Journal of Horticultural Research 2014, vol. 22(2): 63-70
DOI: 10.2478/johr-2014-0023

THE ASSESSMENT OF THE RISK OF ALLERGENICITY OF 'SABINA' AND 'DEBRECENI BÖTERMÖ' SOUR CHERRY CVS (*PRUNUS CERASUS* L.) IN A GUINEA PIG MODEL

Magdalena JASIŃSKA-STROSCHEIN[1]*, Piotr SZCZEŚNIAK[1], Jacek OWCZAREK[1], Krzysztof P. RUTKOWSKI[2], Jarosław MARKOWSKI[2], Elżbieta ROZPARA[2], Daria ORSZULAK-MICHALAK[1]

[1]Department of Biopharmacy, Medical University of Lodz, Muszyńskiego 1, 90-151 Lodz, Poland
[2]Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100, Skierniewice, Poland

Received: July 17, 2014; Accepted: November 25, 2014

ABSTRACT

The allergic reactions to fruits are lesser known among food sensitivities. The most common fruits belonging to the *Rosaceae* family that might cause allergic reactions are apples, pears and peaches. However, little is known about the potential allergic reactions caused by another member of the Rosaceae, the cherry. The aim of this study was to assess the risk of any allergic reaction or food hypersensitivity resulting from topical application and chronic oral administration of cherry fruits. The cherry fruits 'Sabina' cv. were produced in the orchard in Dąbrowice according to the principles of integrated (IFP) and organic (OR) productions. Fruits of 'Debreceni Bötermö' cv. were produced in Dąbrowice (IFP), and in the orchard in Nowy Dwór (OR). The experiments were performed on 65 outbred young, adult, white albinotic guinea pigs (Dankin Hartley). Three procedures were applied: I. Guinea-Pig Maximization Test (GPMT); II. Chronic administration of fruits and III. Skin prick (Dreborg) test. The skin reactions based on GPMT or Dreborg tests revealed no differences between the two cherry cultivars 'Sabina' and 'Debreceni' obtained from integrated or organic production. Similarly, it was not observed of any effect of cultivars of cherries nor the type of fruits production on the guinea pig skin reaction as a result of chronic feeding with fruits.

Key words: sour cherry, allergy, guinea-pig, organic farming, integrated fruit production

INTRODUCTION

Food allergy (FA) affects 2.4-3.7% of adults and the most common 'major food allergens' are: milk, egg, peanut, tree nuts, shellfish, fish, wheat and soy (Zuberbier et al. 2004; Osterballe et al. 2005; Schafer et al. 2001). Adverse reactions to apple, kiwi, pear, peach or cherry are listed among fruit-related FA (Le et al. 2008). The most frequently observed clinical manifestations in fruit-allergic patients involve oropharyngeal, skin and respiratory symptoms, as well as rhinitis and conjunctivitis. Patients affected mainly by birch fruit syndrome due to the presence of Bet v 1 homologous allergens, by latex fruit syndrome due to sensitisation to hevein-like domains in food, or by lipid transfer protein (LTP) syndrome have been found to demonstrate fruit allergies (Pastorello & Ortolani 2003). The growth in the number of people suffering from FA in the past decades has presented researchers the important task of preparing food products that lack allergic potential. This in turn demands the assessment of the largest possible number of food allergens or estimating their stability to processing (Primavesi et al. 2006). Some authors report the existence of a relationship between expression of major fruit allergens and type of production or storage conditions (Matthes & Schmitz-Eiberger 2009; Sancho et al. 2006; Schmitz-Eiberger & Matthes 2011; Botton et al. 2008). Hence, the potential impact of cultivation system should also be considered with respect to allergenicity.

*Corresponding author:
e-mail: magdalena.jasinska-stroschein@umed.lodz.pl

Due to its system of cultivation with limited usage of synthetic pesticides or readily soluble mineral fertilisers, organic cultivation responds to this goal and it is believed that it is a way to ensure a safe farming and production of healthy fruits. Although food products from organic origin are thought to be healthier than the corresponding conventional foods, clear experimental evidence supporting this assumption has not yet been acquired, and the nutritional or allergic potential of these products requires further research. The aim of this study was to assess the risk of any allergic reaction or food hypersensitivity resulting from topical application and chronic oral administration of cherries of the cultivars 'Sabina' and 'Debreceni Bötermö', derived from both integrated and organic production.

