

## The Bioactivity of byproducts from the blackberry juice industry

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### INTRODUCTION & AIM

Blackberry (*Rubus sp.*) is a red fruit with great potential as a functional ingredient thanks to its composition rich in antioxidants. This work aimed to characterize and study the bioactivity of two byproducts (skins and seeds) from the blackberry juice industry. To achieve this objective, "in vitro" gastrointestinal digestion and colonic fermentation were carried out on the byproducts. Phenolic composition, antioxidant capacity, genotoxicity and effect on the microbiota of the colonic fermentation fractions have been analyzed.



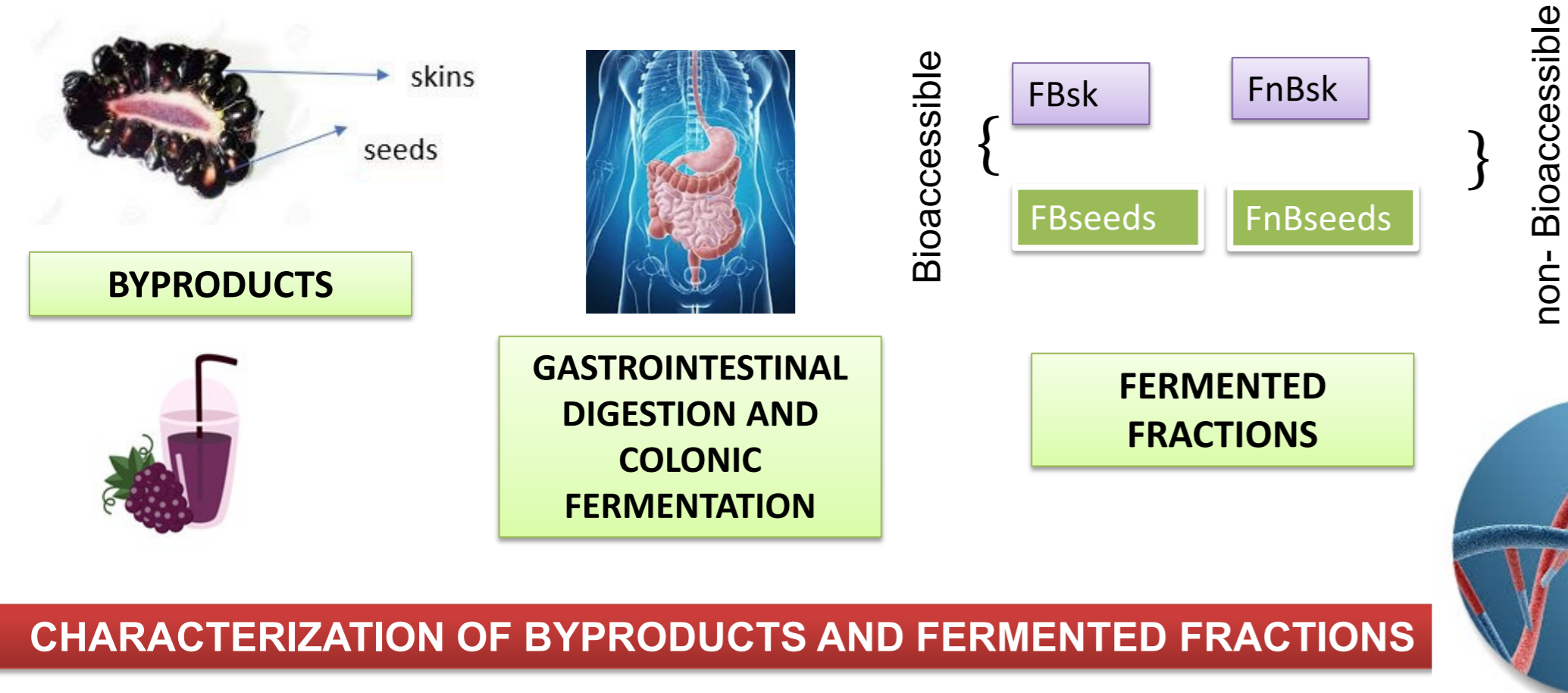
### RESULTS & DISCUSSION

Blackberry by-products contain phenolic compounds that exhibit antioxidant activity. The transformations suffered after gastrointestinal digestion and subsequent fermentation imply an increase in the level of polyphenols and antioxidant capacity, especially in non-bioaccessible fermented fractions (Fig. 1). Non-bioaccessible polyphenols can be high molecular weight compounds such as proanthocyanidins or low molecular weight phenols associated with fiber (Pérez-Jimenez, 2024).

