Nutrition 58 (2019) 30–35

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Pilot study

Influence of polyphenolic content on the in vitro allergenicity of old and new apple cultivars: A pilot study

Josephine Kschonsek M.Sc. [a], Cornelia Wiegand Dr. [b], Uta-Christina Hipler PD, Dr. [b], Volker Böhm PD, Dr. [a,*]

[a] Institute of Nutritional Sciences, Friedrich Schiller University Jena, Jena, Germany
[b] Department of Dermatology, University Medical Center Jena, Jena, Germany

ARTICLE INFO

Article History:
Received 31 May 2018
Received in revised form 3 July 2018
Accepted 4 July 2018

Keywords:
Apple polyphenols
HPLC
Enzymatic browning
Birch pollen allergy
Mal d 1
CAST
BAT
IgE

ABSTRACT

Objectives: More than 70% of birch pollen–allergic individuals are affected by a cross-allergy from apples. The aim of this study was to determine if an increased polyphenolic content of apples is inversely related to clinical allergic reactions in sufferers.
Methods: The polyphenolic content of two old and two new apple cultivars was analyzed using high performance liquid chromatography. The in vitro concentration of sulfidoleukotrienes and the CD63 basophil activation of 27 birch pollen sufferers with cross-reactivity to apples were determined with cellular antigen stimulation and basophil activation tests after incubation with different apple cultivars.
Results: The flesh of old cultivars was characterized by significantly higher total polyphenolic content (86.1 ± 5.5 μg/g) than that of new cultivars (24.7 ± 7.2 μg/g). The concentration of sulfidoleukotrienes and the CD63 basophil activation of old apple cultivars was up to 62% lower than new ones and decreased as the degree of enzymatic browning increased.
Conclusion: Old apples cultivars are better tolerated than new ones by birch pollen-allergic individuals. The in vitro allergenicity (activation of effector cells) of apples depends on the total polyphenolic content and the degree of enzymatic browning.

Introduction

Birch (*Betula verrucosa*) pollen-allergic individuals often develop intolerance to apples (*Malus domestica*). The birch pollen-related apple allergy (BP-RAA) is a result of primary sensitiszation to Bet v 1 (*Betula verrucosa* 1) followed by immunoglobulin (Ig)E cross-reaction to homologous allergens in apples [1–3]. In northern and central Europe >70% of birch pollen-allergic individuals are affected by this cross-allergy because of the major apple allergen Mal d 1 (*Malus domestica* 1) [4,5]. Mal d 1 is an 18 kDA protein of the pathogenesis-related protein family 10 [6]. It is induced in peel and flesh by pathogen attack, abiotic stress factors, and fungal elicitors [7–9].

Over the past decades, BP-RAA has become the most important fruit allergy in Germany with about 4 million individuals showing clinical reactions to apples. This increasing number of allergic individuals is assumed to be associated with a higher consumption of new apple cultivars like Braeburn, Elstar, Golden Delicious, Granny Smith, and Jonagold. The allergen level differs between cultivars as demonstrated by enzyme-linked immunosorbent assay (ELISA) and immunoblotting [7,10–12]. Allergenic differences between apple cultivars are mainly related to expression levels of Mal d 1 [13]. Studies further showed that other internal factors like polyphenolic content, polyphenol oxidase activity, and antioxidant capacity affect apple allergenicity [8,13]. New apple cultivars are characterized by being bred from the same apple strains (Golden Delicious, McIntosh, Jonathan, Cox Orange, Red Delicious, and James Grieve) [14]. It is asserted that new apple cultivars have lower polyphenolic content than old cultivars as polyphenolic compounds were largely bred out, reducing the astringent taste and rapid enzymatic browning. It is thought that the lower polyphenolic content of new apple cultivars is responsible for their increased allergenicity.

Polyphenols are a class of bioactive compounds characterized by aromatic ring(s) with one or more hydroxyl moieties [15,16]. Flavanols, hydroxycinnamic acids, dihydrochalcones, flavonols, and anthocyanins are major classes of polyphenols that are commonly found in apples. Polyphenols are able to influence the development of allergic immune responses on two critical phases: during allergic sensitization and after re-exposure to the allergen [17]. The interaction of polyphenols with allergens can influence the sensitization by forming insoluble complexes. Furthermore,

* Corresponding author: Tel.: +49 3641 949633; Fax: +49 3641 94970.
E-mail address: volker.boehm@uni-jena.de (V. Böhm).

https://doi.org/10.1016/j.nut.2018.07.001
0899-9007/

polyphenols have an inhibitory effect on the secretion of mediators by effector cells (mast cells, basophils) [18]. The most studied group of polyphenols are phenolic acids and flavonoids, which are known to possess an anti-inflammatory and antiallergic potential [19].