Integrated fruit production (IFP) is defined as the economical production of high quality fruits, giving priority to ecologically safer methods, minimising the undesirable side effects and use of agrichemicals, to enhance the safeguards to the environment and human health (Cross & Dickler, 1994). Organic fruit production (OFP) relies on natural mechanisms controlling the growth, yield and health status of the plants. The principles of OFP are not using readily soluble mineral fertilisers, herbicides and synthetic chemical pesticides (Lind et al. 2003). The natural fertilisers, composts, manures, green manures, mulches, varied crop rotation are used for keeping the soil fertility instead. Weeds are eliminated mechanically. The basis of OFP is developing of resistant cultivars and using natural active agents.

MATERIAL AND METHODS

Biological material

The plant materials of study were the fruits of two sour cherry cultivars: 'Sabina' and 'Debreceni Bötermö', which were obtained from orchards located in central part of Poland where cultivation was carried out by IFP or by OFP. The fruits of both cultivars obtained by integrated method came from Experimental Orchard of Research Institute of Horticulture in Dąbrowice (5 km from Skierniewice). Fruits of 'Sabina' (SOP) derived from organic production were obtained also in Dąbrowice, meanwhile fruits of 'Debreceni Bötermö' (DOP) came from Experimental Organic Orchard of InHort, located in Nowy Dwór-Parcela (15 km from Skierniewice). Fully ripe fruits after harvest were frozen at –25 °C disintegrated in the frozen state followed by fine grinding in solid CO_2 with Blixer 3 (Model 712033, Robot Coupe, France) and then were packed into 120-g portions, called thereafter fruit preparations. Such portions were kept frozen until ready to use. Fresh fruits characteristics 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) were used.

Animals

The experiments were performed on 65 outbred young, adult, white albinotic guinea pigs (Dankin Hartley), both sexes, weighing 200-500 g, which were fed on granulated fruits with free access to water. The temperature of the experimental animal room was 20 °C (±3 °C), the relative humidity 30-70% and the sequence of lighting – 12 h light, 12 h dark. The animals were housed in standard cages, 2-3 animals per cage. During the experiments all guinea pigs were receiving an adequate amount of ascorbic acid. The animals were weighed before the test commences and at the end of the test.

The experimental procedures were carried out in accordance with the international guidelines for care and use of laboratory animals. All efforts were made to minimise animal suffering and to 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 (ŁB 460/2009; 3/ŁB 591/2012).

Table 1. Fruit characteristics of the evaluated cultivars depending on the cultivation method, average ± SD

Cultivar	'Sabina'		'Debreceni Bötermö'	
Type of production	Integrated	Organic	Integrated	Organic
Total solids (%)	14.4 (± 0.1) a*	15.8 (± 0.3) b	16.5 ± (0.2) b	15.6 (± 0.9) b
Total soluble (%)	12.3 (± 0.1) a	14.6 (± 0.2) c	14.8 (± 0.2) c	13.6 (± 0.8) b
Titratable acidity (%)	1.75 (± 0.00) b	1.74 (± 0.03) b	0.97 (± 0.04) a	0.92 (± 0.05) a
Anthocyanins (mg·100 g^{-1})	89.9 (± 14.2) b	99.8 (± 14.4) b	49.1 (± 1.1) a	48.1 (± 1.8) a
Polyphenols content (mg·100 g^{-1})	284 (± 9) b	326 (± 2) c	242 (± 11) a	293 (± 31) bc
Malic acid (mg·100 g^{-1})	20.2 (± 0.17) b	20.1 (± 0.83) b	12.7 (± 0.4) a	11.9 (± 0.7) a
Ascorbic acid (mg·100 g^{-1})	< 1	< 1	< 1	< 1
Saccharose (g·kg^{-1})	< 0.01	< 0.01	< 0.01	< 0.01
Glucose (g·kg^{-1})	53.1 (± 1.0) c	60.4 (± 1.2) d	51.8 (± 0.5) bc	49.0 (± 3.6) a
Fructose (g·kg^{-1})	40.3 (± 0.4) a	49.2 (± 1.0) c	47.4 (± 0.5) bc	45.8 (± 2.7) b
Glucose:fructose	1.3 (± 0.0) c	1.2 (± 0.0) b	1.09 (± 0.01) a	1.07 (± 0.05) a
Sugar content (simple sugar plus saccharose) (g·kg^{-1})	93.3 (± 1.3) a	109.6 (± 0.8) b	99.2 (± 1.0) a	94.8 (± 5.9) a
Sorbitol (g·kg^{-1})	12.0 (± 1.0) a	22.9 (± 0.5) c	23.8 (± 0.8) c	17.8 (± 1.7) b

