Molecular Nutrition & Food Research
www.mnf-journal.com

Analyzing Bioaccessibility of Polyphenols in Six Commercial and Six Traditional Apples (*Malus domestica* Borkh.) during In Vitro and Ex Vivo Oral Digestion

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Scope: Apples are an important polyphenol (PP) source. To compare the health benefits of traditional and commercial varieties, the phenolic contents and profiles as well as their release from the matrix (bioaccessibility) during oral digestion are determined. Furthermore, based on these data the proposed beneficial effect of PP on the variety specific allergenicity is discussed.
Methods and results: Phenolics are quantified by HPLC-DAD. Total phenolic contents (TPC) are in the range of 111–645 and 343–1950 mg 100 g^{-1} dry weight for flesh and peel, respectively. Matrix release during oral digestion is investigated ex vivo, with centrifuged and non-centrifuged human saliva and in vitro with simulated saliva fluid (SSF). The overall bioaccessibility is similar in all digestion media, ranging between 40–80% and 39–65% of the TPC in flesh and peel, respectively. Analyzing the correlation among Mal-d 1-allergen-content, unoxidized PP, and the allergenic potential for the samples reveals a negligible effect of phenolics.
Conclusion: Due to higher phenolic contents in combination with a similar release, increased PP concentrations in the oral phase and an improved uptake of PP from traditional varieties are assumed. However, the proposed beneficial effect of phenolics on allergenicity cannot be confirmed.

1. Introduction

Polyphenols (PP) are secondary plant metabolites. To this structural diverse group of compounds various different health benefits are attributed.[1–3] Apples are an important source of PP in the Western diet.[4] The main apple phenolics belong to the subgroups hydroxycinnamic acid derivatives, dihydrochalcone-glycosides, and the flavonoids: flavanols, flavonol-glycosides, and anthocyanin-glycosides.[5–9] Although phenolic structures, identified in different apple varieties are quite similar, marked differences in profiles and total contents have been reported.[5–9]

It is estimated that 50–70% of all individuals with an allergy to the main birch pollen allergen Bet v 1 develop a pollen associated cross allergy against the apple allergen Mal d 1 during their lifetime.[10,11] The allergen is thermo- and proteolytically labile[12] and manifests itself as the oral allergy syndrome with typical symptoms like swelling and itching of lips, palate, and tongue as well as throat irritations.[13] Therefore, affected individuals often avoid the consumption of fresh apples.[11] This deprives the patients of an important source of vitamins, fibers, and secondary plant metabolites. In consumer surveys,[14] clinical studies[15–17] and in vitro experiments[18] a variety specific allergenic potential was observed. In particular, commercially relevant breeds are reported to be highly allergenic.[14,19–21] So far, the variety dependent allergenic potential cannot solely be explained by different Mal d 1 contents.[16] Because commercially relevant varieties are also characterized by lower PP contents than traditional varieties, often grown in local orchard meadows,[7–9,22] an impact of phenolics on the allergenic potential is hypothesized. The low phenolic contents in commercial breeds reflect the consumers' demands for sweet and less or even non-browning apples. Therefore, this led to selective breeding, focused on varieties being poor in PP, to reduce the formation of browning products by the polyphenol oxidase.[23] It is further known that PP bind to proteins, which might reduce the allergenic potential by masking of the IgE epitope regions or reducing of the allergen concentration by protein precipitation.[24,25] Hence, a correlation between allergenicity and polyphenol content is proposed.[21,23,26]

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202300055

DOI: 10.1002/mnfr.202300055

The allergic reaction occurs usually exclusively in the oral cavity[13] and, since only free PP might interact with the allergen during oral digestion, it is important to consider their release during consumption. However, studies assessing the PP bioaccessibility during oral digestion for a wide range of different apple cultivars are missing.

Besides a preliminary study of our group, using an ex vivo and in vitro approach with centrifuged saliva and simulated saliva fluid (SSF), only Tenore et al. studied the release of phlorizin, quercetin-rutinoside, and procyanidins (PC) from five apple cultivars during oral digestion.[27,28] Our previous study revealed, that the total release of PP differed between peel and flesh. Furthermore, these data indicated that the individual phenolic structure affects the release more than individual apple matrix characteristics. To increase our knowledge about the bioaccessibility of PP, we extended the study and analyzed the phenolic profiles and release during oral digestion for six commercial and six traditional cultivars applying SSF and saliva. Since saliva is a very complex biofluid containing potentially bacteria from the oral cavity but also epithelial cells, for which a β-glycosidic activity is described, both, centrifuged and non-centrifuged saliva, were included into the current study.[29–31] We also tested the proposed correlation between the allergenic potentials and the Mal d 1 contents with either total phenolic contents (TPC) or phenolics released during oral digestion. Mal d 1 contents for the same apple samples were previously reported by us[32] and the variety specific allergenicity was collected in a consumer survey published by the BUND Lemgo.[14]

2. Experimental Section

2.1. Reagents

2.1.1. Polyphenol Standards

As standards (+)catechin, cyanidin-3-glucoside, isorhamnetin-3-rutinoside, and the procyanidins PC B1, PC B2, and PC C1 were acquired from PhytoLab (Vestenbergsgreuth, Germany). Chlorogenic acid, (-)epicatechin, phlorizin dihydrate, *p*-coumaric acid, and quercetin-3-glucoside were bought from Sigma-Aldrich (Schnelldorf, Germany). Stock solutions of the PP standards for the quantification mixes were prepared in methanol/water (50/50, v/v) except for cyanidin-glucoside, which was dissolved in 0.01% hydrochloric acid. The concentrations of the stock solutions were quantified using quantitative NMR (*q*-NMR) according to the method published previously.[33] For cyanidin-glucoside and PC C1 *q*-NMR failed; therefore, quantification of these standards was based on weight.

The study found significant differences for quantification of the quercetin-glucoside stock solution using balance (weight) and *q*-NMR.[33] Therefore, the flavonol-glycoside content of the stock solution was calculated based on both quantification methods. All data in the main text were based on the calibration by weight. Individual phenolic contents for all apple varieties based on weight were provided in Tables S1A and S2A, Supporting Information. In Tables S1B and S2B, Supporting Information the contents quantified by *q*-NMR were listed.

