Study of olive pomace antioxidant dietary fibre powder throughout gastrointestinal tract as multisource of phenolics, fatty acids and dietary fibre

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ABSTRACT

Pulp-enriched powder (POPP) was obtained from olive pomace solid fraction, a derived from the new value chain established for olive by-products. As a multifunctional powder, POPP retains several bioactive compounds (fatty acids, dietary fibre and phenolics) under potential synergic interaction, even more, reactive throughout the digestion. So, in this study, the potential multifunctionality of POPP was evaluated after the gastrointestinal tract. A significant loss of phenolics occurred during oral digestion (62.48%). However, the potential role of dietary fibre as phenolics' carrier and its possible liberation in the stomach allowed recovering a significant amount of phenolics (77.11%) and a bioaccessibility index of at least 50% (mainly for tyrosol and its glucoside). POPP also provides high content of dietary fibre mainly insoluble fibre (69.68 g/100 g dry weight) linked to a substantial amount of bound phenolics (7.63 mg of gallic acid equivalents/g fibre dry weight), with a positive effect on the fatty acids bioaccessibility [decreased the saturated (5–6%) and facilitated the unsaturated fatty acids bioaccessibility (4–11%)]. PCA analysis became evident the negative effect of simulated gastrointestinal digestion upon POPP as mainly linked to phenolics' loss. Despite all negative effects of the simulated digestion on POPP bioactive composition, phenolics and unsaturated fatty acids showed to be bioaccessible in significant amount, and the amount of bound phenolics associated to fibre retained in the colon have the potential to exert gut health benefits.

1. Introduction

Olive oil industry produces a large volume of by-products annually. Nowadays, the most implemented two-phase system, where no water is added, allowed to reduce the wastewater production and disposal verified in the past three-phase system (Lafka, Lazou, Sinanoglou, & Lazos, 2011). However, it is obtained a semisolid mixture of water, olive pulp, skin and stones, called olive pomace (OP), challenging to treat due to its organic and moisture content (≥65%). Only in the Andalucía, from the 5.8 million tonnes of olives annually processed, more than 4 million tonnes of OP is generated, representing approximately a 65% of the initial weight (AGAPA, 2015).

Severe environmental problems and waste management costs have

been associated with OP (Moreno-Maroto et al., 2019). However, this by-product and its solid fraction have been highlighted by its composition rich in dietary fibre, phenolic compounds and substantial antioxidant capacity (AOX) (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2015; Ribeiro, Oliveira, Costa, et al., 2020). These characteristics seem to be aligned to the concept of antioxidant dietary fibre proposed by Saura-Calixto (1998), i.e. antioxidant dietary fibre should contains over 50% (dry weight, DW) of dietary fibre and high AOX. Antioxidant dietary fibre combines the health and technological benefits of DF and phenolics together (Beres et al., 2016; Silva, Oliveira, Ribeiro, Madureira, & Pintado, 2018). Hence, the search for natural antioxidant dietary fibre sources to the food industry has been emerging and could be an excellent opportunity of moving olive oil industry towards a sustainable

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circular economy model (Campos, Gómez-García, Vilas-Boas, Madureira, & Pintado, 2020; Quirós-Sauceda et al., 2014).

A new promising antioxidant dietary fibre source was obtained from OP solid fraction using a fractionation approach. The pulp-enriched powder (POPP) obtained in our previous study revealed a rich composition in DF (68.14 ± 0.54 g/100 g DW), mainly insoluble dietary fibre (IDF) (52.17 ± 0.01 g/ 100 g DW), but also a significant amount of free phenolic compounds (extractable) and bound phenolic compounds linked to the lipids, proteins and dietary fibre (Ribeiro, Oliveira, Coelho, et al., 2020). POPP also exhibited a high level (about 19 g/ 100 g DW) of unsaturated fatty acids (UFAs), principally in monounsaturated form (>16%), but also in polyunsaturated form (>1%) Oleic acid was the UFA detected in higher amount followed by linoleic acid (Ribeiro, Oliveira, Coelho, et al., 2020). Both these UFAs are known for their beneficial effects on the reduction of cholesterol and triglycerides (Lopez-Huertas, 2010). Regarding POPP bound phenolics, it was estimated that more than half of its total bound phenolics (≈ 54%) were linked to dietary fibre (Ribeiro, Oliveira, Coelho, et al., 2020). In literature, the bound phenolic compounds have been described as significant contributors of the health-related properties attributed to dietary fibre, due to its capacity to pass through the gastrointestinal tract almost intact reaching the colon linked to dietary fibre (González-Sarrías, Espín, & Tomás-Barberán, 2017; Liu, Jia, Chen, Wan, Dong, Nie, Xie, & Yu, 2019). In the colon, they can be liberated exerting potential health antioxidant benefits which have been neglected (Silva et al., 2018).

In our previous work, the complete chemical characterisation of POPP was achieved showing that POPP is an attractive add-value powdered product with the advantage of retaining several functional compounds, namely UFAs, dietary fibre and phenolics (Ribeiro, Oliveira, Coelho, et al., 2020). The retention of these functional compounds together could interact with each other synergically ascribing multifunctional properties to food (García-Lomillo, González-SanJosé, Del Pino-García, Rivero-Pérez, & Muñiz-Rodríguez, 2014; Saura-Calixto, 1998). However, the digestive tract has been described as a releaser of bioactive compounds, causing negative and positive effects in bioactive compounds bioaccessibility. Indeed, the phenolics and fatty acids (FAs) that are released by chemical hydrolysis may differ from those liberated during gastrointestinal tract, i.e. mastication, acidic pH and digestive enzymes can trigger the release of food matrix compounds more efficiently than aqueous-organic solvents (Gouw, Jung, & Zhao, 2017; Jakobek, 2015). However, studies showed also that only a low amount of phenolics reach the intestine, and even a minor portion can pass the gut barrier. Even in the case of dietary fibre, its amount and composition measured by chemical methods could diverge from those reaching the gut (Gouw et al., 2017). Besides that, phenolics, dietary fibre and lipids showed to interact positively and negatively with each other throughout the gastrointestinal tract (Jakobek, 2015).

Dietary fibre can act as carriers of phenolics throughout the gastrointestinal tract, protecting the phenolics from oxidative degradation, but also affecting its bioaccessibility by entrapping bound and free phenolic compounds and restricting the diffusion of digestive enzymes (Bohn, 2014; Jakobek, 2015; Jakobek & Matić, 2019). Several studies showed the role of dietary fibre as uptake enhancers of some phenolics (Schramm et al., 2003; Serra et al., 2010). Nevertheless, even non-released phenolics could have a potential decisive role in gut health, as mentioned above (González-Sarrías et al., 2017). Similarly, lipids-phenolics interactions have been linked with positive effects on the inhibition of lipid oxidation, lipase activity and fat absorption. On the other hand, lipids could improve the stability of phenolics during gastrointestinal tract by their "capture" protecting them (Jakobek, 2015). For example, in a rat model study, tyrosol and hydroxytyrosol absorption from a lipid-rich matrix (olive oil) were higher (≈ 25%) than that from an aqueous solution (Bohn, 2014). However, other works suggested a small impact of lipids on phenolics absorption (Schramm et al., 2003).

As described above, the content and interaction of dietary fibre,

lipids and phenolics throughout the gastrointestinal tract are considered essential factors for the healthiness impact of functional ingredients like POPP. Therefore, the present study intends to assess the impact of *in vitro* simulation of gastrointestinal digestion (SGD) on the POPP composition (antioxidant dietary fibre, soluble sugars, FAs, phenolics) and AOX. The information obtained in this work may help to clarify the potential health benefits of multifunctional ingredients as POPP.

2. Materials and methods

2.1. Chemicals and reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl, D9132), ABTS diammonium salt (2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid, A1888), 2,2′-azo-bis-(2-methylpropionamidine)-dihydrochloride (AAPH, 440914), fluorescein (46955), methanol and potassium persulfate (379824) were purchased from Sigma-Aldrich (Sintra, Portugal). Folin-Ciocalteu's reagent (1090010100) and sodium carbonate (1063920500) were purchased from Merck (Algés, Portugal). Standards of Trolox (238813), gallic acid (398225), p-coumaric acid (C9008) and caffeic acid (C0625) were obtained from Sigma-Aldrich (Sintra, Portugal), whereas hydroxytyrosol (4999 S), tyrosol (4949 S) and luteolin (1125 S) were purchased from Extrasynthese (Lyon, France).

2.2. Preparation of pulp-enriched olive pomace powder

OP was collected from an olive mill from Oliveira do Hospital, Portugal. Homogenous samples were packed in polyethene flasks and kept on a freezer at − 80 °C until use to avoid the phenolics damage.

Pulp-enriched olive pomace powder (POPP) samples were obtained according to previous work, briefly described as follows: OP was centrifuged (10,000g for 10 min), solid fraction was oven-dried (90 °C, water activity < 0.4, 90 min), milled using a coffee grinder and sieved (mesh 40). All the pieces of stones were removed to obtain a potentially food-grade ingredient free of physical hazards such as small stones.

2.3. In vitro digestion

SGD of POPP was performed according to the method described by Costa et al. (2019) with dialyses process, to simulate the intestinal and blood absorption (Ribeiro, Oliveira, Campos et al., 2020). The complete procedure was described in the supplementary material.

2.3.1. Recovery and bioaccessibility index
The results of each extract determination (sample, after mouth, gastric and intestinal digestion) were reported in 100 g of DW of POPP.

Recovery index (RI %) and bioaccessibility index (BI %) were performed to study the effect of digestion on a multifunctional ingredient as POPP evaluating its principal nutritional/ bioactive compounds throughout SGD. The values of all nutritional, bioactive compounds and AOX of POPP before digestion were assumed as 100% (Gullon, Pintado, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2015; Lucas-Gonzalez et al., 2016).

