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P123

Impact of *in Vitro* Gastrointestinal Digestion on Upcycled Blackcurrant Dried Extract: Anthocyanins Profile and Antioxidant Activity Behaviour

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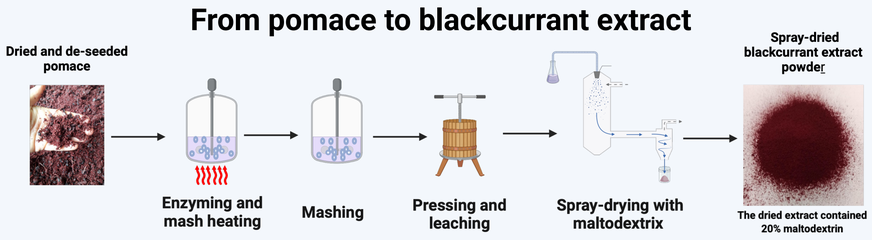
INTRODUCTION

Blackcurrant (*Ribes nigrum L.*) pomace is a **by-product** of the fruit **juice processing** industry that contains a high amount of polyphenols. These **polyphenols have the potential to provide numerous health benefits** such as improved cardiovascular health, reduced risk of diabetes and inflammation, and promotion of intestinal microbiota health. **Blackcurrant is an antioxidant berry that is rich in anthocyanins**, a class of polyphenols that give the fruit its black-purple colour. **For these polyphenols to have a positive effect on health,** they must be bioaccessible, meaning they **must be released from the food matrix during gastrointestinal digestion** (GID) and made available for absorption in the gut[1].

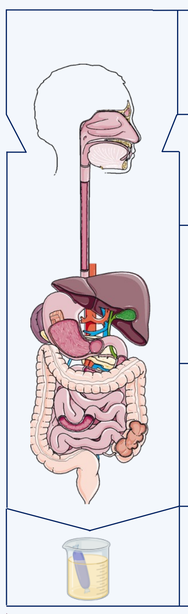
This work aims to raise awareness about the potential use of blackcurrant pomace polyphenols as functional food ingredients that offer health benefits while promoting sustainability in the agri-food processing chain.

METHODS

From pomace to blackcurrant extract



Gastrointestinal tract simulation (INFOGEST 2.0)



In vitro digestion experiment was performed with extract at 5% (w/w) in water

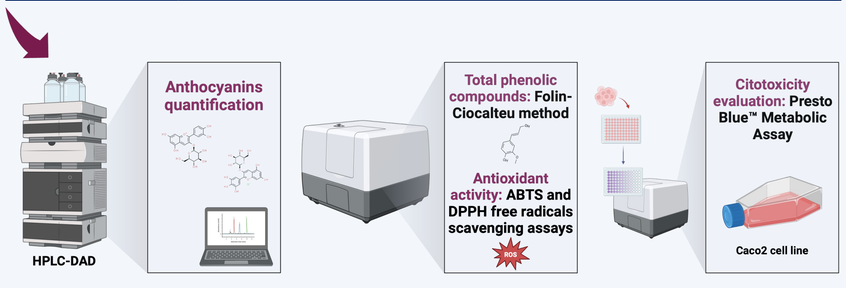
SAMPLE PREPARATION — **5 g of food**: 0.25 g of blackcurrant extract; 4.75 g of distilled water

ORAL PHASE
- Mix food with 4 mL of simulated salivary fluid (SSF) (1:1 (w/w))
- Included 0.025 mL of $CaCl_2$ (1.5 mM in SSF)
- Add salivary 0.632 mL of α-amylase (118.6 U/mg of power)
- Add the remaining water to achieve a 1× concentration of the SSF
- Incubate while mixing (2 min, 37 °C, 200 rpm)
- Store an aliquot of 1.5 mL at - 80 °C

GASTRIC PHASE
- Mix oral bolus with 6.8 mL simulated gastric fluid (SGF) (1:1 (v/v))
- Add 0.00425 mL of $CaCl_2$ (0.15 mM in SSF)
- Add 0.68 mL of lipase and pepsin (15 U/mg of power)
- Verify the pH and adjust to 3.0 if necessary
- Add the remaining water to achieve a 1× concentration of the SGF
- Incubate while mixing (2 h, 37 °C, 200 rpm)
- Store an aliquot of 1.5 mL at - 80 °C

INTESTINAL PHASE
- Mix Food with 4 mL of simulated intestinal fluid (SIF) (1:1 (v/v)
- Add 0.031 mL of $CaCl_2$ (1.5 mM in SSF)
- Add 2.32 mL of bile salts
- Add 3.875 mL of pancreatin (6 U/mg of power)
- Verify the pH and adjust to 7.0 if necessary
- Add the remaining water to achieve a 1× concentration of the SIF
- Incubate while mixing (2 h, 37 °C, 45 rpm)
- Store an aliquot of 3 mL at - 80 °C