### METHOD

#### SAMPLES

The samples were byproducts generated in the production of blackberry juice (skins and seeds), that were ground and dried. From these products, in vitro gastrointestinal digestion (Minekus *et al.*, 2014) and subsequently colonic fermentation (Pérez-Burillo *et al.*, 2018) were carried out. Obtaining the fermented bioaccessible (FB) and non-bioaccessible (FnB) fractions of skin and seeds.



#### CHARACTERIZATION OF BYPRODUCTS AND FERMENED FRACTIONS

##### Folin-Ciocalteu assay (FC)

Total polyphenol content was determined (Singleton and Rosi, 1965) and the results were expressed as mmol Trolox Equivalent /100 g.

##### Antioxidant capacity (ABTS assay)

ABTS (2,2'-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid) method (Re *et al.*, 1999) was evaluated and the results were expressed as mmol Trolox Equivalent /100 g.

##### GENOTOXICITY

The inhibition of oxidative damage to DNA was evaluated by inducing the generation of hydroxyl radicals (OH<sup>•</sup>) and subsequent agarose gel electrophoresis (Muñiz *et al.*, 1995).

##### MICROBIOTA ANALYSIS

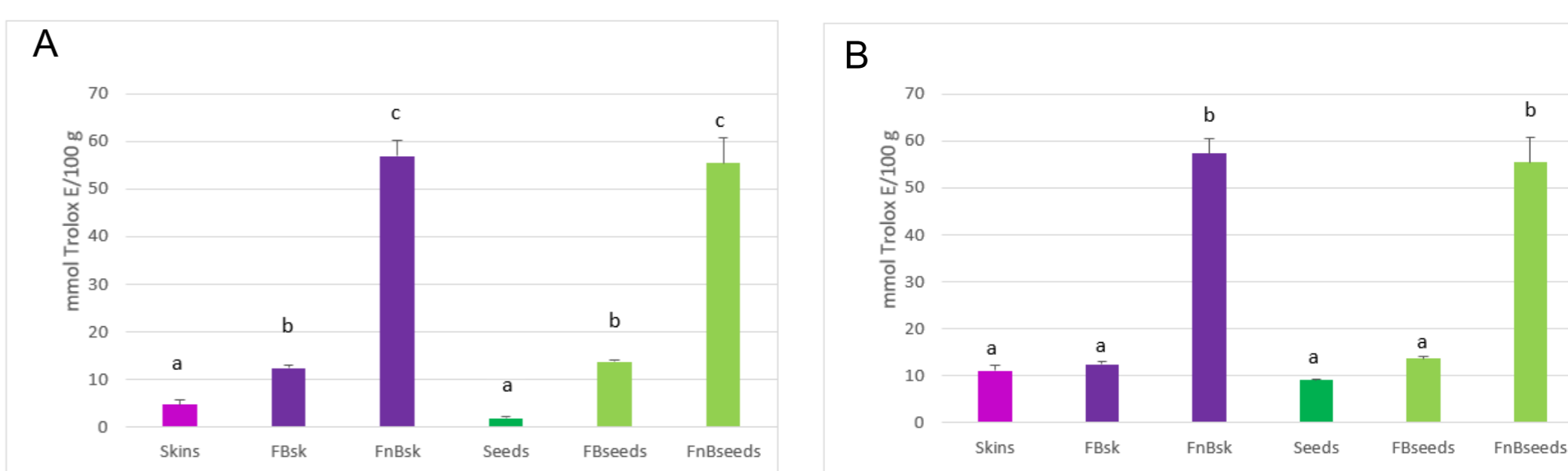
Total DNA was isolated from non-bioaccessible fermented fractions using the QIAamp Mini DNA kit (Qiagen, West Sussex, UK). Eluted DNA was treated with RNase and DNA concentration was measured spectrophotometrically by using a NanoDrop (BioTek, Vermont, USA). Six groups of bacteria were analyzed by PCR and the results were expressed as log copy number per ng of DNA.