According to the methodology of Lucas-Gonzalez et al. (2016) the recovery index allows determining the amount of a given main components' group of the tested food present in the digested sample after oral, gastric and intestinal digestion, according to:

$$Recovery\ index(\%) = \left(\frac{BC_{DF}}{BC_{TF}}\right) \times 100$$

where BC_{DF} is the bioactive content (mg) in the digested sample, and BC_{TF} is the bioactive content (mg) quantified in the test matrix.

The bioaccessibility index is defined as the percentage of the bioactive compound that is solubilised after intestinal dialysis step. Thus, this index defines the proportion of the bioactive compound that could

become available for absorption into the systematic circulation:

$$Bioaccessibility\ index(\%) = \left(\frac{BC_s}{BC_{DF}} \right) \times 100$$

where BC_S is the bioactive content (mg) in the digested sample after the dialysis step (IN) and BC_{DF} is the total bioactive content (mg) in the digested sample after the dialysis step (IN + OUT).

At this point, it is essential to define the term "bioaccessibility" to avoid the confusion with "bioavailability" or "permeability". Bioaccessibility is described as the amount of a compound that is released from its matrix in the digestive tract, becoming available for bloodstream absorption. Bioavailability expresses the fraction of ingested bioactive compound or nutrient that reaches the systemic circulation and finally utilised. Before becoming bioavailable, bioactives must be released from the food matrix and modified in the gastrointestinal tract. Thus, bioavailability includes the term bioaccessibility (Torres-Palazzolo et al., 2018). In this work, bioaccessibility was evaluated using the *in vitro* SGD, and permeability will be assessed in future using Caco-2 cell cultures.

2.3.2. Dietary fibre composition

The digested sample was filtered using a sintered glass crucible (no. 2). The IDF was retained in the crucible, and the supernatant was saved for soluble dietary fibre (SDF) analysis. The procedure used to determine the total amount of IDF, SDF and its composition was described in the supplementary material.

2.3.3. Bioactive compounds determination and quantification: Sugars and organic acids, fatty acids and phenolic compounds

All bioactive compounds were determined in lyophilised samples. The analysis procedure for each POPP component throughout SGD (sugars, organic acids, FAs, total phenolic compounds (TPC) and individual phenolics) was enlightened in the supplementary material.

2.3.4. Nutritional fatty acids quality indices

Nutritional fatty acids (FAs) quality indices of POPP after and before each step of SGD were analysed from FAs composition data. The indices of thrombogenicity (TI) and atherogenicity (AI) were calculated using Eqs. (3) and (4), respectively. Other nutritional quality indices, namely PUFA/SFA and Saturation Index (SI) (Eq. (5)) were also determined (de Alba, Pérez-Andrés, Harrison, Brunton, Burgess, & Tiwari, 2019).

$$TI = \frac{[C14:0 + C16:0 + C18:0]}{\left[0.5 \times \left(\sum MUFA + \sum n6 \right) + 3 \times \sum n3 + \frac{\sum n3}{\sum n6} \right]}$$

$$AI = \frac{[C12:0 + 4 \times C14:0 + C16:0]}{\left[\sum MUFA + \sum PUFA \right]}$$

$$SI = \frac{[C14:0 + C16:0 + C18:0]}{\left[\sum MUFA + \sum PUFA \right]}$$

2.3.5. Antioxidant capacity: ABTS, DPPH e ORAC

The antioxidant capacity (AOX) of POPP during SGD was achieved according to DPPH (Alexandre et al., 2019), ABTS and ORAC (Costa et al., 2019) methods, using a multidetection plate reader (Synergy H1, Vermont, USA). The radical stock solutions were freshly prepared. Lyophilised samples were dissolved in methanol to obtain a concentration comprised between 20 and 200 mg/mL. All analyses were performed in triplicate and expressed in μM of Trolox-equivalents (TE)/ g DW.

2.4. Statistical analysis

All experiments were carried out in triplicates, and data were reported as mean ± standard deviation. Software R was used to carry out

statistical analyses. The Shapiro - Wilk test was used to test the normality of data distribution and then analysed by one-way analysis of variance (ANOVA). Tukey's post hoc test was applied for comparison of means; differences were considered significant at $p < 0.05$. Correlation analysis (Pearson correlation analysis) and principal component analysis (PCA) were performed to evaluate the potential associations between the bioactive compounds of POPP and its AOX through throughout SGD. Correlations between different parameters were considered significant at $r > 0.95$ ($p < 0.05$).

3. Results and discussion

3.1. Dietary fibre

The amount of dietary fibre, its composition (neutral sugars, uronic acids, TPC and individual phenolics) and AOX measured after SGD are presented in Fig. 1, together with the previous results of dietary fibre profile of POPP obtained using the AOAC (Association of Official Analytical Chemists) method (Ribeiro, Oliveira, Coelho, et al., 2020). The comparison between the results achieved using the AOAC methodology and SGD allowed to understand the possible effects of digestion in POPP dietary fibre content and composition, but also to estimate their potential beneficial effects on human health.

After SGD, POPP maintained its higher content of IDF *versus* SDF. Indeed, the amount quantified after SGD (69.68 ± 0.79 g/100 g DW) was higher than that estimated by AOAC (52.17 ± 0.01 g/100 g DW) and the SDF content decreased after SDG (4.49 ± 0.24 g/100 g DW) in comparison to AOAC results (9.89 ± 0.54 g/100 g DW) as can be seen in Fig. 1. Regarding IDF composition (Fig. 1), some significant differences were detected ($p < 0.05$) after SGD. The content of uronic acids and lignin from SGD were significantly ($p < 0.05$) higher than those obtained by the AOAC method, and lower for neutral sugars (mainly to xylose). The higher amount of lignin could be explained by the complex macromolecular structure of lignin and consequent resistance to digestive enzymes action (Gouw et al., 2017). The lower content of neutral sugars (mainly xylose, but also glucose) was probably related with the release of a higher level of compounds by chemical and mechanical reactions occurred during SGD (Grundy et al., 2016).

The higher amount of lignin reported could be an advantage in terms of gut health benefits. Lignified fibres have been described as potent *in vitro* source of antioxidants and adsorbers of hydrophobic carcinogens in the whole intestine (Mudgil, 2017; Sato et al., 2011). Lignin, and more generally IDF, have been claimed as carriers of phenolics throughout the gastrointestinal tract. The principal bound phenolic compound associated with IDF and SDF after AOAC and SGD were identified by LC-ESI-UHR-QqTOF-MS ***(Supplementary Material) and quantified by HPLC (Fig. 1).

IDF revealed a significantly higher content of total bound phenolic compounds after SGD ($p < 0.05$), but also a higher amount of the individual phenolics caffeic, *p*-coumaric acid and luteolin which appeared to be related to IDF higher amount of lignin detected after SGD (Fig. 1). Hydroxycinnamic acid derivatives like p-coumaric and caffeic acid are commonly found linked to cell-wall cellulose and lignin through ester bonds (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014; Calvache, Cueto, Farroni, de Escalada Pla, & Gerschenson, 2016).