2.1.2. Chemicals

All chemicals were of analytical grade. Analytic solvents and formic acid for mass spectrometry were MS-grade. Acetonitrile and methanol were obtained from Fisher Scientific (Loughborough, UK). Formic acid for the HPLC-DAD measurements and hydrochloric acid (HCl, 37%) were bought from Grüssing (Filsum, Germany). Calcium chloride, potassium chloride, and potassium dihydrogen phosphate were bought from Roth (Karlsruhe, Germany). Sodium hydrogen carbonate, magnesium chloride hexahydrate, ammonium carbonate, and formic acid for mass spectrometry were acquired from Merck (Darmstadt, Germany). Ultrapure water (ELGA PurLab flex, Veolia Waters, Celle, Germany) was used throughout all experiments.

2.1.3. Fruit Material

The peel and flesh of 12 different apple varieties, six traditional (Altländer Pfannkuchenapfel, Bohnapfel, Brettacher, Ingrid Marie, Kaiser Wilhelm, and Berlepsch, also known as Goldrenette) and six commercially relevant varieties (Granny Smith, Golden Delicious, Fuji, Gala, Elstar, and Jonagold) harvested in 2019, were investigated. Further information about the apples, e.g., water content of peel and pulp, proportion of peel or pulp to the whole apple, supplier, country of production, genetically descent, and year of first description[34] were available in the Table S3, Supporting Information.

Sample preparation was performed according to Kaeswurm et al.[28] The lyophilized and milled samples were stored under argon at room temperature in the dark until further use.

2.2. Methanolic Extraction and Simulated Oral Digestion

To quantify the TPC of the different apple varieties, a methanolic extraction was carried out in duplicate as described by Kaeswurm et al.[28] The aqueous extracts were stored at −22 °C. Before analysis by HPLC-DAD, samples were diluted 1:5 with 0.1% methanolic HCl.

For ex vivo experiments saliva was collected from two healthy females (age 25 and 26) in the morning before breakfast and brushing teeth. One half was centrifuged at 10 410 rcf for 10 min to obtain centrifuged saliva. The simulated saliva fluid (SSF) was prepared according to the COST model.[35] The proceedings to simulate the oral digestion were similar to the previous study, allowing comparison of the data.[28] In accordance with the regulations of the ethic commission of the University Stuttgart, each proband was only allowed to work with her own saliva.

2.3. Characterization and Quantification of Polyphenols in Methanolic Extracts and Oral Digestion Samples

PP were characterized using a 1260 Agilent HPLC System (Agilent, Santa Clara, USA), equipped with a binary pump (1260 ALS) including a degasser, an auto sampler, a UV-detector (Agilent

Table 1. Total polyphenolic content (TPC) [mg 100 g^{-1} DW/FW] in peel and flesh of different apple varieties.

Variety		Total phenolic content (TPC)				Ratio of mass in flesh to peel in the FW	Ratio of TPC content in flesh to peel in the FW	Calculated TPC for cored model apple [mg 100 g^{-1} FW][b)]
		In the dry weight (DW) [mg 100 g^{-1} DW]		In the fresh weight (FW)[a)] [mg 100 g^{-1} FW]				
		Flesh	Peel	Flesh	Peel			
Traditional varieties	Altländer Pfannkuchenapfel	388 ± 6	1011 ± 72	60 ± 1	196 ± 14	5.1:1	1:3.3	82 ± 3
	Bohnapfel	645 ± 16	1950 ± 33	125 ± 3	492 ± 8	4.6:1	1:4.0	191 ± 3
	Brettacher	332 ± 1	1067 ± 17	49 ± 0	206 ± 3	6.8:1	1:4.2	69 ± 0
	Berlepsch/ Goldrenette	143 ± 1	447 ± 38	22 ± 0	96 ± 8	5.0:1	1:4.3	35 ± 2
	Ingrid Marie	213 ± 7	695 ± 14	37 ± 1	153 ± 3	5.3:1	1:4.1	56 ± 1
	Kaiser Wilhelm	364 ± 8	840 ± 107	64 ± 1	190 ± 24	5.8:1	1:3.0	82 ± 4
Commercial varieties	Elstar	124 ± 0	343 ± 9	19 ± 0	65 ± 2	5.4:1	1:3.6	26 ± 0
	Fuji	255 ± 28	711 ± 38	36 ± 4	128 ± 7	5.1:1	1:3.6	51 ± 4
	Gala	131 ± 0	508 ± 3	19 ± 0	97 ± 1	5.8:1	1:5.0	31 ± 0
	Golden Delicious	152 ± 3	433 ± 8	21 ± 0	79 ± 1	6. 0:1	1:3.7	29 ± 0
	Granny Smith	164 ± 1	461 ± 34	22 ± 0	84 ± 6	6.1:1	1:3.7	31 ± 1
	Jonagold	111 ± 9	406 ± 7	15 ± 1	65 ± 1	5.3:1	1:4.4	23 ± 1

[a)] Contents are calculated based on the polyphenol contents in the DW and the water content (Table S3, Supporting Information); [b)] Contents are calculated based on water content and the ratio of peel and flesh to the whole weight of the fruit (Table S3, Supporting Information). TPC, total phenolic content; mean ± average deviation ($n = 2$).

1260 Infinity II VWD G7114 A) and a time of flight mass spectrometer (Bruker Impact II, Bruker, Billerica, USA) with parameters described in detail by Kaeswurm et al.[28] PP separation was achieved on a C18 Nucleodur Gravity-SB column (150 × 2 mm, ID Ø 2 µm [Machery & Nagel, Düren, Germany]) with formic acid/acetonitrile/water (1/3/96, v/v/v) as eluent A and formic acid/acetonitrile/water (1/90/9, v/v/v) as eluent B at a flow rate of 0.2 mL min^{-1} and a column temperature of 35 °C according to the previously published gradient.[28]

PP quantification was performed with a HPLC-DAD system (Agilent 1260 series) equipped with a quaternary pump (G1311B), autosampler, degasser, and a DAD (G1329B) using similar chromatographic conditions as described for the characterization. Hydroxycinnamic acid derivatives were quantified at 320 nm, flavonols at 370 nm, anthocyanins at 520 nm, and further PP at 280 nm using a five-point external calibration with 11 different standard compounds in five standard mixes. Further details were available in Kaeswurm et al.[28] Data processing was performed by ChemStation CDS software (edition C01.07 SR3, Agilent Technology). TPC was calculated by summing up the content of the individual PP structures.