*according to Duncan's test; means followed by the same letter do not differ at p = 0.05

The animals were randomly allocated into groups, as following:

1. Animals being exposed to cherry 'Sabina' from integrated production (SIP), (n = 10); 2. Animals being exposed to cherry 'Sabina' from organic production (SOP), (n = 10); 3. Control group (n = 5); 4. Animals being exposed to cherry 'Debreceni Bötermö' from integrated production (DIP), (n = 10); 5. Animals being exposed to cherry 'Debreceni Bötermö' from organic production (DOP), (n = 10); 6. Control group (n = 5); 7. Animals being exposed to benzocaine during validation process (B), (n = 10); 8. Control group (n = 5).

Experimental

I. Guinea-Pig Maximization Test (GPMT)

The sensitivity and reliability of the experimental technique was performed by using benzocaine as substance of mild-to-moderate skin sensitisation properties. All procedures were made according to OECD guideline for skin sensitisation test (406) (OECD Guideline 1992). In brief, the test animals were initially exposed to the fruit preparations, by intradermal injection (total volume per one injection: 0.1 ml; vehicle: *Agua pro injectione*) – day 0 (*induction exposur*e) followed by topical application – day 6 (10%; vehicle: Vaseline). Following a rest period of 10-14 days (*induction period*), during which an immune response may develop, the animals were exposed to a challenge. The extent and degree of skin reaction to the challenge exposure in the test animals is compared with that demonstrated by control animals, which undergo same treatment during induction and receive the challenge exposure. That day animals were exposed to fruit preparations (10%; vehicle: Vaseline) according to animal group (i.e. SIP, SOP, DIP, DOP) or vehicle alone (Vaseline) being applied on the skin test areas. Approximately 48 and 72 h after this procedure, the skin reactions were observed and recorded according to the OECD guideline scaling, by two independent investigators (Table 2).

II. Chronic fruit administration

Next, the same animals were tested to assess if chronic feeding with fruit preparations can result in any skin reaction. For this purpose, fruits were administrated *per os* (10 g/day/animal), according to

animals from groups: SIP, SOP, DIP or DOP for 30-day period. In this time, the animals were observed toward the potential occurrence of skin reactions, considering control group, as well.

III. Skin prick test (Dreborg test).

Finally, the animals being used in 1st and then in 2nd phase of the experiments were examined by performance of 'confirmatory' skin prick test. 0.9% of physiologic saline solution was served as a negative control, whereas aqueous histamine hydrochloride solution (1 $mg \cdot ml^{-1}$) was used as positive control throughout all tests. A drop of saline solution and histamine solution were pipetted on the intact animal skin in the shoulder region and pricked with lancet (M Mediware, Blutlanzette, sterile, Premium Quality, REF B2 01). Skin prick tests were read after 10 and 20 min and quantified on the basis of wheal diameter as compared to negative and positive control (Dreborg et al. 1989). 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). Testing, i.e. the assessment of skin reactions was performed by two independent investigators.

