comparing the two growing methods organic *vs* conventional of the same year [15]. Assuming that the Bet v 1-homologous Fra a 1 is recognized by IgE from strawberry allergic patients we could find the previous results comparable with present data as in our study the average IgE level being detected in animals that were exposed to both cultivars derived from organic and integrated production were not significantly differ among each other.

This study does have several limitations. Firstly, the allergenic potential of strawberry cultivars was assessed based only on visual skin changes, without any further analysis of the expression of selected allergenic proteins. Secondly, the prick-test method might not be reproducible due to different pricking positions and prick depth, the amount of allergen taken up by the needle, variations of skin reactivity among subjects or differences between individual fruits from the same cultivar batch [1]. Finally, the fruits used in this and other studies were taken from only one harvest. Seasonality can also exert a strong influence on the phytochemical content of fruits.

CONCLUSION

In conclusion, the organic method of strawberry production was not found to be indifferent in aspect of the allergenicity potential as compared to integrated one. Further human trials are needed to confirm such results. Any strict relationship between the type of cultivar and selected macro-, micronutrients contents or fruit characteristics on the possible increase in allergenicity risk was also not found.

REFERENCES

[1] Bolhaar S.T., van de Weg W.E., van Ree R., Gonzalez-Mancebo E., Zuidmeer L., Bruijnzeel-Koomen C.A., Fernandez-Rivas M., Jansen J., Hoffmann-Sommergruber K., Knulst A.C., Gilissen L.J.: In vivo assessment with prick-to-prick testing and double-blind, placebo-controlled food challenge of allergenicity of apple cultivars. J. Allergy Clin. Immunol., 2005; 116: 1080–1086

[2] Candir E., Kamiloglu M., Üstün D., Kendir G.T.: Comparison postharvest quality of conventionally and organically grown 'Washington Navel' oranges. J. Appl. Bot. Food Qual., 2013; 86: 59–65

[3] Crespo J.F., Rodríguez J., James J.M., Daroca P., Reaño M., Vives R.: Reactivity to potential cross-reactive foods in fruit-allergic patients: Implications for prescribing food avoidance. Allergy, 2002; 57: 946–949

[4] Cross J.V., Dickler E.: Guidelines for integrated production of pome fruits in Europe: IOBC Technical Guideline III. IOBC wprs Bulletin, 1994; 17: 1–8

[5] Dreborg S., Backman A., Basomba A., Bousquet J., Dieges P., Malling H.J.: Skin tests used in type I allergy testing. Position paper prepared by the subcommittee on skin tests of the European Academy of Allergy and Clinical Immunology. Allergy, 1989; 44: 11–59

[6] Focke M., Hemmer W., Wöhrl S., Götz M., Jarisch R.: Cross-reactivity between Ficus benjamina latex and fig fruit in patients with clinical fig allergy. Clin. Exp. Allergy, 2003; 33: 971–977

[7] Food and Agriculture Organization of the United Nations. http://faostat3.fao.org/ (01.02.2018)

[8] Hsieh L.S., Moos M. Jr., Lin Y.: Characterization of apple 18 and 31 kd allergens by microsequencing and evaluation of their content during storage and ripening. J. Allergy Clin. Immunol., 1995; 96: 960–970

[9] Jasińska-Stroschein M., Szcześniak P., Owczarek J., Rutkowski K.P., Markowski J., Rozpara E., Orszulak-Michalak D.: The assessment of the risk of allergenicity of 'Sabina' and 'Debreceni Bötermö' sour cherry cvs (Prunus cerasus L.) in a Guinea pig model. J. Hort. Res., 2014; 22: 63–70

[10] Jorge A., Soares E., Sarinho E., Lorente F., Gama J., Taborda-Barata L.: Prevalence and clinical features of adverse food reactions in Portuguese children. Allergy Asthma Clin. Immunol., 2017: 13: 40

[11] Karlsson A.L., Alm R., Ekstrand B., Fjelkner-Modig S., Schiött A., Bengtsson U., Björk L., Hjernø K., Roepstorff P., Emanuelsson C.S.: Bet v 1 homologues in strawberry identified as IgE-binding proteins and presumptive allergens. Allergy, 2004; 59: 1277–1284

[12] Kelly S.D., Bateman A.S.: Comparison of mineral concentrations in commercially grown organic and conventional crops – Tomatoes (Lycopersicon esculentum) and lettuces (Lactuca sativa). Food Chem., 2010; 119: 738–745

[13] Khalil H.A., Hassan S.M.: Ascorbic acid, β-carotene, total phenolic compound and microbiological quality of organic and conventional citrus and strawberry grown in Egypt. Afr. J. Biotechnol., 2015; 14: 272–277 [14] Krebitz M., Wagner B., Ferreira F., Peterbauer C., Campillo N., Witty M., Kolarich D., Steinkellner H., Scheiner O., Breiteneder H.: Plant-based heterologous expression of Mal d 2, a thaumatin-like protein and allergen of apple (Malus domestica), and its characterization as an antifungal protein. J. Mol. Biol., 2003; 329: 721–730

[15] Kurze E., Kock V., Lo Scalzo R., Olbricht K., Schwab W.: Effect of the strawberry genotype, cultivation and processing on the Fra a 1 allergen content. Nutrients, 2018: 10: 857

[16] Lind K., Lafer G., Schloffer K., Innerhofer G., Meister H.: Organic Fruit Growing. CABI Publishing, Wallingford 2003

[17] Magnusson B., Kligman A.M.: The identification of contact allergens by animal assay. The guinea pig maximization test. J. Invest. Dermatol., 1969; 52: 268–276

[18] OECD Guideline for Testing of Chemicals. Skin Sensitization. Adopted by the Council on 17th July 1992. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg406.pdf (01.08.2016)

[19] Reganold J.P., Andrews P.K., Reeve J.R., Carpenter-Boggs L., Schadt C.W., Alldredge J.R., Ross C.F., Davies N.M., Zhou J.: Fruit and soil quality of organic and conventional strawberry agroecosystems. PLoS One, 2010; 5: e12346

[20] Ryan M.H., Derrick J.W., Dann P.R.: Grain mineral concentrations and yield of wheat grown under organic and conventional management. J. Sci. Food Agric., 2004; 84: 207–216

[21] Tõnutare T., Moor U., Mölder K., Põldma P.: Fruit composition of organically and conventionally cultivated strawberry 'Polka'. Agron. Res., 2009; 7: 755–760

[22] Vanek-Krebitz M., Hoffmann-Sommergruber K., Laimer da Camara Machado M., Susani M., Ebner C., Kraft D., Scheiner O., Breiteneder H.: Cloning and sequencing of Mal d 1, the major allergen from apple (Malus domestica), and its immunological relationship to Bet v 1, the major birch pollen allergen. Biochem. Biophys. Res. Commun., 1995; 214: 538–551

[23] Winter C.K., Davis S.F.: Organic foods. J. Food Sci., 2006; 71: 117–124

[24] Woese K., Lange D., Boess C., Bögl K.W.: A comparison of organically and conventionally grown foods – results of a review of the relevant literature. J. Sci. Food Agric., 1997; 74: 281–293

[25] Zuidmeer L., Salentijn E., Rivas M.F., Mancebo E.G., Asero R., Matos C.I., Pelgrom K.T., Gilissen L.J., van Ree R.: The role of profilin and lipid transfer protein in strawberry aller gy in the Mediterranean area. Clin. Exp. Allergy, 2006; 36: 666–675

The authors have no potential conflicts of interest to declare.