2.4. Statistical Analysis

The significance of data was tested using ANOVA, if data did not fulfill the demands for homogeneity of variance and normal distribution, Kruscal–Wallis test ($\alpha = 0.05$) was employed. Variance homogeneity and normal distribution were checked by Leven's test and Shapiro-Wilk test. Statistical analysis was performed with Excel 2016 utilizing the add-in Real Statistics.[36] For principal component analysis (PCA) Origin 2019b was used.

3. Results

3.1. Differences in the Total Phenolic Content (TPC) between the Varieties Quantified in the Methanolic Extracts

The contents for each individual phenolic compound in the different apple varieties are available in the Tables S1A and S2A (Supporting Information) for peel and flesh, respectively. If quantification of the quercetin derivatives was based on *q*-NMR instead of weight, 22% lower contents were quantified (S1B and S2B, Supporting Information). Due to insignificant amounts of flavonol-glycosides in the flesh this resulted in negligible variations of the TPC (<3 mg 100 g^{-1} dry weight (DW). However, in peel this difference in quantification lead to a decrease of the TPC by 4–8%.

Among the apple varieties investigated, Bohnapfel contained the highest TPC in flesh with 645 mg 100 g^{-1} DW (125 mg 100 g^{-1} fresh weight [FW]), which was 6-fold higher than the PP content in Jonagold (111 mg 100 g^{-1} DW; 15 mg 100 g^{-1} FW), the variety with the lowest TPC (**Table 1**). In peel, Bohnapfel also showed the highest TPC with 1950 mg 100 g^{-1} DW (492 mg 100 g^{-1} FW) and a marked difference to Brettacher, the apple with the second highest TPC with 1067 mg 100 DW^{-1} (206 mg 100 g^{-1} FW), was found. The lowest TPC in the peel was quantified in Elstar with 343 mg 100 g^{-1} DW (65 mg 100 g^{-1} FW).

To roughly estimate the PP intake when a whole cored apple is consumed, the data were transferred to the whole apple for each variety based on the TPC of peel and flesh, water content and proportion of peel to flesh (Table S3, Supporting Information). The calculated values ranged from 23 to 191 mg 100 g^{-1} FW, underlining the marked differences among the varieties. It was evident that traditional varieties often, but not always, showed a higher TPC than commercial breeds. Surprisingly Fuji,

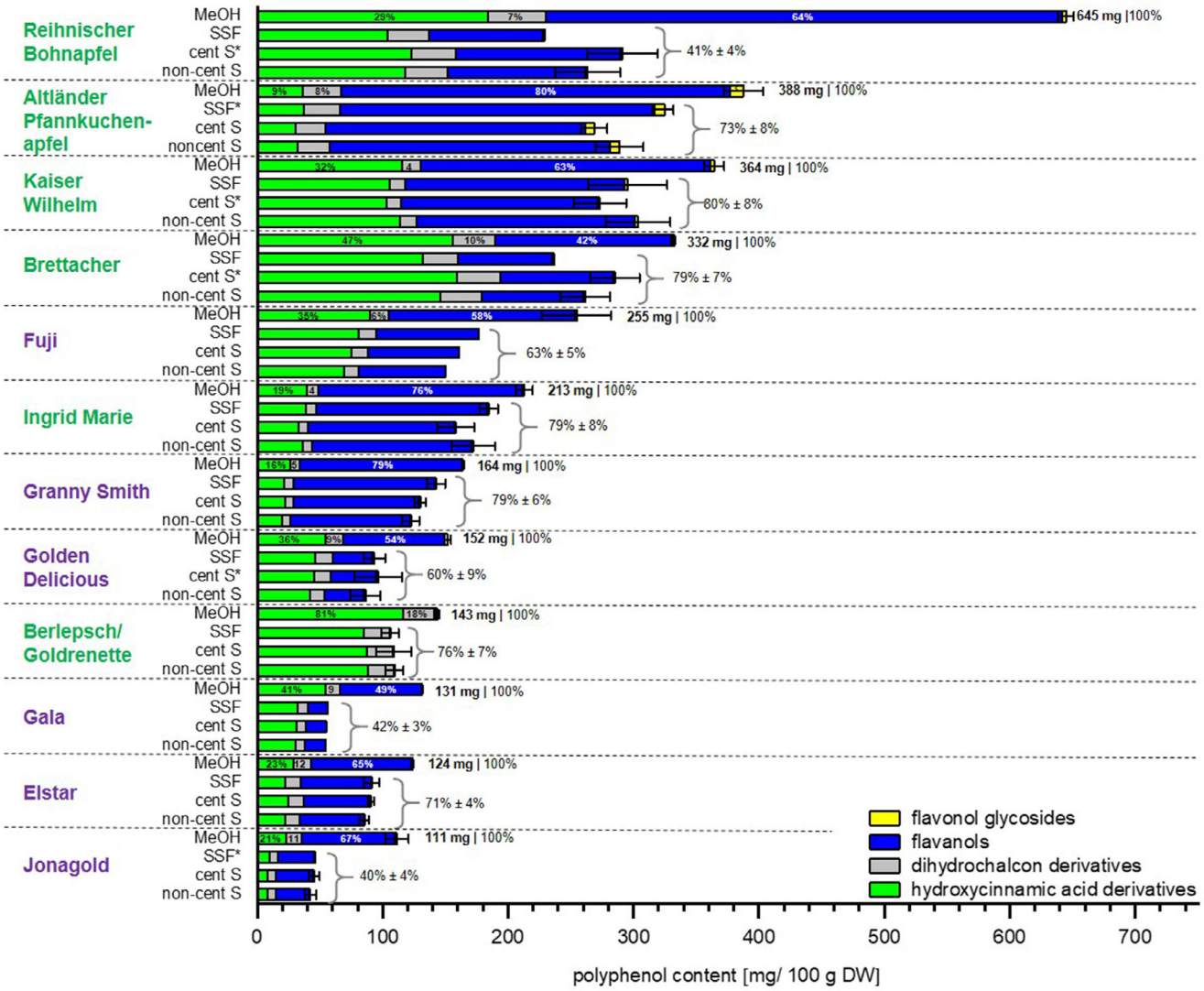

Figure 1. Release of phenolics during oral digestion from the flesh for simulated saliva fluid (SSF), centrifuged (cent. S), and non-centrifuged saliva (non-cent S) for commercial (violet) and traditional apple varieties (green) based on the TPC of the initial methanolic extraction (MeOH). The ratios of phenolic groups are included for the initial extraction (MEOH). Values below 3% were omitted. No significant differences between the saliva of the probands were observed, therefore, data for both probands were combined ($n = 4$ for cent S and non-cent S). For MeOH and SSF $n = 2$. The mean value ± standard deviation for the release was calculated from all digestion fluids ($n = 10$). *One sample was excluded from the average, due to high deviations compared to other samples.