Between the bound phenolics associated with IDF, hydroxytyrosol seemed to be the only bound phenolic negatively modified by SGD. Hydroxytyrosol linked to IDF exhibited a significantly lower amount after SGD compared to AOAC data ($p < 0.05$), which could explain the lower AOX reported by ABTS and DPPH results (Fig. 1). The amount of hydroxytyrosol and electron transfer methods showed to be strongly correlated [hydroxytyrosol/ABTS ($r^2 = 0.80$) and hydroxytyrosol/DPPH ($r^2 = 0.70$)]. On the other hand, the ORAC values of IDF were not negatively influenced by SGD. Overall, the AOX results of IDF from POPP revealed that a substantial bound phenolic compounds amount could hypothetically reach the colon intact and further be liberated to
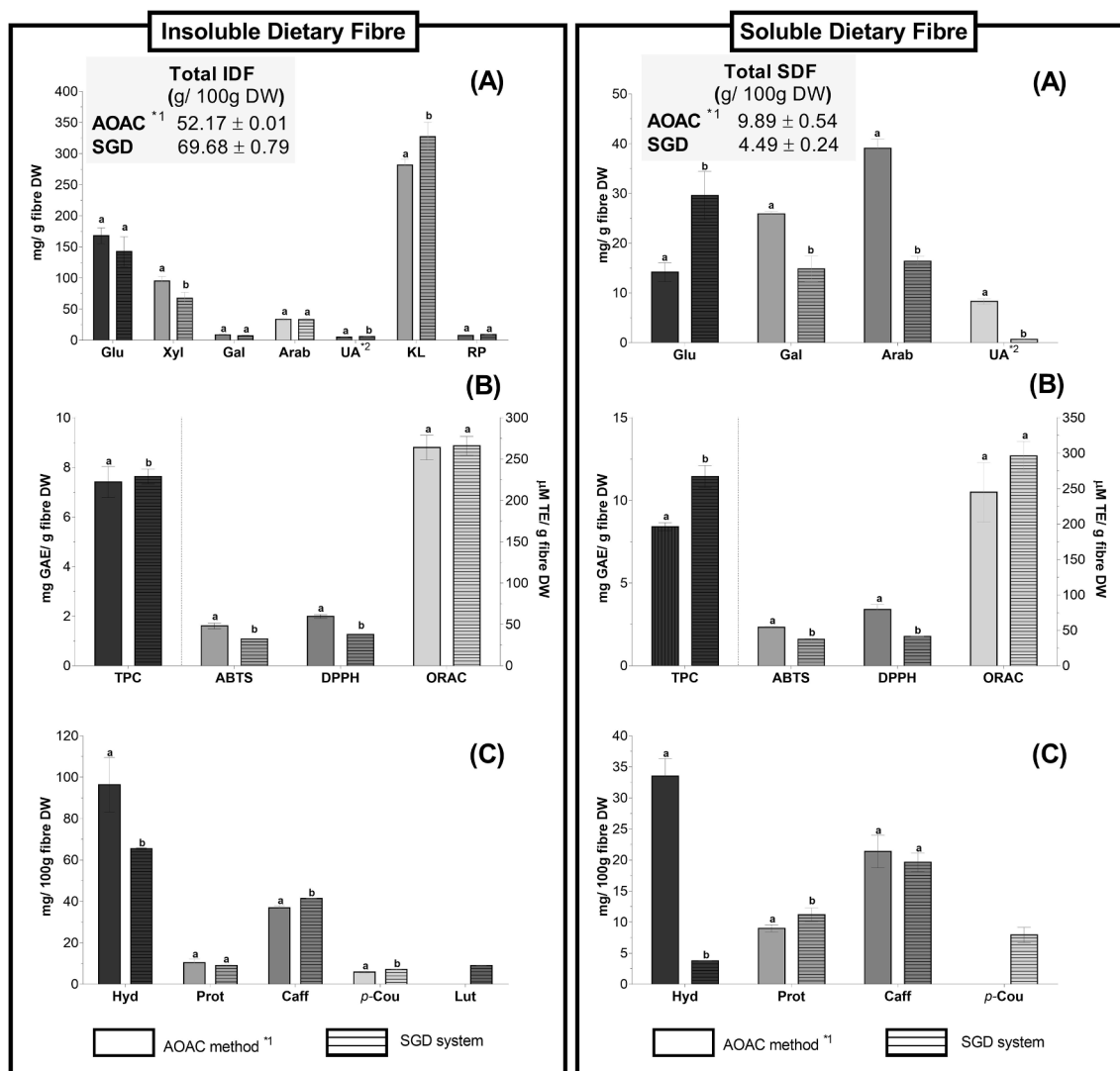
Fig. 1. Dietary fibre composition of POPP using modified AOAC dietary fibre analysis method and simulated gastrointestinal system. (A) Determination (g/ 100 g DW) and profile (mg/g fibre DW) of dietary fibre; (B) Total phenolic compounds (TPC) expressed as mg GAE/ g fibre DW and antioxidant capacity using ABTS, DPPH and ORAC methods of phenolics associated to fibre fraction (μM TE/ g DW); (C) Concentration of main individual phenolics (mg/ 100 g DW) associated to fibre fraction and *DW – dry weight; Glu – Glucose; Xyl – Xylose; Gal – Galactose; Arab – Arabinose; UA – Uronic acids; KL – Klason lignin; RP – Resistant protein; IDF – insoluble dietary fibre; SDF – Soluble dietary fibre; GAE - gallic acid equivalents; TE – Trolox equivalents; Hyd - Hydroxytyrosol; Prot - Protocathecuic acid; Caff - Caffeic acid; p-Cou - p-Coumaric; Lut - Luteolin. [1] Data from previous paper Ribeiro, Oliveira, Coelho, et al. (2020); [2] mg galacturonic acid equivalents (GUAE)/ g fibre DW. Results are the means of three determinations ± standard deviation. Different letters in the same column are significantly different, as determined by ANOVA (p < 0.05).*

exert their potential gut health benefits.

The profile of SDF was negatively changed by SGD (Fig. 1), principally the neutral sugars. All neutral sugars were significantly lower ($p < 0.05$) after SGD than those determined by the AOAC method; only glucose was significantly higher ($p < 0.05$). Total phenolic compounds (TPC) and the individual phenolics protocatechuic and p-coumaric acid amounts were also significantly higher after SGD. The higher concentrations of glucose and phenolics as p-coumaric acid after SGD system might be related as reported in previous studies of p-coumaric acid extraction (Jiang, Li, Long, & Ding, 2016). Equally, the higher concentration of protocatechuic acid could be associated with the lower detection of hydroxytyrosol, i.e. the higher release of hydroxytyrosol from fibre after SGD system could explain a possible consecutive decarboxylation (α-oxidation dihydroxylation) into protocatechuic acid (López de las Hazas et al., 2016). Concerning the AOX of bound phenolic compounds present in SDF, the values were higher using the AOAC method than after the SGD system. The ORAC was the only AOX methodology that not detected negative changes in AOX of bound

phenolics linked to SDF after SGD. The higher TPC detected for bound phenolic compounds after SGD could be related to higher ORAC values estimated after SGD. ORAC was strongly correlated with TPC ($r^2 = 0.86$) than ABTS and DPPH ($r^2 = 0.76$).

3.2. Bioactive compounds through in vitro simulation of the gastrointestinal tract

3.2.1. Soluble sugars and organic acids

The soluble sugars and organic acids RI and concentration throughout SGD are presented in Tables 1 and 2, respectively.

After SGD, the soluble sugars amount changed significantly ($p < 0.05$). Fructose was the sugar most affected (Table 2). In the mouth, a higher release of fructose was verified (RI = 106.32 ± 8.96%), that is maintained until the intestine (RI = 104.14 ± 3.46%) and increased during the simulated intestinal absorption (dialysis) as shown in Table 1. RI higher than 100% was registered. A liberation of compounds from POPP by the action of pH and digestive enzymes; and chemical

Table 1
Recovery index (RI %) and bioaccessibility index (%) of POPP bioactive compounds/ antioxidant capacity throughout simulated gastrointestinal digestion (SGD).

	Recovery index (%)					Bioaccessibility index (%)
	Oral	Gastric	Intestinal	IN (absorbable)	OUT (non-absorbable)	
Sugars & Organic acids						
Glucose	69.02 ± 4.40 [a]	70.07 ± 0.47 [a]	75.08 ± 2.58 [a]	62.6 ± 7.36 [a]	19.55 ± 1.99[b]	75.94 ± 3.99 [ab]
Fructose	106.32 ± 8.96 [ab]	95.37 ± 1.00 [ab]	104.14 ± 3.46 [ab]	121.90 ± 22.31 [a]	62.94 ± 6.94[b]	63.25 ± 6.58[b]
Mannitol	66.83 ± 4.37 [a]	56.05 ± 0.61 [a]	65.01 ± 2.31 [a]	61.17 ± 8.58 [a]	24.77 ± 2.19[b]	70.83 ± 4.71 [ab]
Formic acid	94.01 ± 2.99 [a]	78.64 ± 4.40 [a]	101.20 ± 16.58 [a]	83.12 ± 17.10 [a]	16.11 ± 0.58[b]	83.22 ± 3.24 [a]
SFA						
C14:0	nd	nd	nd	nd	nd	0.00 ± 0.00 [d]
C16:0	20.80 ± 0.41[b]	22.75 ± 0.22 [ab]	23.81 ± 2.33 [ab]	1.38 ± 0.26[c]	27.39 ± 1.20 [a]	5.06 ± 0.76 [bc]
C18:0	21.92 ± 0.44[b]	24.00 ± 0.46[b]	26.68 ± 2.50 [ab]	1.82 ± 0.19[c]	30.73 ± 1.09 [a]	5.76 ± 0.49 [bc]
C20:0	22.27 ± 0.74[b]	23.98 ± 0.54[b]	24.77 ± 2.51 [ab]	0.00 ± 0.00[c]	31.56 ± 4.04 [a]	0.00 ± 0.00 [d]
MUFA						
C16:1 c9	20.99 ± 0.35 [a]	23.17 ± 0.46 [a]	22.85 ± 2.21 [a]	1.30 ± 0.24[b]	27.11 ± 2.08 [a]	4.83 ± 0.53[c]
C16:1 t9	22.86 ± 0.41 [a]	24.67 ± 0.78 [a]	23.90 ± 3.08 [a]	0.00 ± 0.00[b]	28.64 ± 2.24 [a]	0.00 ± 0.00 [d]
C17:1 c10	nd	nd	nd	nd	nd	0.00 ± 0.00 [d]
C18:1 c9	20.83 ± 0.39 [a]	23.29 ± 0.39 [a]	23.12 ± 2.22 [a]	1.29 ± 0.16[c]	9.93 ± 0.49[b]	11.89 ± 0.90 [a]
C18:1 c11	20.57 ± 0.60[b]	23.52 ± 0.51[b]	23.39 ± 2.34[b]	0.99 ± 0.19[b]	385.34 ± 48.14 [a]	0.27 ± 0.04 [d]
C20:1 c9	23.81 ± 0.49 [a]	25.21 ± 0.47 [a]	21.76 ± 2.16 [a]	0.00 ± 0.00[b]	27.38 ± 2.44 [a]	0.00 ± 0.00 [d]
PUFA						
C18:2 c9c12	20.99 ± 0.36 [a]	23.50 ± 0.36 [a]	23.31 ± 2.22 [a]	2.00 ± 0.31[b]	27.61 ± 2.27 [a]	7.06 ± 0.56[b]
α C18:3 c9c12c15	30.98 ± 0.54 [a]	34.96 ± 0.44 [a]	33.99 ± 2.86 [a]	1.56 ± 0.35[b]	39.98 ± 3.21 [a]	4.00 ± 0.53[c]
Phenolic compounds						
TPC	37.52 ± 3.85[c]	77.11 ± 1.40 [a]	46.08 ± 1.80[b]	14.48 ± 2.71 [d]	14.12 ± 2.21 [d]	51.39 ± 3.34 [d]
Hydroxytyrosol glucoside	27.32 ± 4.13[c]	60.81 ± 2.77 [a]	49.29 ± 2.86[b]	33.63 ± 3.49[c]	26.74 ± 1.75[c]	54.93 ± 1.74 [cd]
Hydroxytyrosol	17.88 ± 2.90[b]	58.33 ± 4.83 [a]	54.27 ± 9.93 [a]	4.14 ± 0.80[c]	4.74 ± 0.85[c]	45.80 ± 3.54 [de]
Tyrosol glucoside	24.52 ± 4.55[b]	22.68 ± 4.51[b]	26.73 ± 4.22[b]	48.81 ± 1.27 [a]	18.97 ± 3.44[b]	69.36 ± 3.22[b]
Tyrosol	49.90 ± 1.32 [a]	53.74 ± 8.92 [a]	42.07 ± 2.30 [ab]	45.39 ± 6.36 [a]	28.02 ± 3.92[b]	63.06 ± 7.92 [bc]
Caffeic acid	42.92 ± 7.26 [a]	39.78 ± 3.18 [ab]	31.98 ± 2.27 [bc]	24.27 ± 2.97[c]	3.42 ± 0.56 [d]	87.68 ± 0.73 [a]
p-coumaric acid	29.02 ± 2.56 [bc]	31.41 ± 3.91[b]	55.43 ± 6.35 [a]	12.55 ± 2.22 [d]	22.45 ± 1.61 [cd]	35.20 ± 3.40 [e]
Luteolin	14.09 ± 0.50 [a]	16.85 ± 2.45 [a]	16.37 ± 1.39 [a]	0.00 ± 0.00[c]	10.80 ± 0.87[b]	0.00 ± 0.00[f]
Antioxidant capacity						
ABTS	52.90 ± 9.42[b]	92.84 ± 7.55 [a]	37.12 ± 5.09[c]	35.61 ± 6.95[c]	47.59 ± 5.37 [bc]	43.00 ± 8.99 [a]
DPPH	21.36 ± 8.82[c]	41.46 ± 1.58[b]	43.82 ± 2.27[b]	65.40 ± 3.03 [a]	5.98 ± 0.42 [d]	91.63 ± 0.17[b]
ORAC	70.22 ± 8.61[b]	104.14 ± 10.40 [a]	65.61 ± 3.16[b]	10.16 ± 1.31 [e]	40.73 ± 3.12[c]	24.93 ± 4.20 [d]