It has been suggested that old apple cultivars are more tolerated than new ones, presumably because of higher polyphenolic content [20]. To our knowledge, this statement has not yet been scientifically confirmed. Thus, the present study focused on the investigation of the relation between the polyphenolic content and allergenic reactions to old and new apple cultivars. In a pilot study, in vitro allergenicity (activation of effector cells) of four different apple cultivars was determined in birch pollen-allergic individuals with a cross-allergy to apples using the cellular antigen stimulation test (CAST) and the basophil activation test (BAT). Apple polyphenols were quantified using high performance liquid chromatography with diode array detector, and the degree of enzymatic browning was determined spectrophotometrically.

Materials and methods

Chemicals

All solvents used were of HPLC grade and reagents of analytical grade. Chlorogenic acid, caffeic acid, and (+)-catechin were purchased from Carl Roth (Karlsruhe, Germany). Procyanidin B1, procyanidin B2, (-)-epicatechin, phloretin-2′-*O*-glucoside (phloridzin) dihydrate, and phloretin were from Sigma-Aldrich (Taufkirchen, Germany). Dulbecco's phosphate-buffered saline (PBS) was purchased from Pelobiotech (Planegg, Germany) and ethylenediaminetetraacetic acid-free Protease Inhibitor Cocktail from Roche (Basel, Switzerland). Water for solutions was obtained from a Milli-Q Purification System (Millipore, Merck, Darmstadt, Germany).

Selection of apple cultivars and preparation

Four different apple cultivars were chosen, including two old apple cultivars (Dülmener Rosenapfel and Ontario) and two new cultivars (Braeburn and Granny Smith). The apple cultivars were selected according to the reporting list of the BUND Lemgo from the year 2015, which is based on individual reports of allergic individuals about the tolerance of different apple cultivars. After peeling and coring, the flesh of each apple cultivar was homogenized using a mill (Retsch, Haan, Germany) and stored at −25°C until use. A fresh weight of 20 ± 0.1 g of apple flesh was weighed into a 100-mL Erlenmeyer flask. Samples were extracted by addition of 20-mL Dulbecco's PBS and two tablets of ethylenediaminetetraacetic acid-free Protease Inhibitor Cocktail and a shaking process overnight (4°C) [21]. The resulting extracts were filtrated and centrifuged (5 min, 16 000*g*). The supernatants were used for all following analyses.

Protein and Mal d 1 determination

Total protein concentration was determined with the Pierce BCA Protein Assay Kit from Thermo Fisher Scientific (Darmstadt, Germany). Color absorption was measured in a microplate reader (Fluostar Galaxy, BMG Labtech, Offenburg, Germany) at 595 nm. Bovine serum albumin was used as standard protein (25–2000 μg/mL).

Mal d 1 concentration was determined by competitive ELISA using a previously described method [3]. Human sera from birch pollen-allergic individuals for competition were provided by the Department of Dermatology, University Medical Center Jena.

Pilot study

Participants

The study involved 34 patients with allergy due to birch pollen with cross-reactivity to apples. Patients were recruited on the basis of their clinical history by completing a questionnaire and demonstration of specific IgE to rBet v 1 and rMal d 1 (ImmunoCAp, Phadia GmbH, Freiburg, Germany). Those patients who had undergone specific immunotherapy 4 wk before recruitment were excluded from the study. The other exclusion criteria were the use of drugs (antihistamines, antiallergic drugs, corticosteroids) and multiple sensitizations that might affect the study. Blood sampling for the investigation of in vitro allergenicity took place at the Department of Dermatology Jena. The study protocol including justification and details on blood sampling procedures was reviewed and approved by the Ethical Committee of the Friedrich-Schiller-Universität Jena at the Medical Faculty (Jena, Germany).

Sample preparation

Extracts of apple samples were diluted in PBS depending on the total protein content. Final concentrations of 0.1 mg/mL (CAST) and 1 mg/mL (BAT) were used, which was determined in preliminary experiments (data not shown). Each approach consisted of a negative control, stimulation control(s), and rMal d 1 (CAST: 200 ng/mL; BAT: 225 ng/mL) as positive control.

Cellular antigen stimulation test

The concentration of sulfidoleukotrienes ($sLTC_4$, $sLTD_4$, $sLTE_4$) was determined by ELISA with the CAST assay kit from Bühlmann (Schönenbuch, Switzerland). Color absorbance was measured spectrophotometrically at 405 nm. $sLTD_4$ was used for four-parameter fit regression (50–3200 pg/mL). Allergen stimulation has been considered positive at ≥200 pg sLT/mL after subtraction of the negative control.

Basophil activation test

Determination of CD63 basophil activation was carried out by flow cytometry with the Flow CAST assay kit from Bühlmann (Schönenbuch, Switzerland). A flow cytometer (BD FACSCanto^{M}, BD Biosciences, Heidelberg, Germany) was used with a 488-nm argon laser diode (blue green excitation light) and the following four parameters: forward scatter, side scatter, channels for the fluorochromes fluorescein (FITC), and phycoerythrin. Individual cell populations in a sample were identified depending on size and granularity due to scattered light. Basophils were selected from the cell population by anti-CCR3-phycoerythrin. Subsequently, activated basophils were determined by anti-CD63-FITC. The upregulation of the activation marker CD63 was calculated by the percentage of the CD63-positive cells compared with the total identified basophilic cells. Activation has been considered as positive at a percentage of ≥15%.