a commercial breed, had a considerable higher PP content with 51 mg 100 g^{-1} FW than all other commercial breeds. On the other hand, Berlepsch, also known as Goldrenette, a traditional variety, exhibited a low TPC (35 mg 100 g^{-1} FW), analogous to the commercial breeds Granny Smith (31 mg 100 g^{-1} FW) and Gala (31 mg 100 g^{-1} FW).

3.2. Polyphenol Profiles in Flesh and Peel

While individual phenolic structures were similar in the different varieties, the phenolic profiles, more precisely the ratios among the individual contents, differed markedly (**Figure 1**, Tables S1A and S2A, Supporting Information). In the flesh flavanols were the main subgroup of PPs and made up 40–80% of the TPC. Surprisingly in Berlepsch flavanols were absent. In general, epicatechin was the major monomer, whereas PC B2 and PC C1 were the most important procyanidins in the flesh. In addition, oligomeric PCs with up to seven monomer units were detected. The amounts of hydroxycinnamic acid derivatives were very variable, ranging between 9% of the TPC in Altländer Pfannkuchenapfel and 81% in Berlepsch. Nevertheless, chlorogenic acid was generally the most important hydroxycinnamic acid derivative and one of the most important phenolics in apple flesh. In addition, different coumaroylquinic acid derivatives, feruloyl-hexoside, and a further unknown hydroxycinnamic acid derivative were identified. The latter showed a fragmentation pattern similar to chlorogenic acid and a maximum absorption at

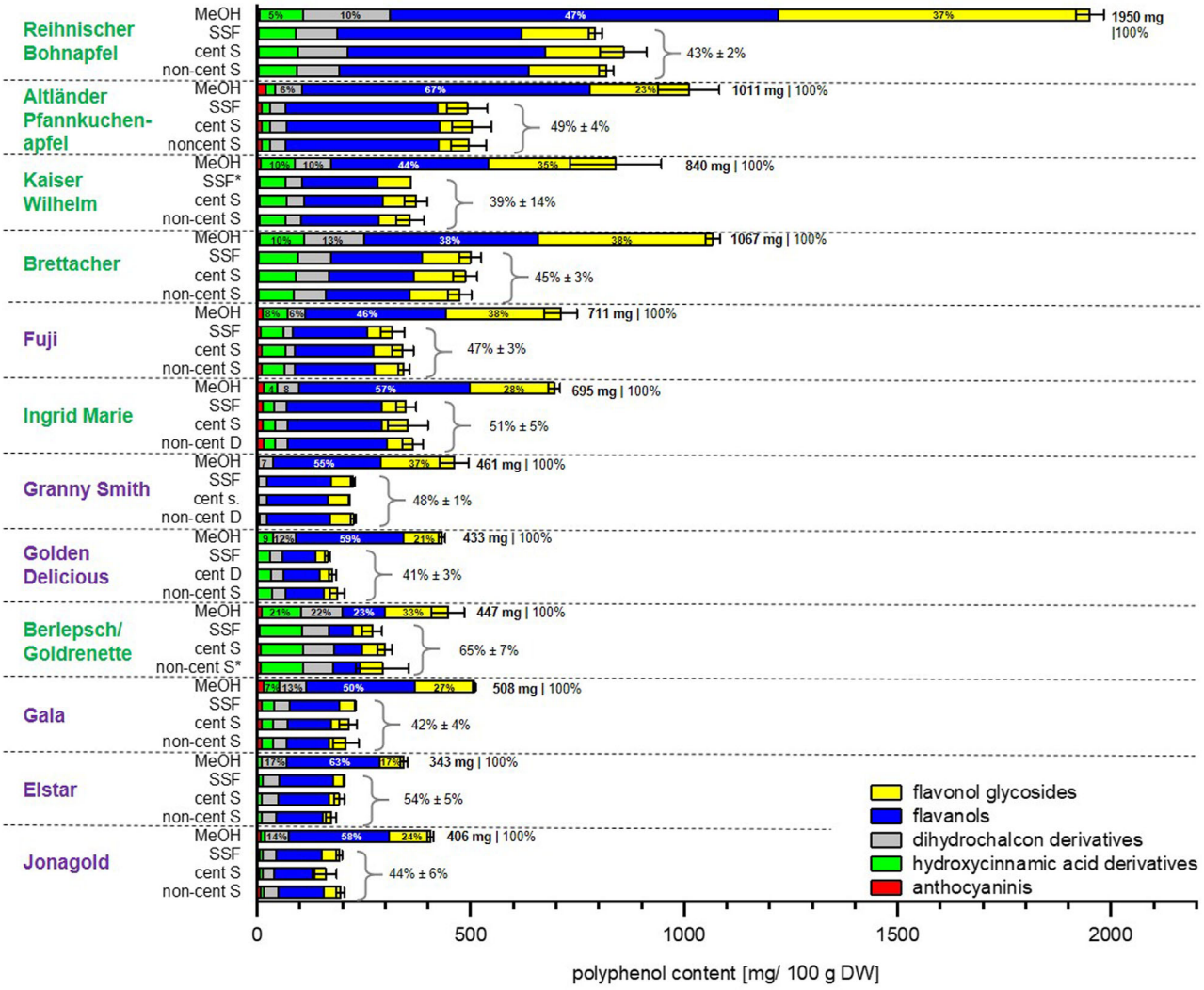

Figure 2. Release of phenolics during oral digestion from the peel for simulated saliva fluid (SSF), centrifuged (cent. S), and non-centrifuged saliva (non-cent S) for commercial (violet) and traditional apple varieties (green) based on the TPC of the initial methanolic extraction (MeOH). The ratios of phenolic groups are included for the initial extraction (MEOH). Values below 3% were omitted. No significant differences between the saliva of the probands were observed, therefore, data for both probands were combined ($n = 4$ for cent S and non-cent S). For MeOH and SSF $n = 2$. The mean value ± standard deviation for the release was calculated from all digestion fluids ($n = 10$). *One sample was excluded from the average due to high deviations compared to other samples.