Nd- non-detected; SFA - Saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids. C14:0 – Myristic acid; C16:0 – Palmitic acid; C18:0 – Stearic acid; C20:0 – Arachidic acid; C16:1 c9 – Palmitoleic acid; C16:1 t9 - trans-palmitoleic acid; C17:1 c10 - cis-10-heptadecenoic acid; C18:1 c9 – Oleic acid; C18:1 c11 - cis-Vaccenic acid; C20:1 c9 - cis-Eicosanoid acid; C18:2 c9c12 – Linoleic acid; α C18:3 c9c12c15 - α-linolenic acid. *Results are the means of three determinations ± standard deviation. Values with different letters in the same line to RI (%) and the same column to BI (%) are significantly different, as determined by one-way ANOVA test (p < 0.05), respectively.*

modifications in these compounds, such as sugar isomerisation, could have taken place, thus justifying this RI value.

Glucose and mannitol exhibited similar behaviour through SGD (Table 1). In the mouth, the RI of these soluble sugars decreased (glucose: RI = 69.02 ± 4.40%; mannitol: RI = 66.83 ± 4.37%) and increased significantly after simulated intestinal absorption (dialysis) (*p* < 0.05). As mentioned above, a higher release of carbohydrates throughout SGD was observed in the dietary fibre profile, which possibly influenced positively the recovery of the soluble sugar detected. This higher soluble sugar release was especially evident during the simulated intestinal absorption, perhaps a consequence of the long incubation time (more 2 h) which means that reactions (proteolytic, lipolytic, amylolytic, etc.) between intestinal digestive enzymes used and dietary fibre, as well its contact with water, are much more lasting and thorough (Singh, Berg, Hardacre, & Boland, 2014; Zhou, Theil, Wu, & Knudsen, 2018) Other reactions of isomerisation reactions of glucose into fructose and mannose, and subsequent hydrogenation into mannitol under alkaline conditions (pH of the intestine), could eventually explain the higher amount of fructose and mannitol (Makkee, Kieboom, & van Bekkum, 1985).

The formic acid concentration in POPP also increased considerably after SGD (Table 2), possibly because of the oxidation of FAs that could occur in each step of the SGD, for example, studies reported that acidic pH of stomach enhanced lipid oxidation (Kanner & Lapidot, 2001; Van

Hecke, Van Camp, & De Smet, 2017). A similar increase of formic acid was detected through the storage of the olive oil and also throughout *in vitro* SGD of the liquid-enriched fraction powder from OP of our previous work (Ribeiro, Oliveira, Campos et al., 2020).

Regarding bioaccessibility (Table 1), the BI of soluble sugars and organic acids after SGD were all higher than 60% [Formic acid (83.22 ± 3.24%) > Glucose (75.94 ± 3.99%) > Mannitol (70.83 ± 4.71%) > Fructose (63.25 ± 6.58%)]. All the soluble sugars and formic acid were detected at higher amounts in the absorbable fraction (IN) than in the non-absorbable fraction (OUT) (Table 2). Nevertheless, the higher amount of mannitol (2.47 ± 0.35 g/100 g DW) in the IN fraction in comparison to glucose (2.21 ± 0.26 g/100 g DW) and fructose (0.62 ± 0.11 g/100 g DW) could be related to potential glycaemic control activity of mannitol (Chukwuma, Matsabisa, Erukainure, Ibeji, & Islam, 2019).

3.2.2. Fatty acids

The effect of SGD on the total FA profile of POPP is expressed in Table 2. Significant variations in FAs profile occurred throughout SGD (*p* < 0.05). Fat digestion occurs mainly in the intestine where about 80% of the lipolysis reaction takes place, however oral and gastric digestion could have a preeminent action on the facilitation of lipid intestinal digestion (Ye, Li, Cao, Xu, Cao, Li, & Liu, 2019). This action was noticed by the changes in the FAs profile in mouth and stomach phases. The

Table 2

Soluble sugars/organic acids, fatty acid and polyphenols concentration obtained after the simulated gastrointestinal digestion (SGD) of POPP.

