IN VITRO BIOACCESSIBILITY OF AN ANTHOCYANIN RICH STANDARDIZED EXTRACT FROM BILBERRY AND BLACKCURRANT

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Introduction: Anthocyanins are water soluble pigments belonging to the polyphenols, responsible for the red-purple-blue colour of fruits and vegetables. Epidemiological studies have suggested that the consumption of food rich in anthocyanins is associated with a reduced risk of different diseases. In fact, *in vivo* and in vitro studies have proved that anthocyanins have anti-inflammatory, antioxidant, anticarcinogenic properties. Nevertheless, the bioavailability of these substances is reported to be very low, between 0.2and 1.8% of the ingested food. This could be due to different factors, such as the anthocyanins poor chemical stability, metabolism during digestion and interactions with the food matrix. Hence, the aim of this study was to evaluate, through a static in vitro digestion model, the bioaccessibility and bioactivity of an anthocyanin rich standardized extract (ACN) from bilberry and blackcurrant characterized for its protective activity against LPS- and TNF-α-induced damage of gut epithelial cells.

Materials and methods: For bioaccessibility studies, ACN was digested alone or co-digested with whole and skimmed milk (1:8w/w) and potato starch (1:4w/w). For all in vitro digestions, the Infogest protocol was used: ACN was incubated with α-amylase in a neutral environment; the bolus obtained from the oral phase was then incubated with pepsin at pH3and afterward with pancreatin and bile at pH 7. The activity and the bio-accessibility of the digested and undigested samples were evaluated through anthocyanin content evaluation and colorimetric assays (TEAC and Folin Ciocalteu test). To evaluate the ACN anti-inflammatory activity, differentiated intestinal epithelial Caco-2cells were pretreated with ACN (15μg/ml) for 24h and then exposed to LPS (100μg/ml) or TNF-a (30 ng/ml) for 6h. Cell viability was performed by Erythrosin B assay.

Results: Data obtained with Caco-2cells showed TNF-a- and LPS-induced cell death (30% and 50% respectively). ACN pretreatment exerted cell protection restoring cell viability to control levels. Based on preliminary data obtained on the cells, we focused our attention on in vitro ACN bioaccessibility. The results have shown a slight decrease of the total anthocyanin levels after the gastric phase followed by significant decrease after the intestinal phase with a recovery of 14%. Interesting, this diminution was accompanied by an increase of the reducing and antioxidant activity, as suggested by the Folin Ciocalteu and TEAC assays. Furthermore, the co-digestion with milk and/or starch increased gastric bioaccessibility (> 90%), while only milk plus starch co-digestion slightly improved intestinal bioaccessibility (12% increase) of the ACN. Interestingly, all the co-digestions improved the reducing and antioxidant activity of ACN after intestinal phase.

Discussions and conclusions: Our data demonstrated that ACN exerts in vitro intestinal protective effects against proinflammatory stimuli-induced cell damage. Interestingly this effect was demonstrated at concentrations that are physiologically achievable following dietary supplementation, as demonstrated by the bioaccessibility findings. In fact, results demonstrated that anthocyanin stability after gastric digestion, was improved by food matrices. Conversely, the neutral pH of the intestine converted anthocyanins into a variety of metabolites, probably leading to a rapid degradation to their corresponding phenolic acids and aldehydes. However, according to our results, these metabolites have a higher antioxidant activity than parent anthocyanins. Additionally, food matrices, such as milk and starch, improved bioaccessibility and activity of ACN. In conclusion our results suggest that anthocyanins could provide a wide contribution to intestinal health when introduced through the diet or as a food supplement.