HPLC analysis of polyphenols

Polyphenolic compounds were analyzed using a previously described method [22] with slight modifications in the elution conditions (A: 0.5% acetic acid, B: methanol): 0–2 min (100% A), 2–6 min (100–85% A), 6–12 min (85% A), 12–17 min (85–80% A), 17–35 min (80% A), 35–70 min (80–65% A), 70–110 min (65% A), 110–125 min (65–20% A), 125–135 min (20–100% A). A reversed-phase Kinetex C18 column (250 × 4.6 mm, particle size 5 μm) (Phenomenex, Aschaffenburg, Germany) was used for separation at 20°C with a flow rate of 0.8 mL/min. Samples integration was set at 254 nm (flavonols), 280 nm (flavanols), and 320 nm (hydroxycinnamic acids).

Influence of enzymatic browning

The old apple cultivars (Ontario and Dülmener Rosenapfel) were used to analyze enzymatic browning. The apple cultivars were frozen at −80°C for 48 h. The frozen apples were peeled and the flesh was treated with liquid nitrogen. Subsequently, the homogenized flesh samples were subjected to a time-dependent browning. Extracts were made after 5, 10, and 15 min of enzymatic browning. The preparation of the extracts was carried out as described in section "Selection of apple cultivars and preparation". The degree of enzymatic browning was evaluated by using a previously described method [23]. The absorbance was measured at 420 nm using a spectrophotometer (V 530, Jasco, Groß-Umstadt, Germany). Six patients with BP-RAA were recruited using the criteria described previously to study the influence of enzymatic browning. CAST and BAT were performed with extracts without enzymatic browning and with time-dependent enzymatic browning (5, 10, and 15 min).

Statistical analysis

All analyses were done in duplicate. The data are expressed as mean ± standard deviation and were analyzed using the statistical program SPSS 22.0 (Statistical Package for the Social Sciences, Chicago, IL, USA). Values $P < 0.05$ were considered statistically significant. The homogeneity of variances for all data was assumed by Levene's test. Data without homogeneity of variances were transformed. The one-way factorial analysis of variance was used followed by the Student Newmann-Keuls procedure for assessing differences between all four apple cultivars. Differences between the average of the two old and the average of the two new apple cultivars were analyzed using the unpaired *t* test. For evaluating correlations, either the Pearson procedure (normally distributed data) or the

Table 1
Demographic data, total and specific IgE levels*, and contents of stimulation controls and rMal d 1 of the study population (N = 27)

Characteristics	Mean ± SD	Median	Range
Age (y)]	35 ± 12	32	21–63
Age of first symptoms (birch)	16 ± 12	13	5–56
Age of first symptoms (apple)	20 ± 11	18	6–56
Total IgE (kU/L)	171 ± 171	106	8–544
Birch-specific IgE	24 ± 22	17	1–100
rBet v 1-specific IgE	19 ± 17	16	1–75
rBet v 4-specific IgE	0.4 ± 1.5	0.0	0.0–7.6
Apple-specific IgE	2.6 ± 3.6	1.3	0.1–14.9
rMal d 1-specific IgE	6.2 ± 5.9	4.8	0.6–20.5
rMal d 3-specific IgE	0.04 ± 0.13	0.0	0.0–0.6
CAST (sLT in pg/mL)			
Negative control: Stimulation buffer	144 ± 120	92	59–620
sC: Anti-IgE rReceptor mAb and fMLP	1128 ± 572	1029	411–2731
rMal d 1	705 ± 422	591	167–2004
BAT (% CD63)			
Negative control: Stimulation buffer	0.4 ± 0.4	0.3	0.0 to 1.3
sC a: Anti-FcεRI mAb	61 ± 19	63	14 to 89
sC b: fMLP	21 ± 16	15	3 to 78
rMal d 1	55 ± 20	56	19 to 87

BAT, basophil activation test; CAST, cellular antigen stimulation test; CD63, CD63 basophil activation; fMLP, N-Formyl-methionyl-leucyl-phenylalanine; Ig, immunoglobulin; mAb, mononuclear antibody; rBet v, recombinant allergen of *Betula verrucosa*; rMal d, recombinant allergen of *Malus domestica* 1; sC,: stimulation control; SD, standard deviation; sLT, sulfidoleukotrienes.
*Total and specific IgE levels determined by ImmunoCap analysis.

Spearman procedure (not normally distributed data) was used, in which the *P*-value was considered to be statistically significant at < 0.01.