Bioactive component	Initial	Oral			Gastric			Intestinal			IN	OUT		
		SF	PF	Total	SF	PF	Total	SF	PF	Total	Total	SF	PF	Total
Sugars & Organic acids (g/ 100 g DW)														
Glucose	3.53 ± 0.28 [a]			2.44 ± 0.16[b]			2.47 ± 0.02[b]			2.65 ± 0.09[b]	2.21 ± 0.2[b]			0.69 ± 0.07[c]
Fructose	0.41 ± 0.06 [ab]			0.54 ± 0.05 [a]			0.49 ± 0.01 [ab]			0.53 ± 0.02 [a]	0.62 ± 0.11 [a]			0.35 ± 0.04[c]
Mannitol	4.03 ± 0.49 [a]			2.69 ± 0.18[b]			2.26 ± 0.02[b]			2.62 ± 0.09[b]	2.47 ± 0.35[b]			1.00 ± 0.09[c]
Formic acid	0.47 ± 0.02 [a]			0.42 ± 0.01 [a]			0.35 ± 0.02 [a]			0.46 ± 0.07 [a]	0.37 ± 0.08 [a]			0.07 ± 0.00[c]
Saturated fatty acids (mg/ g DW)														
C14:0	nd	0.07 ± 0.01 [ab]	0.01 ± 0.00 [de]	0.08 ± 0.00 [a]	0.04 ± 0.01 [cd]	0.01 ± 0.00 [de]	0.05 ± 0.00 [bc]	0.02 ± 0.01 [de]	0.01 ± 0.00 [de]	0.03 ± 0.00 [cde]	0.00 ± 0.00 [e]	0.03 ± 0.00 [cde]	0.01 ± 0.00 [de]	0.03 ± 0.00 [cd]
C16:0	33.69 ± 0.39 [a]	0.39 ± 0.08 [g]	6.62 ± 0.09 [cd]	7.01 ± 0.14[c]	0.23 ± 0.05 [g]	7.74 ± 0.03 [c]	7.67 ± 0.09 [bc]	2.89 ± 0.14[f]	5.13 ± 0.65 [de]	8.02 ± 0.79 [bc]	0.46 ± 0.09 [g]	5.26 ± 0.54 [de]	3.97 ± 0.18 [ef]	9.23 ± 0.40[b]
C18:0	4.98 ± 0.07 [a]	0.07 ± 0.01 [h]	1.03 ± 0.02 [de]	1.10 ± 0.02 [cde]	0.03 ± 0.00 [h]	1.15 ± 0.01 [cd]	1.18 ± 0.01 [cd]	0.49 ± 0.02 [g]	0.84 ± 0.10 [ef]	1.33 ± 0.12 [bc]	0.09 ± 0.01 [h]	0.90 ± 0.07 [e]	0.64 ± 0.03 [fg]	1.54 ± 0.05[b]
C20:0	1.10 ± 0.03 [a]	0.02 ± 0.01[f]	0.23 ± 0.00 [cd]	0.24 ± 0.01 [cd]	0.01 ± 0.00[f]	0.26 ± 0.00 [cd]	0.26 ± 0.01 [cd]	0.10 ± 0.01 [ef]	0.18 ± 0.02 [de]	0.27 ± 0.03[c]	nd	0.26 ± 0.04 [cd]	0.13 ± 0.00 [e]	0.39 ± 0.02[b]
Total	38.67 ± 0.46 [a]			8.19 ± 0.15[c]			8.89 ± 0.08 [bc]			9.38 ± 0.91 [bc]	0.55 ± 0.10 [d]			10.80 ± 0.46[b]
Monounsaturated fatty acids (mg/ g DW)														
C16:1 c9	3.75 ± 0.04 [a]	0.06 ± 0.01 [cde]	0.74 ± 0.01 [h]	0.80 ± 0.01 [cd]	0.03 ± 0.00 [bcd]	0.84 ± 0.00 [h]	0.88 ± 0.02 [bc]	0.32 ± 0.01 [g]	0.55 ± 0.07 [ef]	0.87 ± 0.08 [bc]	0.05 ± 0.01 [h]	0.66 ± 0.08 [de]	0.37 ± 0.02 [fg]	1.03 ± 0.08[b]
C16:1 t9	0.21 ± 0.01 [a]	0.01 ± 0.00 [gh]	0.04 ± 0.00 [cd]	0.05 ± 0.00 [bc]	0.00 ± 0.00 [gh]	0.05 ± 0.00	0.05 ± 0.00 [bc]	0.02 ± 0.00 [fg]	0.03 ± 0.00 [de]	0.05 ± 0.01 [bc]	0.00 ± 0.00 [h]	0.04 ± 0.00 [cd]	0.02 ± 0.00 [ef]	0.06 ± 0.00[b]
C17:1 c10	nd	0.01 ± 0.00 [g]	0.13 ± 0.00 [bcd]	0.13 ± 0.00 [bcd]	0.00 ± 0.00 [g]	0.14 ± 0.00 [abc]	0.15 ± 0.00 [abc]	0.05 ± 0.00[f]	0.10 ± 0.01 [de]	0.15 ± 0.02 [ab]	0.00 ± 0.00 [g]	0.11 ± 0.01 [cd]	0.06 ± 0.00 [ef]	0.18 ± 0.01 [a]
C18:1 c9	160.58 ± 1.91 [a]	1.83 ± 0.40 [g]	31.62 ± 0.31[bc]	33.45 ± 0.62[b]	0.92 ± 0.09 [g]	36.12 ± 0.14[b]	37.40 ± 0.63[b]	13.67 ± 0.61 [ef]	23.47 ± 2.95 [cd]	37.14 ± 3.56[b]	2.07 ± 0.25 [fg]	nd	15.95 ± 0.78 [de]	15.95 ± 0.78 [de]
C18:1 c11	7.82 ± 0.06[b]	0.09 ± 0.02[c]	1.52 ± 0.03 [bc]	1.60 ± 0.05 [bc]	0.06 ± 0.03[c]	1.77 ± 0.01 [bc]	1.83 ± 0.04 [bc]	0.66 ± 0.03[c]	1.16 ± 0.15[c]	1.82 ± 0.18 [bc]	0.08 ± 0.01[c]	29.26 ± 3.76 [a]	0.79 ± 0.04[c]	30.06 ± 3.76 [a]
C20:1 c9	0.75 ± 0.01 [a]	0.03 ± 0.00 [ghi]	0.14 ± 0.00 [cde]	0.18 ± 0.00 [bc]	0.02 ± 0.00 [hi]	0.16 ± 0.00 [bcd]	0.19 ± 0.00[b]	0.06 ± 0.00 [gh]	0.11 ± 0.01 [ef]	0.16 ± 0.02 [bcd]	0.00 ± 0.00 [i]	0.13 ± 0.02 [de]	0.07 ± 0.00 [fg]	0.21 ± 0.02[b]
Total	173.24 ± 2.01 [a]			36.21 ± 0.67[b]			40.50 ± 0.70[b]			40.18 ± 3.87[b]	2.20 ± 0.27[c]			47.48 ± 3.82[b]
Polyunsaturated fatty acids (mg/ g DW)														
C18:2 c9c12	14.73 ± 0.17 [a]	0.16 ± 0.03 [h]	2.93 ± 0.03 [cde]	3.09 ± 0.05 [cd]	0.08 ± 0.01 [h]	3.35 ± 0.01 [bcd]	3.46 ± 0.05 [bc]	1.27 ± 0.06 [g]	2.16 ± 0.27 [ef]	3.43 ± 0.33 [bcd]	0.29 ± 0.05 [h]	2.62 ± 0.34 [de]	1.45 ± 0.08 [fg]	4.07 ± 0.33[b]
α C18:3 c9c12c15	1.17 ± 0.03 [a]	0.02 ± 0.00 [h]	0.34 ± 0.00 [cde]	0.36 ± 0.01 [cd]	0.01 ± 0.00 [h]	0.40 ± 0.00 [bc]	0.41 ± 0.01 [bc]	0.15 ± 0.01 [g]	0.25 ± 0.03 [ef]	0.40 ± 0.03 [bc]	0.02 ± 0.00 [h]	0.30 ± 0.03 [de]	0.17 ± 0.01 [fg]	0.47 ± 0.04[b]
Total	16.47 ± 0.19 [a]			3.45 ± 0.06[b]			3.87 ± 0.06[b]			3.83 ± 0.36[b]	0.31 ± 0.05[c]			4.53 ± 0.37[b]
Polyphenolic compounds (mg/ 100 g DW)														
Hydroxytyrosol glucoside	9.44 ± 1.28 [a]	2.15 ± 0.42 [e]	0.41 ± 0.05[f]	2.61 ± 0.40 [e]	5.48 ± 0.26 [bc]	0.26 ± 0.01[f]	5.74 ± 0.26[b]	4.30 ± 0.24 [cd]	0.33 ± 0.04[f]	4.65 ± 0.27 [bc]	3.17 ± 0.33 [de]	2.37 ± 0.15 [e]	0.14 ± 0.03[f]	2.49 ± 0.16 [e]
Hydroxytyrosol	14.73 ± 0.94 [a]	2.64 ± 0.39 [d]	0.10 ± 0.01 [e]	2.63 ± 0.43 [d]	7.90 ± 0.81[b]	0.46 ± 0.09 [e]	8.59 ± 0.71[b]	5.24 ± 0.26[c]	0.07 ± 0.01 [e]	5.39 ± 0.41[c]	0.61 ± 0.12 [e]	0.70 ± 0.13 [e]	0.02 ± 0.00 [e]	0.70 ± 0.13[e]
Tyrosol glucoside	19.42 ± 2.50 [a]	4.37 ± 0.91[c]	0.40 ± 0.02 [d]	4.76 ± 0.88[c]	4.40 ± 0.88[c]	0.00 ± 0.00	4.40 ± 0.88[c]	4.64 ± 0.89[c]	0.55 ± 0.09	5.19 ± 0.82[c]	8.75 ± 1.82[b]	3.60 ± 0.67[c]	0.21 ± 0.03 [d]	3.81 ± 0.69[c]
Tyrosol														

(*continued on next page*)

Table 2 (*continued*)

Bioactive component	Initial	Oral			Gastric			Intestinal			IN	OUT		
		SF	PF	Total	SF	PF	Total	SF	PF	Total	Total	SF	PF	Total
	20.11 ± 1.59 [a]	8.16 ± 1.22 [c]	0.77 ± 0.16 [f]	8.84 ± 1.30 [c]	12.37 ± 2.25 [b]	1.15 ± 0.00 [def]	13.39 ± 2.19 [b]	7.69 ± 0.51 [c]	0.77 ± 0.21 [f]	8.46 ± 0.46 [c]	9.32 ± 1.70 [c]	4.42 ± 0.85 [de]	0.94 ± 0.00 [ef]	4.73 ± 0.61 [d]
Caffeic acid	3.03 ± 0.35 [a]	1.23 ± 0.23 [bc]	0.07 ± 0.01 [g]	1.30 ± 0.22 [b]	0.79 ± 0.06 [d]	0.41 ± 0.06 [ef]	1.21 ± 0.10 [bc]	0.90 ± 0.07 [cd]	0.07 ± 0.01 [g]	0.97 ± 0.07 [bcd]	0.74 ± 0.09 [de]	0.10 ± 0.02 [fg]	0.00 ± 0.00 [g]	0.10 ± 0.02 [fg]
p-coumaric acid	4.04 ± 0.55 [a]	1.01 ± 0.09 [cde]	0.15 ± 0.02 [g]	1.17 ± 0.10 [cd]	0.79 ± 0.13 [def]	0.48 ± 0.05 [fg]	1.27 ± 0.16 [c]	2.12 ± 0.26 [b]	0.11 ± 0.09 [g]	2.22 ± 0.26 [b]	0.51 ± 0.09 [efg]	0.88 ± 0.06 [cdef]	0.02 ± 0.00 [g]	0.89 ± 0.06 [cdef]
Luteolin	15.34 ± 1.00 [a]	0.17 ± 0.02 [d]	1.97 ± 0.11 [bc]	2.07 ± 0.16 [bc]	0.19 ± 0.04 [d]	2.52 ± 0.27 [b]	2.60 ± 0.26 [b]	0.32 ± 0.05 [d]	2.19 ± 0.25 [bc]	2.51 ± 0.21 [b]	0.00 ± 000	0.00 ± 0.00	1.66 ± 0.25 [c]	1.66 ± 0.25 [c]

Results are the means of three determinations ± standard deviation. Values with different letters are significantly different, as determined by one-way ANOVA test ($p < 0.05$). PF – pellet fraction; SF - soluble fraction; DW – dry weight. C14:0 – Myristic acid; C16:0 – Palmitic acid; C18:0 – Stearic acid; C20:0 – Arachidic acid; C16:1 c9 – Palmitoleic acid; C16:1 t9 - trans-palmitoleic acid; C17:1 c10 - cis-10-heptadecenoic acid; C18:1 c9 – Oleic acid; C18:1 c11 - cis-Vaccenic acid; C20:1 c9 - cis-Eicosanoid acid; C18:2 c9c12 – Linoleic acid; α C18:3 c9c12c15 - α-linolenic acid.

negative effect of mastication in the mouth and acidic pH of the stomach, reported before to other fat-rich foods (Kanner & Lapidot, 2001; Van Hecke et al., 2017), appears to have a strong degradation effect on lipid fraction (RI between 20 and 40%). Another factor that could explain the extensive loss of FAs was the presence of oxygen during all steps of SGD system (Tullberg, Vegarud, & Undeland, 2019). The use of N_2 gas at the start of each digestion step of fat-rich foods should be applied to reduce fat oxidation.

The SGD negative effect was noticed in the same degree for all FAs (Table 1). Generally, saturated fatty acids (SFAs) were more stable than polyunsaturated fatty acids (PUFAs), but the presence of phenolics on POPP could explain the better stability of PUFAs detected (Jakobek, 2015).