Statistics

The statistical analysis was carried out using the STATISTICA version 10.0. The analysis of variance (ANOVA) and *post-hoc* comparisons were performed using the Duncan test. If data were not normally distributed or the values of variance were different, ANOVA with the Kruskal–Wallis and Mann–Whitney's *U* test were used. The Wilcoxon signed-rank test was used when comparing two related samples for not normally distributed data. All parameters were considered statistically significantly different if $p < 0.05$.

RESULTS

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

According to Magnusson–Kligmann scale (Table 2), 80% of animals showed skin reactions to benzocaine manifested as discrete or patchy erythema were observed in 70% of animals, while moderate or confluent erythema was seen in 10% (Table 3). Table 4 shows skin reactions evaluated according to the Magnusson–Kligmann test of guinea pigs exposed to fruits: 'Sabina' or 'Debreceni Bötermö' cherries obtained by integrated and organic production. No differences were found among the examined groups: i.e. organic vs. integrated or 'Sabina' vs. 'Debreceni Bötermö'.

II. Chronic fruit administration

Chronic 30-day oral administration of the examined fruit preparations did not cause any skin reactions in groups receiving 'Sabina' or 'Debreceni Bötermö' cultivars from organic or integrated production.

Table 2. Skin reactions according to Magnusson–Kligmann scale (OECD 406)

Change	Points
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Table 3. The severity of skin reactions on benzocaine during validation of GPMT expressed as percentage (%) of animals that revealed any skin reactions and point average according to Magnusson-Kligmann scale, average ± SD

Group	Benzocaine $N = 10$		Control Group $N = 5$	
Time after exposure	24 h	48 h	72 h	24-72 h
Point average (±SD)	0.65 a* (±0.58)	0.3 b (±0.48)	0.0 b (±0.00)	0.0 b (±0.00)
% of animals with skin reactions	80%	30%	0%	0%

*according to Kruskal–Wallis test; means followed by the same letter do not differ at $p = 0.05$

Table 4. The severity of skin reactions on fruits in GPMT as percentage (%) of animals that revealed any skin reactions and point average according to Magnusson–Kligmann scale, average ± SD

Cultivar/group	'Sabina'						Control group *N*=5
Type of production	Integrated *N* = 10			Organic *N* = 10			
Time after exposure	24 h	48 h	72 h	24 h	48 h	72 h	24–72 h
Point average (±SD)	0.3 (±0.67) a*	0.3 (±0.48) a	0.0 (±0.00) a	0.3 (±0.67) a	0.2 (±0.42) a	0.0 (±0.00) a	0.0 (±0.00) a
% of animals with skin reactions	20%	30%	0%	20%	20%	0%	0%
Cultivar/group	'Debreceni Bötermö'						Control group *N* = 5
Type of production	Integrated *N* = 10			Organic *N* = 10			
Time after exposure	24 h	48 h	72 h	24 h	48 h	72 h	24–72 h
Point average (±SD)	0.2 (±0.42) a	0.05 (±0.16) a	0.05 (±0.16) a	0.3 (±0.63) a	0.15 (±0.34) a	0.0 (±0.00) a	0.0 (±0.00) a
% of animals with skin reactions	20%	10%	10%	30%	20%	0%	0%

*according to Kruskal–Wallis test; means followed by the same letter do not differ at p = 0.05

Table 5. The severity of skin reactions in histamine Dreborg's test expressed as wheal diameter (mm), average ± SD

Cultivar	'Sabina'		'Debreceni Bötermö'	
Type of production	Integrated *N* = 10	Organic *N* = 10	Integrated *N* = 10	Organic *N* = 10
Histamine	6.8 (±4.44) a*	5.4 (±3.03) a	8.1 (±2.67) a	9.2 (±3.22) a
Fruit *cv*	(±0.0) b	0.5 (±1.58) b	0 (±0.0) b	0.6 (±1.90) b
Negative control (0.9% NaCl)	(±0.0) b	(±0.0) b	(±0.0) b	(±0.0) b

*according to Wilcoxon signed-rank test; means followed by the same letter do not differ at p = 0.05

III. Skin prick test (Dreborg test)

A significant difference in skin reactions were observed between animals exposed to histamine and fruit preparations, whereas no significant differences were found between animals exposed to fruit preparations from different cultivation systems and cultivars, and the control groups (Table 5).