Results and discussion

In vitro diagnostic results

Of 34 patients, 27 demonstrated allergen-specific IgE levels to rBet v 1 and rMal d 1 by ImmunoCap method and were suitable for the in vitro investigations (Table 1). Of the 27 apple-reactive individuals included, 81.5% reported that symptoms from apples started after (51.9%) or in the same year (29.6%) as birch pollinosis. Thus, clinical reactions were the result of primary sensitization to birch pollen, which can be confirmed by specific IgE levels. The most prevalent symptoms were itching in the oral cavity, the ear, or both (92.6%), followed by swelling of the lips (70.4%); rhinitis (25.9%); and dyspnea (18.5%). Urticaria (7.4%); cramps, nausea, or both (3.7%); and drop of blood pressure (3.7%) were less frequent. Nine of 27 participants showed a response to the heat-stable lipid transfer protein rMal d 3, but to a much lesser extent than rMal d 1. Sensitization to Mal d 3 is a risk factor for systemic reactions [24]. Mal d 3 essentially concentrates in the skin of the apple fruits as a cell surface-exposed allergen [25]. Data on sex, age, total and specific IgE levels, stimulation controls, and rMal d 1 of CAST and BAT of the study population are summarized in Table 1.

All 27 participants showed response to stimulation controls and rMal d 1 in CAST and BAT (Table 1). Therefore, no false-negative results owing to a non-responder status were encountered. Significant differences in the results of CAST and BAT between the four apple cultivars tested were observed. Ontario had the least significant allergic reaction in both assays followed by Dülmener Rosenapfel. The two new apple cultivars, Braeburn and Granny Smith, resulted in a significantly higher release of sLT and a higher CD63 basophil activation (Table 2). Thus, the old apple cultivars were better tolerated than the new apple cultivars. The results of both in vitro tests correlate significantly ($r = 0.767$; $P < 0.001$).

Quantification of apple polyphenols and Mal d 1

To study the influence of the polyphenolic content on the in vitro allergenicity of apples, the polyphenolic profile of the apple fleshes was analyzed. In the apple fleshes investigated, eight polyphenolic compounds of three subclasses (flavanols, hydroxycinnamic acids, and dihydrochalcones) were identified based on retention times and absorption maxima of reference compounds (Table 3).

Types of polyphenols detected in the apple cultivars were similar to previous studies [22,26,27]. The cultivar Ontario featured the highest total polyphenolic content (90.8 ± 1.7 μg/g) followed by Dülmener Rosenapfel (81.5 ± 0.6 μg/g), Granny Smith (31 ± 0.3 μg/g), and Braeburn (18.5 ± 0.3 μg/g). The flesh of the old cultivars had significantly higher total polyphenolic content (86.1 ± 5.5 μg/g) than the new cultivars (24.7 ± 7.2 μg/g; Table 4). Chlorogenic acid was the major compound in the flesh of the old apple cultivars with a percentage of 62.7%, whereas the content of chlorogenic acid was 14 times lower in the new cultivars (Table 4). Procyanidin B2 had the highest percentage at 63.3% in the new apple cultivars. In addition to chlorogenic acid, the polyphenolic profile of the new apple cultivars was characterized by a significantly lower content of procyanidin B2, caffeic acid, and of the two dihydrochalcones, phloridzin and phloretin-2′-*O*-xylosylglucoside than the old ones (Table 4).

The polyphenolic content of new apple cultivars has been reduced by breeding. This fact can be confirmed on the basis of the

Table 2
In vitro diagnostic concentrations of sulfidoleukotrienes and percentage of CD63 basophil activation of the study population (N = 27) after incubation with different apple cultivars

Immunoassays	CAST (sLT in pg/mL)			BAT (% CD63)		
Apple cultivars	Mean ± SD	Median	Range	Mean ± SD	Median	Range
Old						
Ontario	248 ± 304 [a]	104	0 to 1090	24 ± 26 [a]	14	0–84
Dülmener R.	485 ± 379 [b]	387	0 to 1137	44 ± 26 [b]	48	0–91
New						
Braeburn	904 ± 571 [c]	868	23 to 2782	73 ± 23 [c]	80	14–98
Granny S.	1021 ± 616 [c]	884	0 to 2933	76 ± 21 [c]	81	13–97
P_1-value*	**< 0.001**			**< 0.001**		
Old cultivars	367 ± 361	264	0 to 1137	34 ± 28	29	0–91
New cultivars	962 ± 591	878	0 to 2933	74 ± 22	80	13–97
P_2-value†	**< 0.001**			**< 0.001**		

CAST, cellular antigen stimulation test; SD, standard deviation; sLT, sulfidoleukotrienes; BAT, basophil activation test; CD63, CD63 basophil activation.
*P_1-value: Means with different superscript letters differ significantly between apple cultivars ($P_1 < 0.05$; analysis of variance with post hoc test Student-Newmann-Keuls).
†P_2-value: Significant difference between old and new cultivars ($P < 0.05$; unpaired *t* test).
Data highlighted in bold indicate significant differences.