### CONCLUSION

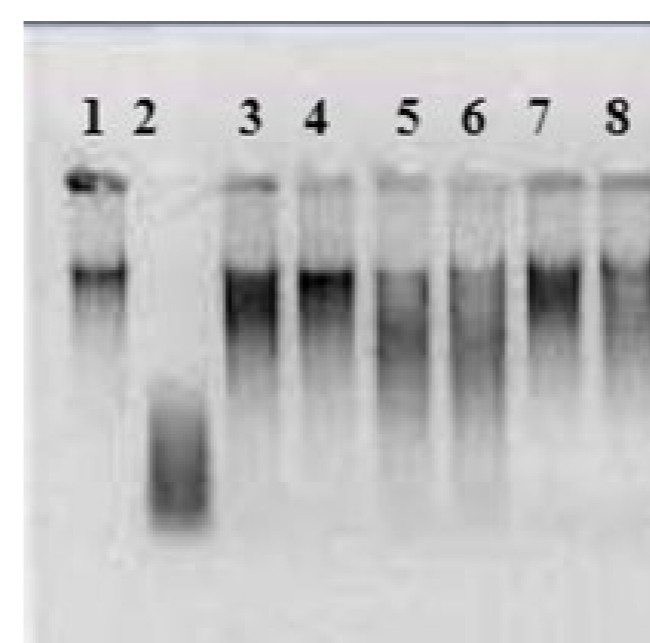
The byproducts of the blackberry industry contain phenolic compounds that exert antioxidant, genoprotective and modulating effects on the intestinal microbiota. These results contribute to giving added value to these byproducts for their potential application as functional ingredients in foods