DIALYSIS
- After GID, a dialysis process with 3.5 kDa membranes was performed
- Add the digested samples inside the membrane and a water on the outside
- Incubate the dialysis samples (overnight, 37 °C, 50 rpm)
- After incubation, both the membrane content (retentate) and the water (permeate) were collected and stored at - 80 °C



CONCLUSION

This study provides valuable insights into the impact of GID on anthocyanins. Anthocyanins remain relatively stable during the gastric phase, but their recovery decreases during the intestinal phase. The study also demonstrates that anthocyanins are transformed into colourless compounds and metabolites (e.g. phenolic acids) under GID. Therefore, blackcurrant pomace contains bioactive ingredients, particularly phenolic compounds, that are available for absorption. Its findings highlight the potential benefits of the circular economy, including promoting sustainability and reducing waste.

RESULTS

Impact of *in vitro* simulated digestion on blackcurrant extract

The main anthocyanins found in blackcurrant

CHEMICAL NAME *COMMON NAME*
Delphinidin-3-*O*-glucoside *Myrtillin*
Cyanidin-3-glucoside *Asterin/Chrysanthemin*
Cyanidin 3-*O*-b-*D*-glucoside *Kuromanin*
Pelargonidin-3-*O*-glucoside *Callistephin*
Cyanidin-3-rutanocide *Betacyanin*

Table 1. Main anthocyanins in blackcurrant extract were quantified by HPLC-DAD during *in vitro* GID simulation using INFOGEST 2.0.

No.	Compound	Gastrointestinal digestion phase — Concentration (mg/L)			
		Non-digested	Oral	Gastric	Intestinal
1	Delphinidin-3-O-glucoside chloride	163.41 ± 1.43	22.95 ± 2.18	14.67 ± 2.18	B.Q.L
2	Cyanidin-3-O-glucoside	535.70 ± 0.81	79.27 ± 0.54	64.19 ± 7.97	B.Q.L
3	Cyanidin 3-*O*-b-*D*-glucoside	25.57 ± 0.48	2.73 ± 0.23	B.Q.L	B.Q.L
4	Pelargonidin-3-O-glucoside	6.14 ± 1.35	26.39 ± 0.73	19.15 ± 5.13	B.Q.L
	Total Anthocyanins by HPLC	987.45 ± 0.89	262.68 ± 4.43	196.92 ± 27.74	-

B.Q.L: Below quantification limit; ND: non-detected.
All determinations were carried out in triplicate and results are shown as mean value ± standard deviation.

The major anthocyanins in blackcurrant pomace extract were cyanidin-3-O-glucoside (54-59%), delphinidin-3-O-glucoside chloride (16-17%), pelargonidin-3-O-glucoside (2-19%), and cyanidin-3-O-rutinoside chloride (2-4%).

The total anthocyanin content in blackcurrant pomace extract was measured, and the results (>900 mg/L) are according to the reported values for blackcurrant juice from different cultivars, which ranged from 498-1173 mg/L. However, the maltodextrin drying process was insufficient to protect and encapsulate the anthocyanins. As a result, the amount of total anthocyanins decreased throughout the GID phases.

Anthocyanins' profile

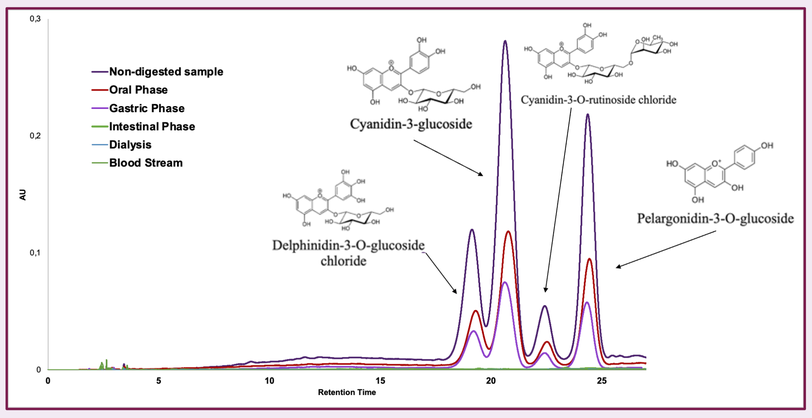


Figure 1. The anthocyanin profile was assessed using HPLC-DAD at 520 nm for the non-digested extract and each GID phase. Results are the means of 3 determinations.