The MUFA oleic acid was identified as the main FA of POPP, being also the most predominant in all digestion phases (Table 2) and the most bioaccessible (BI = 11.89 ± 0.90%) (Table 1). As verified by other *in vitro* studies, the most abundantly released FAs were also the most abundant (Ye et al., 2019). After oleic acid, PUFAs showed higher BI (Table 1). Linoleic (C18:2 *c9c12*) and α-linolenic (α C18:3 *c9c12c15*) exhibit BI values of 7.06 ± 0.56 and 4.00 ± 0.53, respectively. The degree of saturation seems had a relevant impact on the FAs digestion since it was noticed a higher BI of UFAs compared to SFAs (Table 1). The rich dietary fibre composition of POPP could justify the lower SFAs bioaccessibility (palmitic and stearic) due to the oil holding capacity of dietary fibre to retain SFAs, influencing their bioaccessibility. This possible slow down effect of dietary fibre on SFAs bioaccessibility was reported previously in a study with fortified wheat bread (Kurek, Wyrwisz, Karp, & Wierzbicka, 2018). The retention of FAs by dietary fibre was also supported to the detection of the higher amount of FAs (higher proportion of SFAs than UFAs) in the pellet (PF) than in the soluble fraction (SF) (Table 2).

Nutritional quality indices (PUFA/SFA, SI, AI and TI) of POPP throughout SGD are shown in Table 3. To the authors' knowledge, this is the first time that effect of SGD on these indices have been calculated to potential functional ingredients. Alba et al. (2019) explained that AI and TI are a measure of the influence of diet on coronary heart disease. AI relates the risk of atherosclerosis and is based on UFAS (C12:0, C14:0 and C16:0) that can increase or UFAS that can decrease ($\sum$MUFA, $\sum$PUFA) the level of blood cholesterol. TI values relate to the tendency to form clots in the blood vessels, defined as the relationship between the pro-thrombogenic (SFAs) and the anti-thrombogenicity acids (MUFAs, n-6 PUFAs and n-3 PUFAs). In the present study, the low AI and TI values of POPP were maintained throughout SGD (Table 3). Low values for AI and TI are recommended, indicating POPP positive health benefits. POPP revealed similar AI (0.18 ± 0.00), and TI (0.39 ± 0.00) values to the values reported to olive oil (AI = 0.14 and 0.32) (Alba et al., 2019). Other good indicators of the nutritional value of dietary fat

Table 3
Nutritional value of POPP fatty acid profile throughout the SGD.

	Initial	Oral	Gastric	Intestinal	IN	OUT
PUFA/SFA	0.43 ± 0.00 [b]	0.42 ± 0.00 [b]	0.43 ± 0.00 [b]	0.41 ± 0.00 [b]	0.57 ± 0.01 [a]	0.42 ± 0.03 [b]
TI	0.39 ± 0.00 [a]	0.39 ± 0.00 [a]	0.38 ± 0.00 [a]	0.41 ± 0.00 [a]	0.40 ± 0.02 [a]	0.40 ± 0.02 [a]
AI	0.18 ± 0.00 [a]	0.18 ± 0.00 [a]	0.17 ± 0.00 [a]	0.18 ± 0.02 [a]	0.18 ± 0.01 [a]	0.18 ± 0.01 [a]
SI	0.20 ± 0.00 [a]	0.21 ± 0.00 [a]	0.20 ± 0.01 [a]	0.21 ± 0.00 [a]	0.22 ± 0.01 [a]	0.21 ± 0.01 [a]

PUFA/SFA: polyunsaturated fatty acids/saturated fatty acids. SI: saturation index. AI: index of atherogenicity. TI: index of thrombogenicity. Results are the means of three determinations ± standard deviation. Values with different letters are significantly different, as determined by one-way ANOVA test ($p < 0.05$).

are PUFA/SFA ratio and SI index (Table 3). PUFA/SFA ratio in the human diet should be above 0.45. The SI indicates the relationship between the sum of SFAs (pro-thrombogenic) and UFAs (anti-thrombogenic). There are no numerical values assigned to SI, but food with lower values of these SFAs compared to UFAs would be considered healthier food (Alba et al., 2019). SGD modified PUFA/SFA and SI positively and significantly ($p < 0.05$). PUFA/SFA ratio changed from the initial 0.43 ± 0.00 to 0.57 ± 0.01 on the absorbable fraction (IN) and SI maintained a low value throughout SGD (0.20–0.22).

The higher UFAs proportion and the low values of AI, TI, SI and PUFA/SFA above 0.45 detected in absorbable fraction (IN) after SGD makes POPP a highly recommendable food or nutraceutical ingredient from a nutritional standpoint associated to health benefits as reduction of the risk of cardiovascular disease, hypertension or general inflammation even more after SGD (Lopez-Huertas, 2010).

3.2.3. Phenolic compounds

Table 1 showed BI and RI of TPC obtained for POPP after SGD phases. All digestion phases revealed a significant decrease in TPC amount ($p < 0.05$) of POPP (Fig. 2). This negative effect of SGD on olive phenolics was also observed in other studies with table olives (Fernández-Poyatos, Ruiz-Medina, & Llorent-Martínez, 2019). Fernández-Poyatos et al. (2019) reported a loss of approximately 75% of the phenolic content after SGD. The mouth step lowered TPC recovery (RI = 37.52%), but the gastric digestion increased TPC recovery (RI = 77.11%) from POPP. The higher amount of TPC in the stomach phase could be related to the breakdown of bound phenolic compounds from macromolecules, evidencing the phenolics' carriers role of dietary fibre, lipids and protein (Jakobek, 2015). The acidic pH of the stomach also explains this higher release and stability of phenolics (Jakobek & Matić, 2019; Seiquer,
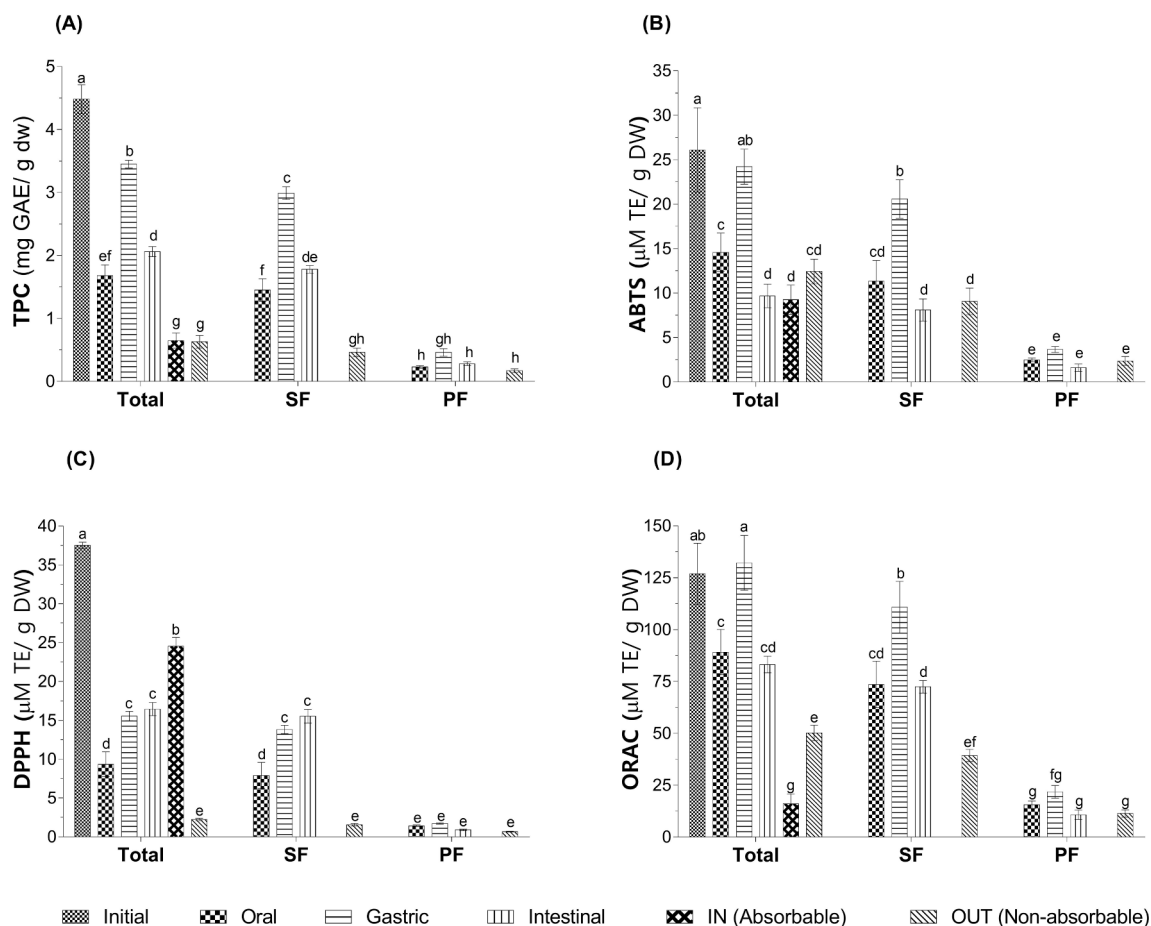
Fig. 2. Effect of *in vitro* gastrointestinal digestion on POPP antioxidant properties after each step of *in vitro* gastrointestinal digestion (oral, gastric, intestinal, after dialysis IN and OUT). (A) Total phenolic compounds (TPC) (mg GAE/ g DW). (B) Antioxidant capacity measured by ABTS (μM TE/ g DW). (C) Antioxidant capacity measured by DPPH (μM TE/ g DW). (D) Antioxidant capacity measured by ORAC (μM TE/ g DW). *PF – pellet fraction; SF - soluble fraction; DW – dry weight; GAE - gallic acid equivalents; TE – Trolox equivalents Results are the means of three determinations ± standard deviation. Values with different letters above are significantly different, as determined by one-way ANOVA test (p < 0.05).*

Rueda, Olalla, & Cabrera-Vique, 2015). On the other hand, the alkaline conditions of intestine influenced significantly TPC recovery (RI = 46.08%) and even more, during intestinal absorption phase (RI ≈ 14% for IN and OUT fraction) (Seiquer et al., 2015). The great and lower stability of olive phenolics to gastric and intestinal digestion respectively were reported in other studies with table olives (Fernández-Poyatos et al., 2019), olive leaf (González et al., 2019) and olive oil (Seiquer et al., 2015). These studies suggested that a proportion of TPC could be transformed during digestion into different structural forms with different chemical properties and bioaccessibility, especially after the intestinal phase (Seiquer et al., 2015). These results supported the evidence that gastrointestinal tract can acts simultaneously as a releaser and damaging agent of phenolics (Gouw et al., 2017).