Table 3
Characterization of reference compounds in HPLC-DAD analysis

Compound	Class	t_R (min)	λ (nm)	λ_{max} (nm)
Procyanidin B1	FLA	20.67	280	281
(+)-Catechin	FLA	24.32	280	281
Chlorogenic acid	HCA	29.07	320	326
Procyanidin B2	FLA	29.87	280	281
Caffeic acid	HCA	32.51	320	324
(-)-Epicatechin	FLA	40.03	280	290
Phloretin-2′-*O*-xylosylglucoside	DHC	80.11	280	287
Phloridzin	DHC	88.03	280	287

λ, absorption used in HPLC-DAD; λ_{max}, absorption maximum determined in HPLC-DAD; DHC, dihydrochalcones; FLA, flavanols; HCA, hydroxycinnamic acids; HPLC-DAD, high-performance liquid chromatography with diode array detector; t_R, retention time.

results of this study, showing that especially a reduction of hydroxycinnamic acids in new apple cultivars took place. In contrast to other published results, contents and percentages of polyphenolic compounds (especially flavanols) in this study are considerably lower because of crushing of the apples without protection against enzymatic oxidation by polyphenol oxidases [28].

The apple cultivars that were tested differed significantly regarding their content of Mal d 1, but not between old and new cultivars (Table 4). For freshly harvested apples, the Mal d 1 content was between 1 to 72.5 μg/g [29,30]. Our results were at the lower end of the range. The Mal d 1 content in apples is highly dependent on the cultivar and on the degree of maturity and storage [11,13,29,30].

Polyphenols and BP-RAA

It has been suggested that polyphenols are able to influence the allergic immune responses [17]. Concentration of sLT and CD63 basophil activation correlated significantly (sLT: $R = -0.547$, $P < 0.001$; CD63 basophils: -0.639, $P < 0.001$) with the total polyphenolic content of the four apple cultivars tested. Based on this inverse correlation, higher total polyphenolic contents are associated with lower allergic responses. One possible reason could be the enzymatic browning of apples. It is hypothesized that oxidation by polyphenol oxidases in the presence of phenolic compounds modifies proteins, resulting in a loss of allergenicity [12].

Therefore, in an additional study the influence of enzymatic browning on the in vitro allergenicity was investigated. As shown in Figure 1, the total polyphenolic content, the concentration of sLT, and the CD63 basophil activation of Ontario and Dülmener Rosenapfel decreased significantly as enzymatic browning increased. For the cultivar Ontario, there was a 25% greater decrease in the total polyphenols (0–5 min) compared with Dülmener Rosenapfel, which was accompanied by a stronger enzymatic browning. In particular, flavanols and hydroxycinnamic acids are very good substrates for phenol oxidases in apples, thus the stronger browning may be due to the higher content of total flavanols (50%) and total hydroxycinnamic acids (15%) in Ontario. This stronger degree of browning was associated with a higher decrease in sLT (−43%) and CD63 basophil activation (−18%) compared with Dülmener Rosenapfel. Correlation analysis showed that chlorogenic acid, epicatechin, and caffeic acid have the strongest effects on the in vitro concentration of sLT and CD63 basophil activation. Correlation coefficients ranged from 0.820 (chlorogenic acid, epicatechin) to 0.335 (phloridzin) in CAST and from 0.633 (caffeic acid) to 0.349 (phloridzin) in BAT.

Garcia et al. showed that the addition of polyphenol oxidase to a Golden Delicious apple extract reduced IgE binding by Mal d 1 and a combination of polyphenol oxidase and catechin resulted in an even stronger inhibition [3]. Chung and Champagne treated protein extracts based on peanut products with monomeric phenolic compounds (caffeic, chlorogenic, and ferulic acids), which resulted in the formation of insoluble complexes and a reduction of the level of soluble major peanut allergens [31]. Gruber et al. reported a decrease of IgE binding activity by the recombinant cherry (*Prunus avium*) major allergen as a consequence of polyphenol oxidase catalyzed oxidation [32]. They hypothesized that the polyphenol oxidase catalyses the reaction of polyphenols to *o*-quinones, which can interact with allergens. This reaction leads to an irreversible change in the tertiary structure of the allergen by modifying nucleophilic amino acid side chains of proteins and results in a loss of conformational epitopes of the allergen [32]. Consequently, polyphenols have an inhibitory effect on the basophil and mast cell activation and on the release of mediators like sLT and histamine [18]. Thus, apple cultivars with a high total polyphenolic content and an equally high polyphenol oxidase activity may have lower allergenicity [13,33]. This in turn can be the reason for the reduced allergenicity of the old apple cultivars (Ontario and Dülmener Rosenapfel) compared with the new ones (Braeburn and Granny Smith). Based on the Spearman correlation, there was no significant correlation between the Mal d 1 content and the results of

Table 4
Contents of apple polyphenols and Mal d 1 in the flesh of different apple cultivars (contents in μg/g of fresh weight)