DISCUSSION

The Magnusson–Kligmann test, performed using benzocaine, a substance with mild-to-moderate skin sensitisation properties, confirmed the sensitivity and reliability of the experimental technique used in the study. In a properly conducted test, a response of at least 30% of subjects should be expected (OECD 406 Guideline 1992). In our study, skin reactions including discrete or moderate erythema were observed in 80% of the examined animals.

Although apples are most commonly known to be the allergenic fruits of the *Rosaceae* family, some reports also describe incidences of cherry-related food allergies (FA). In a study by Le et al. (2008) performed on 218 subjects with FA, cherry FA comprised 22% of total reactions, while apples induced 39% and hazelnut 31%. Some of the major cherry allergens responsible for such sensitivities are

Pru av 1, Pru av 2, Pru av 3 and Pru av 4, which are characterised with different clinical relevance according to geographical area. Pru av 1 (Scheuer et al. 1997) and Pru av 4 (Scheurer et al. 2001) proteins homologous to major birch allergens Bet v 1 and Bet v 2 are responsible for birch and cherry cross-reactivity in the birch pollen/fruit syndrome (Primavesi et al. 2006) in northern and central Europe.

The occurrence of allergic reactions was shown to be determined by several factors including type of fruit cultivar as well as agronomic practices, such as shadowing, elevation, water stress or storage. All these factors were described to potentially exert an impact on the expression of allergen-related genes. However, previous observations have concerned mainly apples and their major allergen, Mal d (Botton et al. 2008; Schmitz-Eiberger & Matthes 2011). For example, shadowing or low elevation can significantly induce the up-regulation of Mal d genes in fruit skin that might result in higher allergenicity risk (Botton et al. 2008; Sancho et al. 2006). Some reports concern the linkage between the presence of allergenic proteins and fruit ripening (Botton et al. 2008; Brenna et al. 2004). Other studies also discuss the influence of storage conditions on allergic gene expression (Sancho et al. 2006; Bolhaar et al. 2005; Marzban et al. 2005).

Another issue is whether the use of organic production impacts allergenicity risk as a result of potential changes of responsible gene expression. Although studies generally indicate a lack of significant differences in allergenic protein concentration related to changes in cultivation method, relevant data are scarce. Prick-to-prick tests revealed that the majority of organically produced apple cultivars did not show higher allergenicity in comparison to ones produced by integrated method. Gene expression studies (Matthes & Schmitz-Eiberger 2009) showed that most apple cultivars from integrated production was characterised with significantly higher Mal d 1 concentrations in comparison to those cultivated according to organic production guidelines. A literature review reveals a lack of similar studies on cherry cultivars. According to the results of the present study, the choice of cultivation system does not exert any significant impact.

As with apple Mal d 1, the majority of previously identified cherry allergens belong to the group of pathogenesis-related (PR) proteins (Buczyłko 2010). Hence, their synthesis may be provoked by various stress factors in both cultivation systems, but in different ways. Biotic stress factors such as fungi, viruses and bacteria may impact PR protein expression in organic fruits, but these are cancelled by the pesticide treatment used in integrated production. However, it is possible that the pesticides might themselves stimulate accumulation of PR proteins. Detailed investigations are needed to assess, which mechanism can induce the activation of allergenic protein synthesis to a greater degree. In this study, the type of production, integrated vs. organic, was not found to significantly impact skin reactions due to topical or chronic feeding.

As previously described, exposure to pathogens in different cultivation systems may also determine the content and profile of phenolics compounds in plants. Although several studies report a higher content of phenolic compounds in organically produced apples (Weibel et al. 2004; Stracke et al. 2009) or kiwi (Park et al. 2012; Tarozzi et al. 2004), but did not reveal any differences in phenolics contents between organically and conventionally produced fruits. The results of the present study indicate that the polyphenols content in the organically-farmed ‘Sabina’ was 14% and in the ‘Debreceni Bötermö’ cultivar was 21% higher than when integrated production was used. This confirms the results of previous studies revealing a tendency for higher phytochemical concentrations in organically produced fruits, which was explained by higher phosphorus uptake and limited nitrogen availability, which provides the necessary energy for the synthesis of phytochemicals (Stracke et al. 2009). However, it should be mentioned that present study, similar to others, was conducted using fruits of only one harvest. Thereby, the influence of seasonality should not be excluded as climate variations were shown to have a great influence on the phytochemical content.

