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Research Article

Bioavailability and Bioactivity of Characteristic Phenolics from Apple Products

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Abstract

In order to test matrix effects on the g.i. absorption and bioavailability of Polyphenolics from apple processing products a single oral dose of raw apples, apple juice and freeze dried juice extract of the same cultivar and harvest season adjusted to equivalent antioxidant activity (TEAC test)were administered in a human study to 12 probands in cross over design. In regular intervals the concentration pattern of single phenolics as well as antioxidant values were analyzed in plasma and urinary excretion. In apples highest contents of p-coumaroylquinic acid were found whereas in juice and juice extract chlorogenic acid dominated. The plasmatic antioxidant capacity, tested by using the TEAC assay, reached C_{max} values 1 hour after intake and declined within the following 4 to 5hours. No significant differences existed in the plasmatic TEAC values or in the phenolics content between the apple products. In contrast to plasma, the excretion of phenolics after apple juice extract exceeded the values after apples and juice intake when related to equivalent antioxidant doses, with p-coumaroylquinic acid and dihydrochalcones as most dominating single phenolics. The excretion pattern ranges from 8 % up to 36 % suggesting an impaired absorption of polyphones due to matrix effects mostly evident in the pulp rich apples

Introduction

The health protective potential of plant phenolics is generally accepted. The natural composition of polyphenolics within fruits and fruit products is to be considered as an optimal combination of synergistically active antioxidants with health protective properties. [1-4]. Bioavailability and bioactivity as well as metabolism of polyphenolic subgroups are, however, matter of intensive studies since years. Extensive data on absorption and metabolism wiredrawn for flavonoids such as quercetin, anthocyanins and the catechism [5-11]. For quercetin, an active absorption mechanism could be revealed with partial participation of the sodium dependent glucose co transporter SGLT1 and the intestinal lactase-phlorrhidzin hydrolase as well. This may also be apply to anthocyanins [7,8,11]. The uptake of phenol carbonic acids from the intestine uses likewise a sodium dependent mechanism, as could be shown at least in animal experiments [12-14].

The antioxidative potential is the predominant and verifiable effect of polyphenolics and is suitable as biomarker for testing their bioactivity in vitro and in vivo [15]. Nevertheless, the bioavailability and-activity of plant phenolics was up to now predominantly tested with single substances, isolated from plant material [5-7,10]. Sparse information exists to date on the interference with matrix effects of the special plant food. Relevant differences may arise when fruits and vegetables were processed to juices, concerning the transfer as well as the availability of these phenolics for man. Persons with impaired digestibility, as e.g. the growing group of elderly persons, will prefer fruit juices in order to comply with the recommendation soft health claims for fruit and vegetable consumption. In order to evaluate those matrix effects we have compared the availability and -activity of phenol carbonic acids as the predominating phenol compounds from raw apples, apple juice and apple juice extract. Apple juice is the most popular fruit juice in Germany and dominates the worldwide consumption with 33 l per capita, followed by the Netherlands and the USA [21].

Materials and methods

Test objects were apples, cultivar “RoterBoskop”, rich in polyphenolics which were processed to juice as well as to juice extract. In a human study with 12 volunteers (10♀, 2♂) a single oral dose of apples, apple juice and juice extract adjusted to equivalent antioxidant activity (TEAC-test) was administered after a 12-hour fasting in cross over design. Study design and anthropometric data are shown in (Figure 1) and (Table 1).

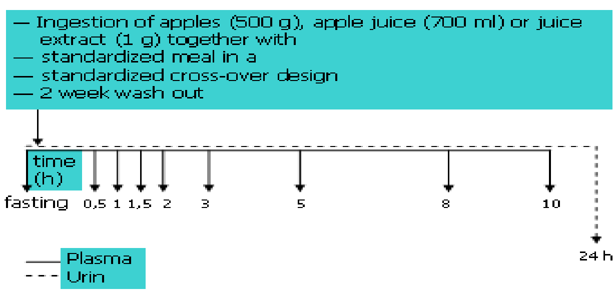

Figure 1: Study design.

♀	n = 10
♂	n = 2
age (years)	range: 21-25 (ø 23,4)
BMI (kg/m²)	17,5-23,8 (ø 20,7)
healthy	n = 12
non-smoker	n = 12

Table1: Anthropometric data of volunteers.

In regular intervals up to 24 hours after intake blood and urinary samples were withdrawn. In plasma samples, withdrawn in regular intervals up to 10 hours after intake, and, additionally, in urine samples collected for 24 hours after ingestion, the antioxidant capacity was assessed by using the TEAC-assay as well as the content of polyphenolics and content and pattern of single phenolics in 24 hours urine excretion according to [16-20] (Table 2).

Test products	dose administered	TEAC-value (mmol/ dose)
apples	500 g	4,93
Apple juice	700 ml	4,71
Apple juice extract	1 g	4,80

Table 2: Antioxidant capacity of apples, apple juice and apple juice extract(mmole trolox equiv./applied dose).

Results

In apples highest contents of p-coumaroylquinic acid were found whereas in juice and juice extract chlorogenic acid dominated (Table 3).

polyphenolics	mg/ dose applied		
	apples	Apple juice	Apple juice extract
Chlorogenic acid	21,50	50,54	93,59
Caffeic acid	31,35	-----	6,71
4-p-coumaroyl quinic acid	107,43	16,04	28,61
phloretin-2-xylosylglucosid	29,95	9,92	18,91
phloridzin	68,27	14,24	32,01

Table 3: Concentration of phenolics in apple products.

The plasmatic antioxidant capacity tested by using the TEAC assay increased initially reaching c_{max} values at about 1 hour after ingestion of the test products and declined slowly within the following 4 to 5 hours. Only apple intake inclined to enhanced but not significant antioxidant AUC-values of the plasma compared to juice and extract when adjusted to ascorbic and uric acid level.