Compound	Old Ontario	Dülmener R.	New Braeburn	Granny S.	P_1*	Old cultivars*	New cultivars†	P_2†
Procyanidin B1	2.0 ± 0.02^{b}	1.6 ± 0.04^{c}	0.6 ± 0.01^{a}	2.2 ± 0.1^{d}	<**0.001**	1.3 ± 0.4	1.4 ± 0.9	0.800
(+)-Catechin	1.5 ± 0.1^{d}	1.0 ± 0.01^{b}	1.1 ± 0.1^{c}	0.5 ± 0.02^{a}	<**0.001**	1.2 ± 0.3	0.8 ± 0.4	0.136
Chlorogenic acid	54.0 ± 1.3^{c}	54.0 ± 0.4^{c}	0.6 ± 0.2^{a}	7.0 ± 0.1^{b}	<**0.001**	54.0 ± 0.8	3.8 ± 3.7	<**0.001**
Procyanidin B2	17.2 ± 0.1^{d}	16.3 ± 0.01^{c}	15.4 ± 0.04^{a}	15.9 ± 0.01^{b}	<**0.001**	16.8 ± 0.5	15.7 ± 0.3	**0.017**
Caffeic acid	1.1 ± 0.01^{c}	1.3 ± 0.04^{d}	0.4 ± 0.03^{a}	0.7 ± 0.02^{b}	<**0.001**	1.2 ± 0.1	0.5 ± 0.1	**0.001**
(-)-Epicatechin	2.2 ± 0.01^{b}	1.9 ± 0.1^{a}	n.d.	3.7 ± 0.04^{c}	<**0.001**	2.1 ± 0.2	3.7 ± 0.04	0.181
Phloretin-2′-*O*-xylosylglucoside	3.7 ± 0.1^{d}	1.9 ± 0.02^{c}	0.2 ± 0.01^{a}	0.5 ± 0.01^{b}	<**0.001**	2.8 ± 1.1	0.4 ± 0.2	**0.018**
Phloridzin	10.2 ± 0.3^{c}	3.5 ± 0.1^{b}	0.2 ± 0.01^{a}	0.5 ± 0.04^{a}	<**0.001**	6.9 ± 3.9	0.4 ± 0.2	**0.044**
total PP	90.8 ± 1.7^{d}	81.5 ± 0.62^{c}	18.5 ± 0.3^{a}	31.0 ± 0.3^{b}	<**0.001**	86.1 ± 5.5	24.7 ± 7.2	<**0.001**
Total FLA	21.9 ± 0.1^{c}	20.8 ± 0.13^{b}	17.1 ± 0.1^{a}	22.3 ± 0.1^{d}	<**0.001**	21.3 ± 0.6	19.7 ± 3	0.369
Total HCA	55.0 ± 1.3^{c}	55.3 ± 0.46^{c}	1.0 ± 0.2^{a}	7.6 ± 0.2^{b}	<**0.001**	55.2 ± 0.8	4.3 ± 3.8	<**0.001**
Total DHC	13.9 ± 0.4^{d}	5.4 ± 0.03^{c}	0.4 ± 0.01^{a}	1.0 ± 0.04^{b}	<**0.001**	9.6 ± 4.9	0.7 ± 0.4	**0.036**
Mal d 1	1.7 ± 0.07^{b}	0.7 ± 0.04^{a}	0.8 ± 0.02^{a}	3.3 ± 0.12^{c}	<**0.001**	1.2 ± 0.6	2.0 ± 1.4	0.211

FLA, flavanols; HCA, hydroxycinnamic acids; DHC, dihydrochalcones; n.d., not detected; PP, polyphenols.
Means with different superscript letters differ significantly between apple cultivars.
*P_1-values represent results from one-way factorial analysis of variance after the Student-Newmann-Keuls procedure between all four apple cultivars.
†P_2-values represent results from unpaired *t* test between the average of the two old cultivars and the average of the two new cultivars ($P < 0.05$).
Data highlighted in bold indicate significant differences.