320 nm but could not be identified as neo- or cryptochlorogenic acid by standard addition. Dihydrochalcone-glycosides were only present in limited amounts with 4–18% of the TPC, all containing a phloretin as aglycon.

Flavonol-glycosides played a major role in the peel with a ratio of 17–38% of the TPC (**Figure 2**). These flavonols were identified predominantly as quercetin-glycosides. However, in the peel of Brettacher and Kaiser Wilhelm 5% and 2% isorhamnetin-glycosides were present, respectively. A red cyanidin-glycoside, most likely cyanidin-galactoside,[37] was detected, in traces (0.3–3%) in the peel of all samples, except for the green and yellow skinned varieties Granny Smith and Golden Delicious. A strong impact of illumination during ripening is reported for the anthocyanins and flavonols, which might affect the results.[38] Besides anthocyanins and flavonols no differences were observed between flesh and peel. As in the flesh flavanols were also the most important phenolics in the peel, with a proportion of 38–67%. Berlepsch, the variety with hardly any flavanols present in the flesh, was also characterized by a lower flavanol content in the peel (23%). The proportions of hydroxycinnamic acid derivatives were markedly reduced in the peel (1–21%) compared to the flesh (9–81%). While the ratio of dihydrochalcone-glycosides to the TPC in peel (6–22%) was similar to the flesh (4–18%).

3.3. Bioaccessibility of Polyphenols during Simulated Oral Digestion

Bioaccessibility of PP from the flesh was tested at three different digestion conditions. On average 66 ± 16% of the TPC, based

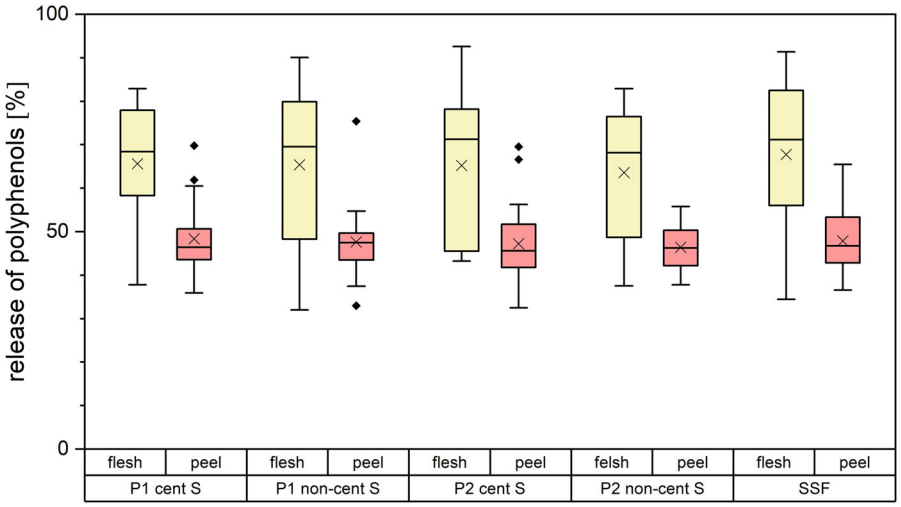

Figure 3. Boxplot of the phenolics [%] released from flesh (yellow) and peel (red) ($n = 24$), quantified in the different saliva fluids (centrifuged [cent S] and non-centrifuged [non-cent S] saliva) from proband 1 and 2 (P) and simulated saliva fluid (SSF).

Table 2. Bioaccessibility of phenolics (%) from flesh and peel.

	Bioaccessibility from flesh [%][a)]	Bioaccessibility from peel [%][a)]
Hydroxycinnamic acid derivatives	77 ± 17	89 ± 10
Dihydrochalcone glycosides	81 ± 12	57 ± 7
Flavanols	56[b)] ± 20	50 ± 8
Flavonol-glycosides		31 ± 5
Anthocyanin-glycoside		73[c)] ± 17

[a)] The rate of release is based on the initial content in the methanolic extraction (TPC). The mean ± standard deviation of all analyzed digestion samples over all varieties is shown ($n = 60$); [b)] The data do not contain values for Berlepsch, since flavanols were only present in traces; [c)] No cyanidin-glycosides were present in the green and yellow skinned varieties Granny Smith and Golden Delicious; therefore, no anthocyanin-glycoside release was determined ($n = 50$).

on the initial content, were released, but significant differences among the varieties were observed ranging from 40% to 80% (Figure 1). ANOVA or Kruscal–Wallis revealed no significant differences among the three different oral digestion fluids (SSF, centrifuged and non-centrifuged saliva), which is further illustrated in **Figure 3**. However, differences in the release depending on phenolic structure were found (Table S1, Supporting Information). Thus, hydroxycinnamic acid derivatives (77 ± 17%) and dihydrochalcone-glycosides (81 ± 12%) were markedly better released than the flavanols (56 ± 20%) (**Table 2**). Compared to the flesh of the other apple varieties a reduced bioaccessibility of PP (60–80%) was determined for the flesh of Bohnapfel (41 ± 4%), Jonagold (40 ± 4%), and Gala (42 ± 3%) (Figure 1). In particular the flavanol bioaccessibility was low with 25–38% in these varieties.

In peel no significant differences among SSF and centrifuged and non-centrifuged saliva were observed either (Figures 2 and 3, Table S2, Supporting Information). The overall release from the peel (48 ± 7%) was significantly lower than from the flesh (66 ± 16%). In particular, flavonol-glycosides were poorly bioaccessible (31 ± 5%) (Table 2). The release of anthocyanin-glycosides was 73 ± 17%. Compared to the flesh the release of dihydrochalcone-glycosides was significantly reduced (57 ± 7%), while the average release of flavanols (50 ± 8%) was similar between flesh and peel. The highest release was observed for the hydroxycinnamic acid derivatives (89 ± 10%), being even higher than the release from the flesh.