Moreover, the response to any stress factors might be cultivar dependent. In contrast to other members of the *Rosaceae*, very little is known about

differences in allergenic potential characterising cherry (*Prunus cerasus* L) cultivars. Nevertheless, Primavesi et al. (2006) did not identify any significant differences in the allergenic pattern of six varieties of sweet cherry (*Prunus avium*): 'Mora di Vignola', 'Durone Nero I di Vignola', 'Napoleon', 'Rainier', 'Adriana' and 'Grace Star'. Similarly, the results of the present study reveal no differences between two cultivars of *P. cerasus*, with respect to skin reactions resulting from topical (GPMT, Dreborg test) or chronic oral exposure.

This study does have three key limitations. The first was that the allergenic potential of cherry cultivars was assessed based only on the visual skin changes, without any further analysis of the presence of selected allergenic proteins. Secondly, the fruits used in this and other studies were taken from only one harvest. The seasonality can exert a strong influence on the phytochemical content of fruits. Finally, the prick-test method might not be reproducible due to such factors, as different pricking position and depth of needle, 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, which was pointed by Bolhaar et al. (2005).

Acknowledgements

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

REFERENCES

Bolhaar S.T., van de Weg W.E., van Ree R., Gonzalez-Mancebo E., Zuidmeer L., Bruijnzeel-Koomen C.A., et al. 2005. In vivo assessment with prick-to-prick testing and double-blind, placebo-controlled food challenge of allergenicity of apple cultivars. J. Allergy Clin. Immunol. 116: 1080-1086. DOI: 10.1016/j.jaci.2005.07.004.

Botton A., Lezzer P., Dorigoni A., Barcaccia G., Ruperti B., Ramina A. 2008. Genetic and environmental factors affecting allergen-related gene expression in apple fruit (*Malus domestica* L. Borkh). J. Agric. Food Chem. 56: 6707-6716. DOI: 10.1021/jf800813d.

Brenna O.V., Pastorello E.A., Farioli L., Pravettoni V., Pompei C. 2004. Presence of allergenic proteins in different peach (Prunus persica) cultivars and dependence of their content on fruit ripening. J. Agric. Food Chem. 52: 7997-8000. DOI: 10.1021/jf0491052.

Buczyłko K. 2010. Roślinne białka stresu jako alergeny dla człowieka. Alergia 45: 53-58. [in Polish]

Cross J.V., Dickler E., eds. 1994. Guidelines for Integrated Production of pome fruits in Europe: IOBC Technical Guideline III. IOBC/WPRS Bulletin 17(9): 1-8.

Dreborg S., Backman A., Basomba A., Bousquet J., Dieges P., Malling H. 1989. Skin tests used in type I allergy testing. Position paper of the European Academy of Allergy and Clinical Immunology. Allergy 44: 1-69.

Le T.M., Lindner T.M., Pasmans S.G., Guikers C.L., van Hoffen E., Bruijnzeel-Koomen C.A., Knulst A.C. 2008. Reported food allergy to peanut, tree nuts and fruit: comparison of clinical manifestations, prescription of medication and impact on daily life. Allergy 63: 910-916. DOI: 10.1111/j.1398-9995.2008.01688.x.

Lind K., Lafer G., Schloffer K., Innerhofer G., Meister H. 2003. Organic Fruit Growing. CABI Publishing. 1-281.

Marzban G.P., Behringer V., Day R., Brynda S., Ma Y., Martinelli A., et al. 2005. Localisation and distribution of major apple allergens in fruit tissue. Plant. Sci. 169: 387-394. DOI: 10.1016/j.plantsci.2005.03.027.