A significant loss of phenolics occurred throughout SGD of POPP, but at least 50% of the TPC amount that reaches the intestine were bioaccessible (BI = 51.39 ± 3.34%) to be absorbed, metabolised and exert their potential beneficial effects as shown in Table 1 (Lucas-Gonzalez et al., 2016). Similar results of olive phenolics bioaccessibility (>50%) were reported for olive leaf (González et al., 2019) and olive oil (Seiquer et al., 2015). The detection of higher TPC content on the soluble (SF) than in pellet fraction (PF) also demonstrated that POPP phenolics were released from solid matrix and available to be metabolised. A similar proportion of TPC on SF and PF fractions were described to other by-products' powders (Lucas-Gonzalez et al., 2016).

Individual phenolics of POPP before and after SGD were identified using LC-ESI-UHR-QqTOF-MS analyses (Supplementary Material). The main individual phenolics were quantified by HPLC-DAD (Table 2). Regarding individual phenolics, hydroxytyrosol, tyrosol and its derivatives were the predominant phenolics. Correlations between individual phenolics and TPC (Supplementary Material) validated the majority role of hydroxytyrosol glucoside ($r^2 = 0.90$), hydroxytyrosol ($r^2 = 0.95$) and tyrosol ($r^2 = 0.87$) among all phenolics detected on POPP throughout SGD.

SGD had a negative effect in all phenolics, principally in mouth step (RI varies between 14 and 50%) (Table 1). Similar results were reported in the literature that estimates that 90% of phenolics are digested before reaching the intestine (Costa et al., 2019). An interdependent relation could also be expected between phenolics and lipids. Not only the lipids could protect the phenolics, but also phenolics could create a positive antioxidant environment or react with harmful products of the lipid peroxidation to neutralise them (Gorelik, Kanner, Schurr, & Kohen, 2013).

Tyrosol was the most abundant and the most stable POPP phenolic throughout SGD (Table 2). After the significant loss on the mouth (RI = 49.90%), the variation on the RI value for each digestion was minimal (50–40%) as presented in Table 1. The good stability of tyrosol has been reported in previous works. Tyrosol has less tendency to react with other medium macromolecular components and remains stable during the digestion with no dramatic changes in its structure and properties (Dinnella, Minichino, D'Andrea, & Monteleone, 2007). On the other hand, hydroxytyrosol underwent more changes throughout the SGD (Table 1). Despite the significant loss in the mouth (RI = 17.88%), the

hydroxytyrosol amount increased under the acidic conditions of the stomach (RI = 58.33%). However, during the intestinal absorption step, hydroxytyrosol concentration declined substantially, which may be attributed to its high instability under alkaline conditions (González et al., 2019). Nevertheless, its glucosidic form (hydroxytyrosol glucoside) increased during the intestinal absorption phase. Tyrosol glucoside also increased in this step. LC-ESI-UHR-QqTOF-MS confirmed the presence of hydroxytyrosol glucoside (m/z 315.1085) and tyrosol glucoside (m/z 299.1139). The higher release of these glucosidic compounds during intestinal absorption could be related to the action of α-amylase present in the pancreatin extract used in SGD. This pancreatin is an extract from porcine pancreas composed by different enzymes, which can be classified as proteolytic, lipolytic, amylolytic, and nucleic acid splitting enzymes. α-Amylase (EC 3.2.1.1), the main amylolytic enzyme in pancreatin, is an endohydrolase specific for α-(1 → 4) glycosidic bonds (Singh et al., 2014).

Regarding absorbable fraction (IN), tyrosol and its glucoside exhibited the highest RI values (45.39% and 48.81%, respectively) and elevated BI (63.06% and 69.36%, respectively) as can be seen in Table 1. Tyrosol and its glucoside presented several potential health benefits. Tyrosol glucoside is suggested to act as an anti-ageing, anti-inflammatory and anticancer compound, mostly due to its role as an adaptogen, i. e. a biologically-active compound that is supposed to increase resistance in humans to different stress-related disorders and other diseases. Tyrosol was shown to inhibit the oxidation of LDL and to prevent the risk of reactive oxygen metabolite-mediated diseases inhibiting leukocyte 5-lipooxygenase (Dinnella et al., 2007). Other POPP phenolic with higher BI (87.68%) that also possess a potential inhibition action on leukocyte 5-lipooxygenase was caffeic acid. Caffeic acid is known to exhibit anti-mutagenic, carcinogenic and antioxidant activities *in vitro* (Sato et al., 2011).

Luteolin and *p*-coumaric acid were detected in significant amounts in the non-absorbable fraction (OUT) due to its low BI values (0.00 and 35.20%, respectively). More than 30% of the initial amount of luteolin and *p*-coumaric acid were available in the colon, where they potentially could be metabolised by the microflora, increasing their presumed biological activity (Table 1). Luteolin has been pointed out as a potent intestinal anti-inflammatory agent by different mechanisms using *in vitro* gut inflammation models (Mizuno & Nishitani, 2013). Tyrosol, tyrosol glucoside and hydroxytyrosol glucoside were also present in significant amounts in OUT fraction (more than 70% of the initial amount of these compounds present on POPP), where they could also encourage the growth of healthy bacteria (Liu et al., 2019), act as an anti-inflammatory agent on the gut (González-Sarrías et al., 2017) and protecting the Caco-2 intestinal mucosal cells against the cytostatic and cytotoxic effect of oxidised LDL (Bonechi et al., 2019).

3.2.4. Antioxidant capacity: ABTS, DPPH e ORAC

The AOX of POPP was negatively influenced by SGD (Fig. 2). At the end of the SGD, the AOX measured by ABTS, DPPH and ORAC were lower than the initial AOX of POPP. DPPH/ ABTS assays are examples of electron transfer methods, and ORAC is a hydrogen atom transfer (HAT) method. DPPH was more efficient to measure AOX of less polar compounds, due to its solubilisation only in organic media (Arnao, 2000). On the other hand, the ORAC peroxyl free radical from ROS generator AAPH ((2,2′-azobis(2-methylpropionamidine) dihydrochloride)) only react with water and lipid-soluble substances (Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2010).

The correlation between the different AOX methods could be an excellent approach to help the evaluation of the results (Supplementary Material). Usually, ABTS and DPPH exhibit a strong correlation; however, the less polar nature (generous fat content) of POPP could provoke dissimilar behaviour between these methods (r^2 = 0.53). Similar low correlation (r^2 = 0.69) between ABTS and DPPH were also reported to 33 fruits after SGD (Chen et al., 2014). ABTS was more correlated with ORAC (r^2 = 0.78). DPPH allowed validating the role of the less – polar

composition of POPP and ABTS/ORAC evaluated more the potential of POPP soluble compounds by two different AOX mechanisms.

Phenolics were known to their AOX (Gullon et al., 2015; Lucas-Gonzalez et al., 2016), so Pearson's correlation coefficients between the TPC/ individual phenolics amount and their AOX were analysed (Supplementary Material). The phenolics' contribution to AOX was supported by the good correlations between TPC and AOX. ORAC exhibited a better correlation with TPC (r^2 = 0.86) than ABTS (r^2 = 0.76) and DPPH (r^2 = 0.75). These good correlations of all AOX methods validated the importance of POPP phenolics as antioxidant compounds. These results are in agreement with several previous studies which reported high correlations between phenolics and AOX throughout SGD including other by-products powders and extracts as pomegranate peel flour (Gullon et al., 2015) and *Cinnamomum camphora* seed kernel extracts (Zhang et al., 2020). FAs appeared to have a lower impact on the AOX, influencing in same degree the DPPH (r^2 = 0.59) and ABTS (r^2 = 0.59–0.60) and even less ORAC (r^2 = 0.45–0.46).

During SGD, the mouth phase changed more profoundly the AOX of POPP (Table 1). Only 19.29%, 52.90% and 70.22% of initial DPPH, ABTS and ORAC value of POPP were recovered after oral digestion, respectively. In the stomach phase, AOX increased again as a result of phenolics liberation in the gastric phase (higher TPC); principally in the AOX measured by ABTS (RI = 92.84%) and ORAC (RI = 104.14%). This AOX increase in the stomach supported the importance of bound phenolic compounds linked to macromolecules to the high AOX potential of POPP (Jakobek, 2015; Silva et al., 2018). The phenolics influence on AOX was also noticed in the intestine, where the lower amount of phenolics decreased AOX (Fig. 2). Besides the higher and lower amount of phenolics in stomach and intestine, respectively reported in Table 2, the pH changes that occur during the SGD could be another factor that probably affected phenolics' reactivity (creation of enantiomers with different biological reactivity). Thus, phenolics have been reported as more reactive, i.e. antioxidants, at the acidic pH of the stomach step than at the intestinal pH close to neutrality (Gullon et al., 2015).