4. Discussion

4.1. Differences in Polyphenol Contents and Profiles among Different Apple Varieties

The PP contents in the peel and flesh differed significantly among apple varieties. Generally, phenolic contents were three- to five-times higher in the peel than in the flesh (Table 1), which is in line with literature, reporting a factor of 2–6.[4] The quantified contents of 111–645 mg 100 g^{-1} DW (15–125 mg 100 g^{-1} FW) and 343–1950 mg 100 g^{-1} DW (65-492 mg 100 g^{-1} FW) for flesh and peel of the 12 studied cultivars (Table 1), respectively, were in the same range as reported for the seven other apple varieties in our previous study (112–604 mg 100 g^{-1} DW (flesh) and 378–1224 mg 100 g^{-1} DW (peel)).[28] An outstanding TPC was found for Bohnapfel. Since diverse extraction methods for PP are in use, comparing our TPCs with literature values is limited. However, our data were similar to the results reported by Jakobek et al. ranging from 5 to 129 mg 100 g^{-1} FW and 25–380 mg 100 g^{-1} FW in flesh and peel, respectively.[8,39] In contrast, Kschonsek et al. quantified significant lower values (10–42 mg 100 g^{-1} DW [flesh] and 100–495 mg 100 g^{-1} DW [peel]),[9] while Jakobek et al. reported significant higher TPC contents with 265–686 and 586–1400 mg 100 g^{-1} FW in flesh and peel, respectively.[7] In addition, Wojdyło et al. quantified TPC contents of 523–2724 mg 100 g^{-1} DW but they did not distinguish between flesh and peel.[6] The latter two studies had in common that particular attention was paid to procyanidin quantification by implementing a hydrolysis step to analyze the resulting monomers. Therefore, much higher proportions of flavanols (>80%) were determined in these studies[6,7] compared

to 40–80% and 23–67% in our study for flesh and peel, respectively. It is well documented that an additional hydrolysis step is required for the entire extraction of high molecular weight PCs, since they are not completely extracted from the matrix with water-organic solvents.[40] Hence, we assume that the flavanol contents in the present study might be underestimated.

Compared to our previous study and further literature, the proportions of the different phenolic structures in the flesh were similar (Figure 1).[6,8,28,39] Nonetheless, contents of hydroxycinnamic acid derivative in the flesh of Altländer Pfannkuchenapfel (9%), Ingrid Marie (19%), Granny Smith (16%), Elstar (23%) and Jonagold (21%) were significantly lower than analyzed for the varieties in our previous study (33–83%),[28] but in the range of the data published by Jakobek et al. (2–85%) and Wojdyło et al. (1–31%).[6,39,8] The proportion of flavanols in Altländer Pfannkuchenapfel (80%), Granny Smith (79%), and Ingrid Maria (76%) was higher than the proportion of these compounds (45–60%) in the varieties investigated in the previous study.[28] The proportions in the peel were analogous to literature data and our previous study.[8,28,39] However, some results reported in literature for flavonol-glycoside contents in peel were significantly higher with up to 80%[8] than the highest proportion quantified by us with 38%.

The trend of higher phenolic contents in non-commercial traditional varieties reported in literature was confirmed in this study for most varieties, except for Berlepsch and Ingrid Marie.[7,9] Both varieties are characterized by very low TPCs in the range or even lower than determined for commercial breeds. The phenolic profile of Berlepsch flesh was similar to the flesh of Santana (analyzed in our previous study), containing only traces of flavanols, while all other apples comprised significant amounts of these compounds.[28]

4.2. Bioaccessibility of Polyphenols during Simulated Oral Digestion

In the flesh of most apple varieties 60–80% of the TPCs were released during simulated oral digestion. No difference was obvious between commercial and non-commercial varieties. These data were in line with the results obtained in our previous study.[28] In contrast to the average of the investigated varieties, Bohnapfel (41 ± 4%), Gala (42 ± 3%), and Jonagold (40 ± 4%) were characterized by a significantly reduced bioaccessibility (Figure 1). Surprisingly, the release of PC B2 in Bohnapfel and Gala was limited to only 10% and 25%, while ordinarily at least 50% of the PC B2 was released during oral digestion. The consequence of this reduced release of PC B2 was particularly pronounced in Bohnapfel since PC B2 was by far the most important PP in the methanolic extract of the flesh (111 ± 4 mg 100 g^{-1} DW). Additionally, the release of CA was also reduced to 36–65% in Gala, Bohnapfel, and Jonagold compared to more than 75% determined in the other varieties. For Bohnapfel this might be explained by the extremely high PP content found in the methanolic extraction and a saturation of the "water based" saliva media. However, this explanation is not transferable to Gala and Jonagold since both varieties show low TPCs. In contrast the PP release from Berlepsch flesh was remarkably high. This might be due to the higher ratio of hydroxycinnamic acid derivatives, which are characterized by good bioaccessibilities in all apple samples.

The PP release from the peel (39–65%) was in the same range as our previous data from different apple varieties.[28] Only for anthocyanins a significant difference for the oral bioaccessibility was observed with 73 ± 18% compared to a release of 42 ± 6% in our previous study.[28]

To the best of our knowledge only one other study about the bioaccessibility of PP from apples during the oral (in vitro) digestion is published.[27] Surprisingly, in that study no significant differences in the bioaccessibility of PP from flesh and peel were observed and phlorezin and PCs were released to a significantly lower extend with 27% and 35%, respectively. Only the release of quercetin-rutinoside, with 35% was similar to the values we determined for flavonols. The differences between our results and the results reported by Tenore et al.[27] might be explained by a different SSF composition (Tenore et al.[27]: 89.6 g L^{-1} KCl, 20.0 g L^{-1} KSCN; 88.8 g L^{-1} NaH_2PO_4, 57.0 g L^{-1} Na_2SO_4, 175.3 g L^{-1} NaCl, 84.7 g L^{-1} $NaHCO_3$, and 25.0 g L^{-1} urea). Furthermore, the high degree of milling of our samples might also have a positive impact on the PP release, because for raw carrots a positive effect on the bioaccessibility of β-carrotin with decreasing particle size was reported.[41] A particle size between 2–4 mm (40%) and 4–6 mm (20%) for raw carrots after chewing was determined in this study. Therefore, if a similar size distribution is assumed for apples, the milled samples were too small to represent the "real" particle size distribution after chewing an apple, which would yield in an overestimation of the bioaccessible phenolics. Hence, our results underline the importance of chewing an apple well to increase the bioaccessibility of PP in the oral phase. Furthermore, an increased consumption of apples rich in PP is encouraged, since health promoting effects for these substances have been reported.[2,3] With few exceptions, traditional varieties exhibited higher TPCs than commercial breeds, while the bioaccessibility was the same. Therefore, it is assumed that, if traditional varieties rich in PP are consumed, the uptake of the nutritional valuable substances is amplified.