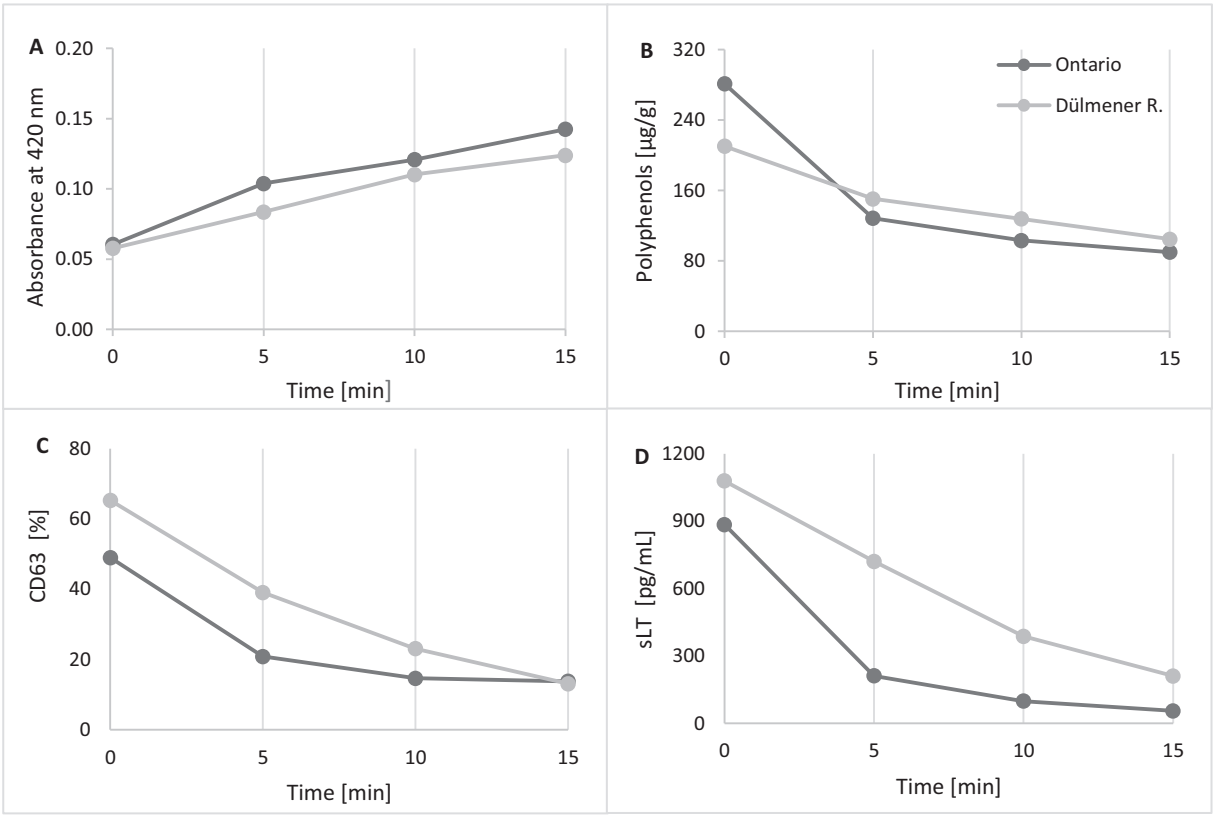

Fig. 1. Influence of enzymatic browning on total polyphenols and in vitro allergenicity of the apple cultivars Ontario and Dülmener Rosenapfel. Course of (A) degree of enzymatic browning; (B) content of total polyphenols by high performance liquid chromatography; (C) percentage of CD63 basophil activation by basophils activation test; (D) concentration of sulfidoleukotrienes by cellular antigen stimulation test.

CAST ($P = 0.091$) and BAT ($P = 0.062$). This confirms our claim that lower CAST and BAT results of old cultivars are due to a significantly higher polyphenolic content.

Conclusion

Old apple cultivars like Ontario and Dülmener Rosenapfel could be better tolerated by BP-RAA allergic individuals than new ones owing to a higher total polyphenolic content and a stronger degree of enzymatic browning. Correlation analysis indicated that a high content of chlorogenic acid, caffeic acid, and epicatechin leads to a lower allergenicity of apples. However, assessment of the allergenicity of apple cultivars is complex, and this study was limited to in vitro assays. Further in vivo studies are necessary for clinical evaluation. This knowledge could help breeders and apple producers in selecting cultivars to provide fruits with a lower allergenicity.