The RI values of ABTS and ORAC reported in stomach decreased significantly to about half in the intestine (37.12 and 65.61, respectively) (p < 0.05). Only DPPH values did not exhibit significant differences between the gastric and intestinal steps. This stability of DPPH from the stomach to intestine digestion could be associated with the increase of the amount of *p*-coumaric acid and stability of hydroxytyrosol, tyrosol, tyrosol glucoside and caffeic acid (Table 2). DPPH revealed reliable and better correlations (r^2 between 0.75 and 0.88) with these phenolics than ABTS and ORAC and luteolin only exhibited a good correlation with DPPH (r^2 = 0.66). Nevertheless, between all phenolics analysed, hydroxytyrosol (r^2 = 0.75) and its glucoside (r^2 = 0.76) showed to be the compounds with better correlation with ORAC and their significant loss during the intestinal absorption (Table 2) could explain the significant decrease of AOX measured by ORAC to the absorbable fraction (IN) of POPP (Fig. 2). Also, the good correlation between DPPH and tyrosol and its derivative could validate the higher DPPH value of absorbable fraction (IN).

Regardless of the AOX loss throughout SGD, a significant AOX was retained in bioaccessible fraction (IN) (Table 1). DPPH exhibited a higher amount of AOX kept in fraction IN (91.63%) followed by ABTS (43.00%) and ORAC (24.93%). The higher retention of AOX in absorbable fraction by DPPH may be related to the high recovery and bioaccessibility of phenolics as hydroxytyrosol glucoside, tyrosol, tyrosol glucoside and caffeic acid, but also mannitol which is also considered an antioxidant agent (Ribeiro, Oliveira, Campos et al., 2020). The good correlation between DPPH and tyrosol, its derivative and mannitol (r^2 ≥ 0.75) validated this higher DPPH value. Borges, Pereira, Cabrera-Vique, and Seiquer (2017) and Seiquer et al. (2015) described similar higher retention of AOX measured by DPPH (>90%) to the bioaccessible fraction (IN) of extra virgin olive oils after SGD.

As stated in previous works, the main POPP phenolics, i.e. tyrosol, hydroxytyrosol and its derivatives, are potent antioxidants that retain

their biological activities after ingestion (Karković Marković, Torić, Barbarić, & Jakobušić Brala, 2019). Besides that, tyrosol and its derivatives are characterised by a lower AOX than hydroxytyrosol (González et al., 2019). However, the potential conversion of tyrosol into hydroxytyrosol reported in vivo in humans allowed to expect an AOX higher than the reported (Boronat et al., 2019). The hydroxytyrosol AOX is very strong due to its potential as free radical-scavenger, metal-chelator and activator of different cellular signalling pathways to increase the defences against oxidative stress (Karković Marković et al., 2019).

The lower AOX retention in the absorbable fraction (IN) than in non-absorbable fraction (OUT) measured by ABTS and ORAC (Table 1) could be related to the affinity of these methods with more polar compounds. Nevertheless, this low bioaccessibility allowed obtaining an OUT fraction with significant AOX with potential health benefits to the gut (González-Sarrías et al., 2017; Liu et al., 2019).

3.3. Interactions between all bioactives throughout in vitro gastrointestinal digestion

A PCA was performed, reducing the multidimensional structure of the data and providing a two-dimensional map to explain the AOX variance of POPP observed throughout SGD. The scree plot of PCA

analysis, the graph of the loadings plot and scores plot of different SGD phases impacting POPP are presented in Fig. 3. The scree plot indicates that the first two principal components account for 73.1% of the total variance (PC1 = 60.1% and PC2 = 13.0%). Most of the variables were essential contributors to PC1 and PC2. A high cos2 indicates a good representation of the variable on the principal component. All variables exhibited a significant high cos2 value (higher than 0.6), except for ORAC that showed an intermediate cos2 value.

The most significant contributors to the PC1 were the amount of the individual phenolics (hydroxytyrosol glucoside, hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, luteolin), TPC, DPPH, mannitol and glucose. As presented in the loadings plot shown in Fig. 3, most parameters are positioned close to each other, which indicates high positive correlations between them.

PCA shows that SGD has a significant effect on the bioactives composition and AOX of POPP. POPP before the digestion was positioned mainly on IV quadrant and all steps of digested POPP were placed mostly on the III and II quadrants. Scores plot allows easy and quick insight into the effect of SGD upon POPP based on the most critical factor (PC1), which contributes with 60.0% of the total variance and contains information about TPC, AOX (mainly DPPH), main individual phenolics and sugars.
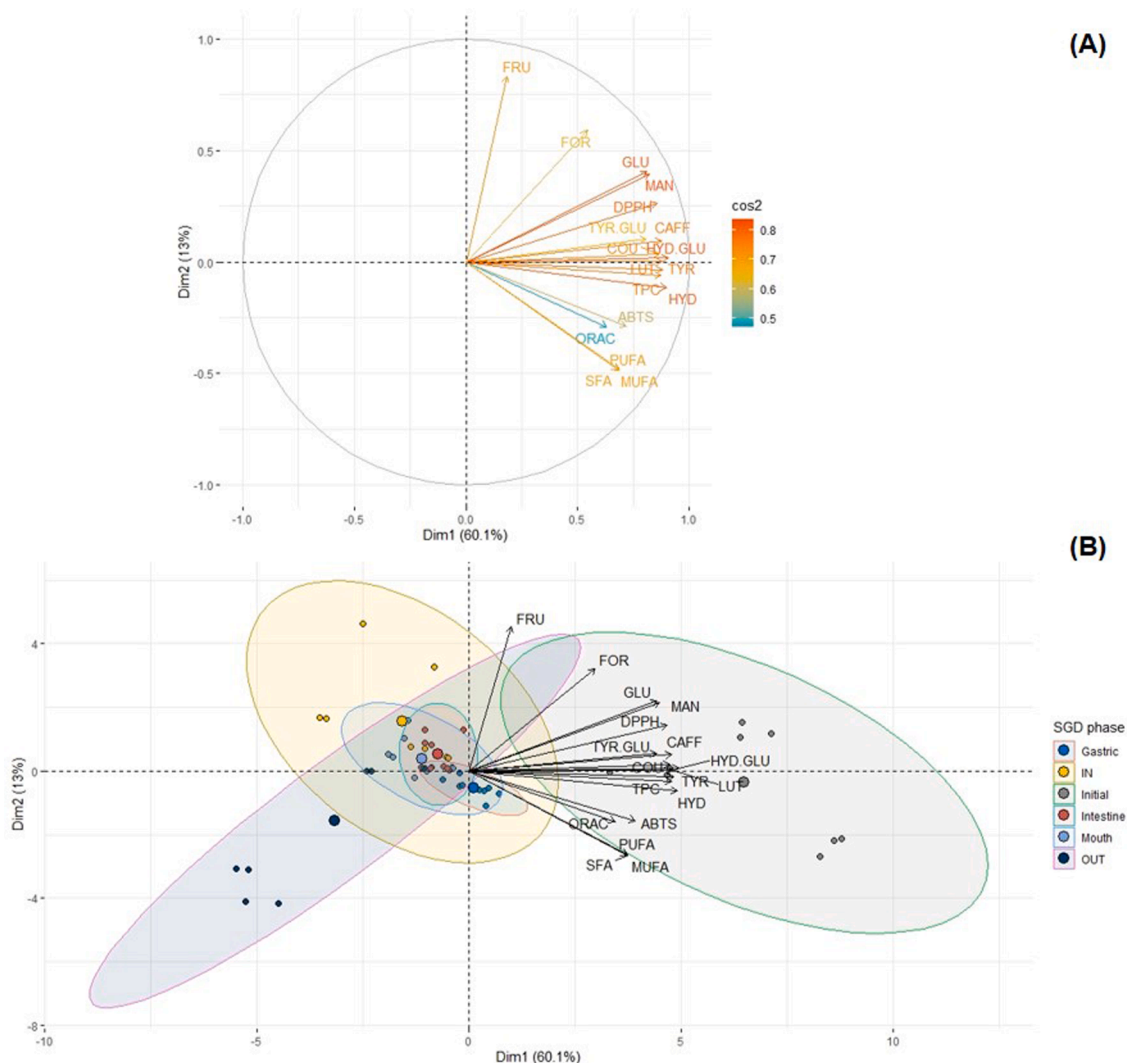

Fig. 3. PCA of POPP digestion. (A) Scree plot of the principal component analysis for POPP SGD phases. (B) Scores plot of different phases of the digestion of POPP.

4. Conclusion

Based on the results obtained, it can be concluded that phenolics' behaviour during digestion is strongly correlated with the potential biological effect of POPP. Despite the higher loss of phenolics at the beginning of digestion, the bound phenolics liberation in the stomach allowed to recover a significant amount of these antioxidant compounds. Dietary fibre and fatty acids appeared to act as phenolic carriers through *in vitro* tract with positive effects on the antioxidant potential of absorbable fraction and non-absorbable fraction. Besides, POPP showed to be an interesting dietary fibre source, which also provides a considerable amount of free and bound phenolics that reach the colon where they could exert potential health benefits (antioxidant, antimicrobial and anti-inflammatory activity). Dietary fibre also demonstrated a potential positive interaction with lipids, decreasing the bioaccessibility of saturated fatty acids and facilitating the absorption of the unsaturated fatty acids. PCA analysis allowed to validate the negative impact of digestion, principally in phenolics and antioxidant capacity. Notwithstanding the negative effect of digestion on POPP bioactive composition and antioxidant capacity, not only dietary fibre, phenolics and unsaturated fatty acids benefits were bioaccessible in a significant amount but also phenolics were retained in the colon where they could exert potential gut health benefits. Further studies need to be developed *in vitro as* transepithelial diffusion across intestinal (Caco-2) cell layers and in vivo or clinical trials, to validate the findings and implications resulting from the *in vitro* experiments described in the present work.

CRediT authorship contribution statement

Tânia B. Ribeiro: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Débora Campos:** Investigation. **Ana Oliveira:** Conceptualization, Validation. **João Nunes:** Supervision, Writing - review & editing. **António A. Vicente:** Supervision, Writing - review & editing. **Manuela Pintado:** Resources, Validation, Supervision, Conceptualization, Writing - review & editing.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.110032.

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