Likewise no differences could be detected in the plasmatic content of polyphenolics after ingestion of the test products, despite in apples highest contents per applied dosage were found, independent of the antioxidant activity (Table 4 and 5).

Test products	AUC (µM × h) TEAC	applied dose (mg GaEq/ dose)	AUC (GaEq/l × h) total phenolics
apples	1040 ± 680	59	666 ± 774
Apple juice	410 ± 790	428	616 ± 832
Apple juice extract	620 ± 720	410	667 ± 758

Table 4: Plasma content of the antioxidant activity (TEAC value) and total phenolics(adjusted to ascorbic and uric acid levels).

Contrasting to plasma, the urinary excretion of total phenolics after ingestion of apple juice extract reached more than twice the excreted content of apples and apple juice. Particularly, p-coumaroylquinic acid and dihydrochalcones dominated in the urinary excretion. Nevertheless, in the antioxidant potential of urinary excretion after apple juice extract intake only a slight but not significant increase was visible by TEAC assay (Table 5).

Testproducts	applied dose(mmoletroloxequiv.)	TEAC activityin 24 h urinaryexcretion
apples	4,93	2,76 ± 1,02
Apple juice	4,71	2,95 ± 1,39
Apple juice extract	4,80	3,51 ± 1,53

Table 5: Antioxidant activity (TEAC) in urinary excretion (mmoleTrolox equiv./24h, adjusted to ascorbic and uric acid excretion).

Discussion

National and international boards recommend regular daily consumption of fruits and vegetables, culminating in the „Five – a - day"campaign, corresponding to the postulated health claims for fruits and vegetables [21]. Bioavailability of antioxidants from food matrices is a precondition for their potential bioactivity in vivo. The bioavailability of orally administered plant phenolics, however, is limited as was estimated from several human studies finding only low plasma levels [5,6,8-10]. It is, however, also conceivable that the low plasmatic concentration of phenolics might be founded by inadequate analytical methods of detection, as was suggested by some authors [7]. Matter of discussion is furthermore the extent of metabolism inside the body and the proportion of metabolites being urinary excreted. Besides the orally given doses may, on the other hand, several matrix components affect the bioavailability and in the following the potential biological efficacy. Lotito and Frey postulated that an increase of the antioxidant potential in plasma after apple consume could be the result of the enhanced uric acid being metabolically formed from the fructose content of the apples [22]. In order to eliminate possible interferences with physiological antioxidants the antioxidant values in plasma and urine war adjusted to ascorbic and uric acid levels. The plasmatic antioxidant activity tested by TEAC assay increased initially after ingestion of the apple processing products enhancing so the bioactivity of apple polyphenolics (not shown). Even though some trends were visible, no significant differences could be detected in the plasmatic antioxidant capacity or in the polyphenolics content of plasma after ingestion of the apple products (Table 4). Obviously, the half life time of unchanged plant phenolics in the plasma is rather short and those will rapidly be removed via urinary excretion, because no differences could be detected neither in the AUC of tested phenolics in plasma nor in its antioxidant potential by TEAC. Eight hours after intake phenolics from plasma had been eliminated and couldn´t be detected.

The urinary excretion of unchanged flavonoles (quercetin e.g.) and flavanoles (catechin group) ranged from 1 % up to 36 % the given dose, as was previously found by several authors [5-7,10]. This is in accordance to our results. The polyphenols excretion of the apple products ranged from 8 % (apples) up to 36 % (extract) the administered dosage (Table 3 and 6). Such an excretion pattern of polyphenolics from apple processing products suggests impaired polyphenols absorption and following the excretion which is mostly evident in the pulp rich apples and least effective in the matrix free extract. The urinary excretion of single phenolics reveals p-coumaroylquinic acid as the main compound in all tested products, followed by caffeic acid in apples and apple juice (Table 6).

Phenolics	mg/24 h		
	Apples	Applejuice	Applejuiceextract
Chlorogenic acid	0,93 ± 0,60	1,10 ± 0,88	1,46 ±0,75
Caffeic acid	2,99 ± 0,95	2,29 ± 0,72	3,72 ± 1,61
Vanillic acid	0,98 ± 0,25	1,25 ± 0,65	0,63 ± 0,78
4-p-coumaroyl quinic acid	13,98 ± 5,20	13,15 ± 5,01	28,63 ± 14,29
phloretin glucuronid	2,12 ± 0,36	1,97± 1,03	18,20 ± 4,42

Table 6: 24 h urinary excretion of single polyphenolics after intake of apple, apple juice and apple juice extract.

Coumaroylquinic acid seems to be either well bioavailable or poorly metabolized. Caffeic acid, though not detectable in the juice, may be generated from chlorogenic acid hydrolysis during processing or intestinally. Both acids were found in minor contents in juice and extract (Table 3). It is conceivable that these compounds could be more rapidly decomposed by polyphenoloxidases because of their more special affinity to hydroxycinnamic acids as described by Guyot et al. [23]. The obviously enhanced availability of dihydrochalcones from the extract compared to apples and juice may point out to specific matrix effects. In apples, phloretin derivatives are tightly bound on cellular components. These are scarcely soluble in water and so the transfer into the juice is low. Only in the matrix free extract these are much better absorbable.

Conclusion

This small study demonstrates that polyphenolic subgroups such as hydroxycinnamic acids from apple species are best available from processing products such as juice and freeze-dried juice extract free of cellular components. After intestinal absorption hydroxycinnamic acids were rapidly removed from plasma via urinary excretion, revealing 4-p-coumaroylquinic acid and caffeic acid as most prominent acids excreted.

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