Analogous to our previous data, we confirmed that SSF is well suited to replace centrifuged saliva in bioaccessibility experiments. However, it was surprising that the phenolic profiles and contents were similar for centrifuged and non-centrifuged saliva. A β-glycosidic activity provoking the degradation of PP-glycosides has been described for bacteria and epithelial cells in non-centrifuged saliva, but high inter-person variations in the activity are known.[30,31] In our samples prepared with non-centrifuged saliva no aglycon formation was detected. These observations indicated that the impact of bacteria and epithelial cells was negligible, probably due to the short incubation time of only 2 min and a high sample dilution prior to analysis.

4.3. Impact of Polyphenols on the Allergenicity of Different Apple Varieties

An effect of PP on the allergenicity of apples is proposed.[14,19] To test this hypothesis, the TPCs of the flesh, determined after methanolic extraction and release during the oral digestion phase were plotted against the Mal d 1 contents published previously

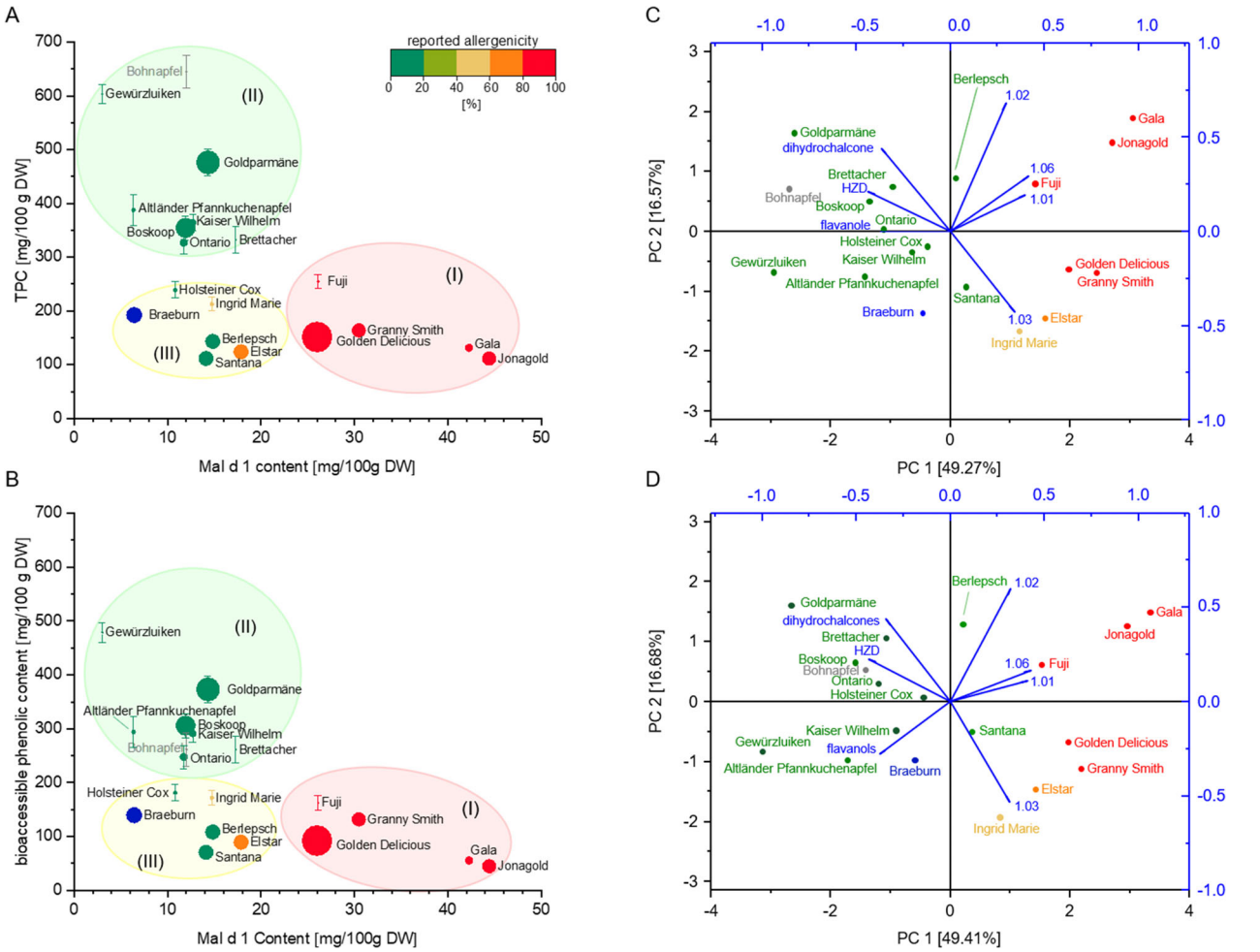

Figure 4. Correlation between total phenolic contents (TPC; A) or released phenolics during oral digestion (B) and Mal d 1 contents in the flesh. Additionally, biplots of PCAs, using the content of the isoallergen specific Mal d 1 markers and the contents of the different phenolic subclasses in the methanolic extract (C) or bioaccessable phenolics (D) in flesh as variables. All data were determined either in this study or published previously.[28,32] The allergenic potential (A–D) is based on the consumer survey by the BUND Lemgo.[14] No data are available for Bohnapfel (grey) and Braeburn (blue) is marked as inconclusive, since clinical studies report contradicting results to the consumer survey.[16,17] Size of the data points indicate sample size in the survey ranging from 6 to 160. HZD, hydroxycinnamic acid derivatives.