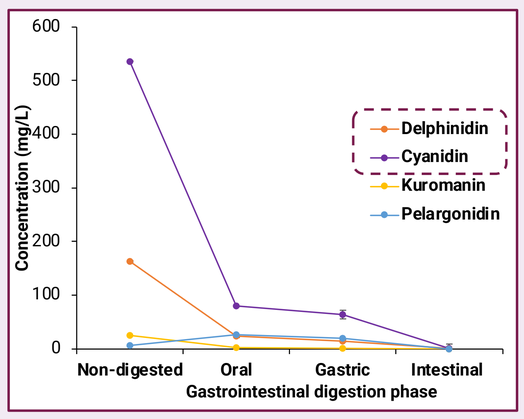


Figure 2. The impact of GID on the main individual anthocyanins in blackcurrant extract using HPLC-DAD. Results are the means of 3 determinations ± standard deviation.

What is the reason for the large decrease in anthocyanins after intestinal digestion?

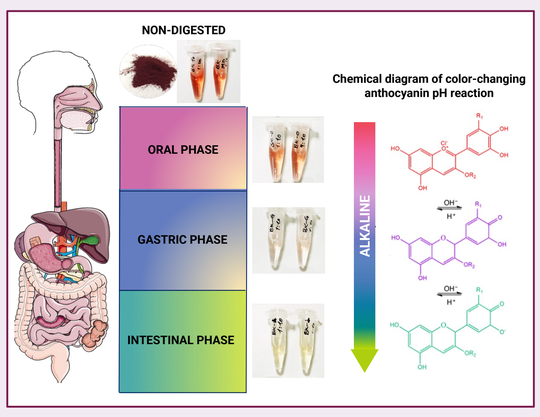


Figure 3. Scheme of GID simulation conditions according to the INFOGEST 2.0 protocol and their impact on colour change on blackcurrant experimental extracts (Dilution 1:10).

Anthocyanins are red pigments that tend to degrade to dark oxidized compounds in basic environments. **This is because the pH shifts from acidic (gastric) to neutral/alkaline (intestinal), causing a break in the anthocyanin B-ring**[2]. However, **cyanidin-3-glucoside and delphinidin-3-O-glucoside chloride have better resistance to these changes**. It is also worth noting that anthocyanins can be absorbed in the glycoside form through the gastric mucosa and even through the mouth. These factors may explain why there is a decrease in anthocyanin content throughout GID.

Bioaccessibility and biocompatible of blackcurrant dried extract

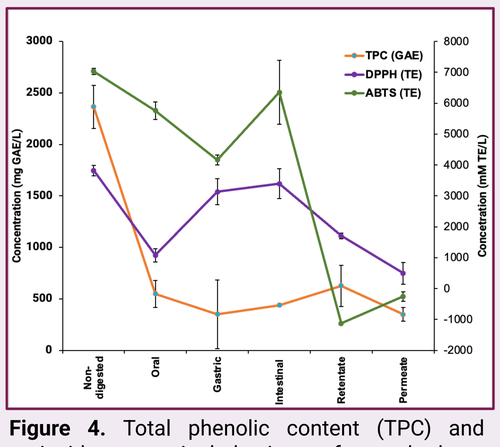


Figure 4. Total phenolic content (TPC) and antioxidant capacity behaviours after each phase of the GID. Results are the means of 3 determinations ± standard deviation GAE: gallic acid equivalents; TE: trolox equivalents.

The extract's TPC decreased during the oral and gastric phases but slightly increased during the intestinal phase, with a 19% recovery index. This is due to the formation of small phenolics resulting from the degradation of anthocyanins[3]. The extract's antioxidant activity decreased, with a 19% and 23% bioaccessibility index for ABTS and DPPH scavenging activities, respectively. Besides, the extract had a cytotoxic effect on Caco-2 cells, with metabolic inhibition exceeding 30%, except at 25 mg/mL.

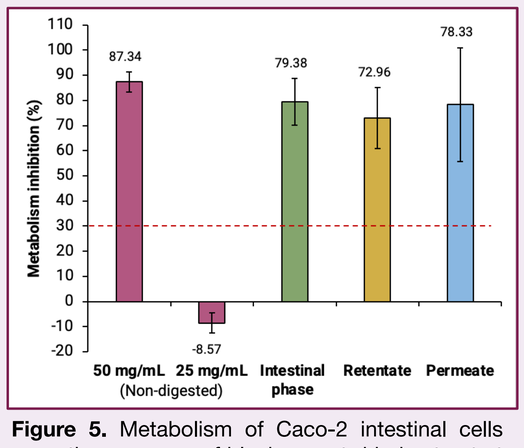


Figure 5. Metabolism of Caco-2 intestinal cells upon the presence of blackcurrant dried extract at concentrations of 25 and 50 mg/mL and after intestinal digestion (n=5). The dashed red horizontal line represents the 30% inhibition limit.

Acknowledgements

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