Letter to the Editor

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Protocatechuic acid: The missing human cyanidins' metabolite

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We read with great interest the special issue on berry fruits. Some articles therein [1–4] included additional evidence to the existing literature, demonstrating that dietary anthocyanins (ACN) have a wide spectrum of potential health benefits against reactive oxygen/nitrogen species, some types of cancer, and cardiovascular and neurodegenerative diseases [5-9]. On the other hand, studies dealing with absorption and metabolism of ACN reported a very low bioavailability of these compounds. In detail, the recovery of known metabolites (glucuronidated and methylated compounds) in urine and plasma was below 1%, whereas 60–90% of the ingested ACN disappeared [10]. Despite this striking evidence, limited efforts have been made to explain this discrepancy. Therefore, the absorption-metabolism process of ACNs resembles a movie: we see the initial scenes (rapid gastric/intestinal absorption and formation of known, although quantitatively negligible, urinary metabolites) and then the happy end (biological effects), but the main part of the movie, *i. e.* how ACNs enter the body, distribute to tissues and exert beneficial health effects, remains a black screen.

In order to exert an effect *in vivo,* a dietary compound has to reach tissues, in the native or metabolised form, in a dose sufficient to exert biological effects. On the other hand, if the bioavailability of a dietary compound corresponds to the ingested amount that is absorbed, a molecule that is poorly absorbed would be found undigested in massive quantities and/or biotransformed by gut microflora [10].

To date none of these conditions have been demonstrated for ACN. Besides, together with the low recovery in 24-hour urine, literature studies showed that 69% of the ACN disappeared from the gastro-intestinal tract within 4 hours after food ingestion [10 and references cited therein]. According to some authors [11–14] this disappearance can be partly due to the degradation of ACN aglycons, leading to the formation of the corresponding phenolic acids and aldehydes, or to a massive accumulation in some organs. While this figure has never been demonstrated, some *in vitro* studies demonstrated the poor stability of ACN aglycons [15]. Taken together, these considerations clearly indicate that any conclusion on ACN bioavailability is still premature and that the physiological effects of dietary ACN (*i. e.* the huge increase of plasma antioxidant activity after ACN-rich foods) were likely to be due to as yet unidentified metabolites. In particular, phenolic compounds could be formed *in vivo* following the ingestion of dietary ACN. Nonetheless, albeit sometimes prudently defined as *apparent* [9, 10], the bioavailability of ACNs has been inexorably described as poor.

In a recent intervention study, where six healthy volunteers consumed 1 L of a commercial Sicilian red orange juice (ROJ) containing 71 mg of total cyanidin glucosides (CyG) as major ACN, protocatechuic acid (PCA) was retrieved as the main metabolite of CyG [16]. Consistently with the previous studies in the literature, less than 1% of native, methylated and glucuronidated ACN was found in blood and urine, while, for the first time, PCA was identified as the major CyG metabolite, accounting for about 72% of ingested CyG, as the sum of 44% in serum and 28% in the faeces (no PCA was detected in urine).

In a previous study performed by Kay *et al.* [17], trace amounts of PCA in both urine and serum samples of humans were found. As the source of the PCA could not be determined, the authors excluded it from their report. On the other hand, finding PCA in human faecal samples is completely new in the literature and it demonstrated for the first time the ability of gut microflora to degrade *in vivo* CyG in PCA.

The metabolic fate of CyG has been well defined in rats: Tsuda *et al.* [14] reported the presence of PCA in plasma of rats fed with CyG. They found that the plasma concentration of PCA was 8 times higher than that of CyG, whereas the cyanidin aglycon (Cy) was not found. Moreover, they found Cy and PCA in the intestine, demonstrating *in vitro* that PCA was formed 15 min after the addition of Cy to plasma. More recently, Seeram *et al.* [15] demonstrated that CyG easily converts to PCA and other hydroxybenzoic acids *in vitro* at physiological pH. Furthermore, in a recent

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in vitro study focused on the pH-dependent stability of various ACN, it was reported that, at pH 7.4, CyG almost completely disappeared after 60 min to form different dimerisation products (*via* the quinoid anhydrobase), PCA and an aldehyde (*via* the a-diketone intermediate) [18].

The discovery of PCA as a major metabolite of CyG in humans changes the scenario of ACN bioavailability and provides two main outcomes:

(i) For dietary compounds, such as polyphenols, whose extensive and rapid biotransformation after ingestion has been demonstrated *in vivo*, it is not adequate to predict the health effects by considering their native structures. The abundant literature data from *in vitro* experiments on these dietary compounds have to be reconsidered and future research should pay more attention to biological properties of known metabolites instead of those of native dietary compounds. To date PCA has been poorly investigated due to its low concentration in foods. Nonetheless it deserves great nutritional interest as a human metabolite. Thus, in order to fully explain the mechanism at the basis of ACN-rich food health benefits, future research should pay more attention to this phenolic acid and to all hydroxybenzoic acids due to their formation *in vivo*.

(ii) The health benefits associated in epidemiologic studies with the consumption of ACN-rich foods have been mainly explained by their antioxidant properties (based on *in vitro* studies). This contradicted the supposed low bioavailability of these compounds but, according to the study by Vitaglione and co-workers [16] an explanation has at last been found. PCA has a marked antioxidant activity [15] and its recovery in faecal samples taken the day after the ingestion of ROJ, as well as the reduction of DNA oxidative damage demonstrated *ex vivo* in fasting plasma lymphocytes of subjects following consumption of ROJ for 28 days [19] supports the hypothesis of PCA absorption through the colon. From this standpoint, health benefits associated with ACN-rich foods may be explained by a slow and continuous release of phenolic compounds through the gut into the bloodstream, thus representing a real defence against body oxidative processes.

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