References

[1] Ballmer-Weber BK. Food allergy in adolescence and adulthood. Chem Immunol Allergy 2015;101:51–8.
[2] Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. Allergy 1983;38:167–72.
[3] Garcia A, Wichers JH, Wichers HJ. Decrease of the IgE-binding by Mal d 1, the major apple allergen, by means of polyphenol oxidase and peroxidase treatments. Food Chem 2007;103:94–100.
[4] Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol 2011;127. 616–22.e1.
[5] Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, Susani M, Ebner C, Kraft D, et al. 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;14:538–51. 214.
[6] Hoffmann-Sommergruber K. Plant allergens and pathogenesis-related proteins. What do they have in common? Int Arch Allergy Immunol 2000;122:155–66.
[7] Marzban G, Puehringer H, Dey R, Brynda S, Ma Y, Martinelli A, et al. Localisation and distribution of the major allergens in apple fruits. Plant Sci 2005;169:387–94.
[8] Vieths S, Janek K, Aulepp H, Petersen A. Isolation and characterization of the 18-kDa major apple allergen and comparison with the major birch pollen allergen (Bet v I). Allergy 1995;50:421–30.
[9] Pühringer H, Moll D, Hoffmann-Sommergruber K, Watillon B, Katinger H, Laimer da Câmara Machado H. The promoter of an apple Ypr10 gene, encoding the major allergen Mal d 1, is stress- and pathogen-inducible. Plant Sci 2000;152:35–50.
[10] Asero R, Marzban G, Martinelli A, Zaccarini M, Machado ML. Search for low-allergenic apple cultivars for birch-pollen-allergic patients: Is there a correlation between in vitro assays and patient response? Eur Ann Allergy Clin Immunol 2006;38:94–8.
[11] Zuidmeer L, van Leeuwen WA, Kleine Budde I, Breiteneder H, Ma Y, Mills C, et al. Allergenicity assessment of apple cultivars: hurdles in quantifying labile fruit allergens. Int Arch Allergy Immunol 2006;141:230–40.
[12] Son DY, Lee SI. Comparison of the characteristics of the major allergen Mal d 1 according to apple varieties. Food Sci Biotechnol 2001;10:132–6.
[13] Schmitz-Eiberger M, Matthes A. Effect of harvest maturity, duration of storage and shelf life of apples on the allergen Mal d 1, polyphenoloxidase activity and polyphenol content. Food Chem 2011;127:1459–64.
[14] Bannier HJ. Moderne Apfelzüchtung - Genetische Verarmung und Tendenzen zur Inzucht. Erwerbs-Obstbau 2011;52:85–110.
[15] Scalbert A, Morand C, Manach C, Rémésy C. Absorption and metabolism of polyphenols in the gut and impact on health. Biomed. Pharmacother 2002;56:276–82.
[16] Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci 2007;8:950–88.
[17] Singh A, Holvoet S, Mercenier A. Dietary polyphenols in the prevention and treatment of allergic diseases. Clin Exp Allergy 2011;41:1346–59.
[18] Pearce FL, Befus AD, Bienenstock J. Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. J Allergy Clin Immunol 1984;73:819–23.

[19] Chirumbolo S. Dietary assumption of plant polyphenols and prevention of allergy. Curr Pharm Des 2014;20:811–39.
[20] BUND Lemgo. Project apple allergy starts. http://www.bund-lemgo.de/download/2017_10_Apfelallergie_Sortenliste_allgemein_int.pdf. Accessed March 28, 2018.
[21] Rudeschko O, Fahlbusch B, Henzgen M, Schlenvoigt G, Herrmann D, Jäger L. Optimization of apple allergen preparation for in vivo and in vitro diagnostics. Allergy 1995;50:262–8.
[22] Kschonsek J, Wolfram T, Stöckl A, Böhm V. Polyphenolic compounds analysis of old and new apple cultivars and contribution of polyphenolic profile to the in vitro antioxidant capacity. Antioxidants 2018;7:E20.
[23] Coseteng MY, Lee CY. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J Food Sci 1987;52:985–9.
[24] Fernández-Rivas M, Bolhaar S, González-Mancebo E, Asero R, van Leeuwen A, Bohle B, et al. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. J Allergy Clin Immunol 2006;118:481–8.
[25] Borges JP, Jauneau A, Brule C, Culerrier R, Barre A, Didier A, Rouge P. The lipid transfer proteins (LTP) essentially concentrate in the skin of Rosaceae fruits as cell surface exposed allergens. Plant Physiol Biochem 2006;44:535–42.
[26] Vrhovsek U, Rigo A, Tonon D, Mattivi F. Quantitation of polyphenols in different apple varieties. J Agric Food Chem 2004;52:6532–8.
[27] Liaudanskas M, Viškelis P, Jakštas V, Raudonis R, Kviklys D, Milašius A, et al. Application of an optimized HPLC method for the detection of various phenolic compounds in apples from Lithuanian cultivars. J Chem 2014;2014:1–10.
[28] Guyot S, Marnet N, Djamel L, Sanoner P, Drilleau JF. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (Malus domestica Var. Kermerrien). J Agric Food Chem 1998;46:1698–705.
[29] Matthes A, Schmitz-Eiberger M. Apple (Malus domestica L. Borkh.) allergen Mal d 1: Effect of cultivar, cultivation system, and storage conditions. J Agric Food Chem 2009;57:10548–53.
[30] Sancho AI, Foxall R, Browne T, Dey R, Zuidmeer L, Marzban G, et al. Effect of postharvest storage on the expression of the apple allergen Mal d 1. J Agric Food Chem 2006;54:5917–23.
[31] Chung SY, Champagne ET. Reducing the allergenic capacity of peanut extracts and liquid peanut butter by phenolic compounds. Food Chem 2009;115:1345–9.
[32] Gruber P, Vieths S, Wangorsch A, Nerkamp J, Hofmann T. Maillard reaction and enzymatic browning affect the allergenicity of Pru av 1, the major allergen from cherry (Prunus avium). J Agric Food Chem 2004;52:4002–7.
[33] Kiewning D, Wollseifen R, Schmitz-Eiberger M. The impact of catechin and epicatechin, total phenols and PPO activity on the Mal d 1 content in apple fruit. Food Chem 2013;140:99–104.