### REFERENCES

- Muñiz *et al.*, (1995). *Free Radical Bio Med*, 18, 747-755.  
Pérez Burillo *et al.*, (2018). *Food Chem*, 239:1253-1262.  
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Re *et al.*, (1999). *Free Radical Bio Med*, 26,1231-1237.  
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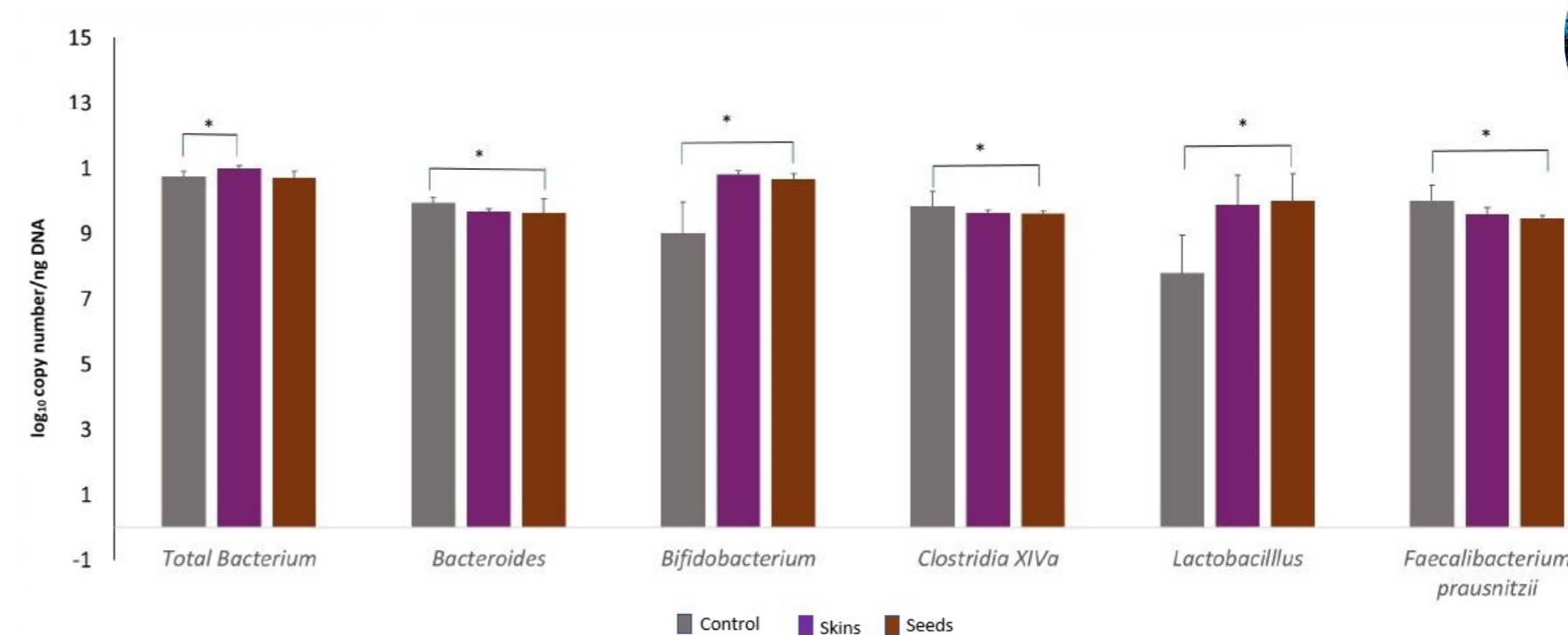
**Figure 1.** (A) Total polyphenols (FC) and (B) antioxidant capacity (ABTS) of skins and seeds byproducts of blackberry juice and their bioaccessible (FB) and non bioaccessible fermented fractions (FnB). Values represents mean (n = 3) ± SD. Differences between samples are indicated by letters for each method.



In lanes 3,4 and 7 the FnB skins show the greatest protection against the oxidative effect (Fig. 2). This greater effect of non-bioaccessible fermented fractions correlates with the greater content of phenolic compounds in these fractions.

**Figure 2.** Genoprotective effect of the non-bioaccessible fermented fractions on DNA damage induced by Cu (II) and ascorbic acid. 1. DNA alone, 2. DNA exposed to Cu (II)-ascorbic acid, 3. and 4. DNA plus Cu (II)-ascorbic acid plus FnB skins, 5. and 6. DNA plus Cu (II)-ascorbic acid plus FB seeds, 7. DNA plus Cu (II)-ascorbic acid plus FnB skins, 8. DNA plus Cu (II)-ascorbic acid plus FnB seeds

Colonic fermentation of blackberry skin and seed samples modulated the gut microbiota (Fig 3), promoting the growth of bacterial *Bifidobacterium* and *Lactobacilli*, which are associated with a beneficial microbiota due to their known anti-inflammatory and immune-regulating effects on the intestinal barrier.



**Figure 3.** Microbiota composition of the fecal content used for the colonic fermentation of the skin and seeds blackberry juice byproducts. \*The asterisks represent significant differences between the samples for each of the groups of bacteria. (t-student p≤0.05), n=3.

### FUTURE WORK

This work is part of a larger study in which the possible effects of different blackberry byproducts on oxidative stress at the cellular level will be analyzed.

#### ACKNOWLEDGMENT

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