Matthes A., Schmitz-Eiberger M. 2009. Apple (*Malus domestica* L. Borkh.) allergen Mal d 1: effect of cultivar, cultivation system, and storage conditions. J. Agric. Food Chem. 57: 10548-10553. DOI: 10.1021/jf901938q.

OECD Guideline For Testing Of Chemicals. Skin Sensitization. Adopted by the Council on 17th July 1992 http://iccvam.niehs.nih.gov/SuppDocs/Fed-Docs/OECD/OECDtg406.pdf (08.2011).

Osterballe M., Hansen T.K., Mortz C.G., Høst A., Bindslev-Jensen C. 2005. The prevalence of food hypersensitivity in an unselected population of children and adults. Pediatr. Allergy. Immunol. 16: 567-573. DOI: 10.1111/j.1399-3038.2005.00251.x.

Park Y.S., Ham K.S., Kang S.G., Park Y.K., Namiesnik J., Leontowicz H., et al. 2012. Organic and conventional kiwifruit, myths versus reality: antioxidant, antiproliferative, and health effects. J. Agric. Food Chem. 60: 6984-6993. DOI: 10.1021/jf3010614.

Pastorello E.A., Ortolani C. 2003. Oral allergy syndrome. In: Food allergy: Adverse reactions to foods and foods additives, 3rd ed.; Metcalfe D.D., Sampson H.A., Simon R.A. (Eds.), Blackwell Scientific Publications, Boston MA. pp. 169-182.

Primavesi L., Brenna O.V., Pompei C., Pravettoni V., Farioli L., Pastorello E.A. 2006. Influence of cultivar and processing on cherry (*Prunus avium*) allergenicity. J. Agric. Food Chem. 54: 9930-9935. DOI: 10.1021/jf0622376.

Sancho A.I., Foxall R., Rigby N.M., Browne T., Zuidmeer L., van Ree R., et al. 2006. Maturity and storage influence on the apple (*Malus domestica*) allergen Mal d 3, a nonspecific lipid transfer protein. J. Agric. Food. Chem. 54: 5098-5104. DOI: 10.1021/jf0530446.

Schäfer T., Böhler E., Ruhdorfer S., Weigl L., Wessner D., Heinrich J., et al. 2001. Epidemiology of food allergy/food intolerance in adults: associations with other manifestations of atopy. Allergy. 56: 1172-1179. DOI: 10.1034/j.1398-9995.2001.00196.x.

Scheurer S., Metzner K., Haustein D., Vieths S. 1997. Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. Mol. Immunol. 34: 619-629.

Scheurer S., Pastorello E.A., Wangorsch A., Kästner M., Haustein D., Vieths S. 2001. Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the in vitro diagnosis of cherry allergy. J. Allergy. Clin. Immunol. 107: 724-731. DOI: 10.1067/mai.2001.113864.

Schmitz-Eiberger M., Matthes A. 2011. Effect of harvest maturity, duration of storage and shelf life of apples on the allergen Mal d 1, polyphenoloxidase activity and polyphenol content. Food Chemistry. 127: 1459-1464. DOI: 10.1016/j.foodchem.2011.01.101.

Stracke B.A., Rüfer C.E., Weibel F.P., Bub A., Watzl B. 2009. Three-year comparison of the polyphenol contents and antioxidant capacities in organically and conventionally produced apples (*Malus domestica* Bork. cv. 'Golden Delicious'). J. Agric. Food Chem. 57: 4598-4605. DOI: 10.1021/jf803961f.

Tarozzi A., Marchesi A., Cantelli-Forti G., Hrelia P. 2004. Cold-storage affects antioxidant properties of apples in Caco-2 cells. J. Nutr. 134: 1105-1109.

Weibel F.P., Bickel R., Leuthold S., Afolldi T. 2000. Are organically grown apples tastier and healthier? A comparative field study using conventional and alternative methods to measure fruit quality. Acta. Hortic. 7: 417-427.

Zuberbier T., Edenharter G., Worm M., Ehlers I., Reimann S., Hantke T., et al. 2004. Prevalence of adverse reactions to food in Germany – a population study. Allergy. 59: 338-345. DOI: 10.1046/j.1398-9995.2003.00403.x.