(**Figure 4**).[32] Data of the methanolic extraction and release of the 12 apples analyzed in this study and the seven apples from a previous one were used.[32] The pooled, freeze-dried apple samples used for PP quantification and release studies were the same as for the Mal d 1 quantification. Data about the allergenicity were taken from a consumer survey (data from 2021) initiated by the BUND Lemgo, where people suffering from a mild apple allergy have been encouraged to report their tolerance levels to different apple varieties online.[14] This survey is not supervised scientifically and does not provide clinical data on the effect level. However, scientifically supervised surveys and clinical studies covering a wide range of traditional and commercial apple varieties are missing so far. An additional weakness of the survey is the disparity in the reported entries between the different varieties ranging from >100 to <5. These data are therefore only a helpful tool to estimate the allergenicity of apples.

Correlating the Mal d 1 contents and TPCs resulted in the formation of three groups (I–III) (Figure 4A). In the first group (I) varieties with high Mal d 1 contents and low TPCs were assembled, covering only commercial varieties. Considering the information about the allergenicity, these apple varieties were all reported as highly allergenic.[14] The second group (II) contained traditional apple varieties, rich in phenolics and low in Mal d 1 contents. For these varieties a low allergenic potential has been observed by consumers. The third group (III), characterized by low TPCs and low Mal d 1 contents, included medium allergenic commercial breeds like Elstar, traditional varieties with a medium allergenic potential such as Ingrid Marie and varieties with a low allergenic potential such as Santana, Holsteiner Cox, and Berlepsch. The consumer survey identified Braeburn to be highly allergenic, however this is in contrast to clinical studies[16,17] and personal reports of allergen sufferers. The grouping was not altered by using data from the release during oral digestion (Figure 4B). Considering only specific phenolic subgroups (initial and bioaccessible content) no correlation was obvious, either (Figure S4, Supporting Information). Analogous

to the flesh no correlation was evident in the peel, neither if TPCs or bioaccessible phenolics (Figure S5, Supporting Information) nor specific phenolic subgroups were considered (Figure S6, Supporting Information).

In general, most commercial breeds, tended to be highly allergenic and exhibited a high Mal d 1 and a low PP content. On the other side most of the traditional varieties showed high phenolic and low Mal d 1 contents and were usually well tolerated. This might lead to the interpretation that apples rich in PP, mostly non-commercial varieties, are less allergenic. But is this reduced allergenic potential due to the high level of PP or due to the low Mal d 1 content? Performing PCA revealed predominantly a separation between most commercial and traditional varieties (Figure 4C,D). Exceptions were the commercial varieties Santana and Braeburn, which were clustered together with the traditional varieties, while the varieties Elstar and Ingrid Marie were not allocated to any of both groups. The loadings revealed opposing impacts of the Mal d 1 content and TPC (Figure 4C) or bioaccessible PP (Figure 4D) on the first principle component (PC 1). Taking the allergenic potential reported by the BUND Lemgo[14] under consideration, it is obvious that the groups formed by PCA analyses are based on the allergenicity. The commercial variety Santana, known for its hypoallergenic potential,[17] is grouped with the traditional varieties with low allergenic potential, while the commercial variety Elstar and the traditional variety Ingrid Marie, both characterized by medium allergenic potentials, are grouped separately.[14] Despite the contradicting results for the allergenicity in literature,[14,16,17] the PCA supports a classification for Braeburn as a rather low allergenic variety.

A PCA performed solely with the isoallergen specific Mal d 1 contents[32] (Figure S7, Supporting Information) revealed an improved separation according to the allergenic potential and a more cohesive clustering, particularly in regards to the non-allergic varieties, with Braeburn clustering within this group. Therefore, we assume that the unoxidized phenolics during apple consumption in the oral digestion phase do not have a relevant impact on the allergenic potential of an apple variety and that the allergenicity is mainly influenced by the total Mal d 1 content and the isoallergen profile. Further investigations into polyphenol oxidase activity is recommended, since interactions between quinones and proteins have been described, which might affect the allergenic potential.[23,24,42]

5. Conclusion

In conclusion, the observed trend of polyphenol-rich varieties having lower Mal d 1 contents might be a coincidence. The phenolic contents and Mal d 1 contents may be inversely related due to apple physiology. Ancestral relations might also be relevant, since it is conspicuous, that the highly allergenic Golden Delicious, which contains low phenolic but high Mal d 1 contents is a common ancestor of many commercial breeds, e.g. of Elstar, Gala, and Jonagold (Table S3, Supporting Information).[34] Kiewning et al. even recommended to avoid varieties with Golden Delicious in the parentage if aiming to breed a well-tolerated variety.[43]

In summery we demonstrated that the phenolic compounds in apples are similar in all varieties, while the profiles vary markedly. In particular, traditional varieties often tend to have higher TPCs than commercial ones, making their consumption highly recommended, due to the proposed health-promoting effects of these compounds. Release and therefore bioaccessibility during oral digestion differed between PP structures and tissues and was higher for flesh than for peel. Furthermore, we could verify that SSF is a good replacement for centrifuged and non-centrifuged saliva in oral digestion experiments, allowing to work with a standardized sample fluid avoiding ethical and hygienically considerations. The absence of β-glycosidic activity in the non-centrifuged saliva was surprising but might be explained by the short incubation time of 2 min.

Correlating the data of the PP content with data published previously about the Mal d 1 content[32] and the proposed allergenic potential,[14] did not reveal a major impact of the PP on the variety specific allergenic potential of apples. However, polyphenol oxidase activity might influence the formation of reactive quinones, which interact with proteins and further phenolics. This might modify the allergenic potential and therefore, requires further investigation.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors are grateful to A. Siegele (Obstbauberatung Stuttgart, Liegenschaftsamt) for donating the apples. S. Richter (Department of Technical Biochemistry, University Stuttgart) is acknowledged for freeze drying of the apple samples. This research was funded by the German Research Foundation (DFG, grant number 3811/1-1),by the Ministry of Science, Research and the Arts Baden-Württemberg (M.B., M. v. Wrangell program), by the Dr. Leni Schöninger Foundation and by the funds of the chemical industry, Germany (FCI).

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.B. and J.K.; sample preparation: F.M. and R.S.; data evaluation: J.K., F.M., R.S., and M.R.B.; writing and editing of the original draft: J.K. and M.B.; reviewing F.M. and R.S.; and acquisition of funding M.B. All authors have read and agreed to the publication of the manuscript.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

Keywords

apple allergy, human saliva, matrix release, phenolics, simulated saliva fluid

Received: January 31, 2023
Revised: May 30, 2023
Published online: September 19, 2023

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