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Incorporation of olive pomace ingredients into yoghurts as a source of fibre and hydroxytyrosol: Antioxidant activity and stability throughout gastrointestinal digestion

Tânia B. Ribeiro [a,b], Teresa Bonifácio-Lopes [a], Pilar Morais [c], Arménio Miranda [c], João Nunes [b], António A. Vicente [d], Manuela Pintado [a,*]

[a] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal
[b] Association BLC3 – Technology and Innovation Campus, Centre Bio R&D Unit, Rua Nossa Senhora da Conceição, 2, Lagares, 3405-155, Oliveira do Hospital, Portugal
[c] Frulact, S.A., Rua do Outeiro, 589, 4475-150, Gemunde, Maia, Portugal
[d] CEB - Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal

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ABSTRACT

Liquid-enriched powder (LOPP) and pulp-enriched powder (POPP) obtained from olive pomace were incorporated into yoghurt, not only, to increase its content in dietary fibre, hydroxytyrosol and unsaturated fatty acids, but also to understand the lipids-phenolics interaction by simultaneous incorporation of olive oil. POPP (2%) and LOPP (1%) addition to yoghurt allowed fulfilling the condition of being a "source of fibre" and provided 5 mg of hydroxytyrosol and derivatives in a standard yoghurt (120 g), respectively. Yoghurts' unsaturated fatty acids profile was positively influenced by the addition of only POPP and olive oil + LOPP or + POPP. All OP powder-fortified yoghurts exhibited higher total phenolic content and antioxidant activity than the control ($p < 0.05$). After in vitro digestion the bioaccessibility of total phenolics (more 25.58%) and hydroxytyrosol (more 68.71%) in LOPP-yoghurts was improved by the addition of olive oil. In conclusion, OP powderś incorporation gave additional and essential healthy properties to yoghurt.

1. Introduction

The current consumers' awareness about the importance of diet to health fostered the development of functional and/or fortified foods (Hashemi Gahruie et al., 2015). Food fortification, defined as the addition of one or more essential nutrients to food to levels higher than usual with the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients, is also a way of enhancing the nutritional value and potential health benefits of food (Hashemi Gahruie et al., 2015).

The consumption of fortified foods has been increasing in the last decade, principally in dairy products (Hashemi Gahruie et al., 2015; Helal and Tagliazucchi, 2018). Fortified dairy products, such as yoghurt, have overrun the appeal of traditional products (Baba et al., 2018). Yoghurt is highly consumed and appreciated for its nutritional value and positive health benefits mainly associated to the presence of bioactive peptides, prebiotics and probiotics (Helal and Tagliazucchi, 2018; Oliveira and Pintado, 2015). The global yoghurt market was valued in 2019 at USD 99,553.38 million, and it is estimated to reach USD 141, 829.95 million by 2025 (CAGR of 6.25%, from 2020 to 2025). The yoghurt market has viewed significant growth due to the rise of health-conscious consumers. In consequence, players in the yoghurt market are coming up with various healthy and flavour options to satisfy consumer preferences (Mordor Intelligence, 2019).

Among the food components that are not present in yoghurt, dietary fibre (DF), phenolic compounds and unsaturated fatty acids (UFAs) can be highlighted. Diets with high DF, phenolics and UFAs content play a significant role in the prevention of several diseases (Román et al., 2019). As a result, natural sources such as fruits and cereals have been used to fortify yoghurts with phenolics (Helal and Tagliazucchi, 2018) and DF (Hashemi Gahruie et al., 2015). However, limited research concerning the fortification of yoghurt with UFAs [monounsaturated (MUFA) and/or polyunsaturated fatty acids (PUFA)] to improve its lipid profile has been reported (Baba et al., 2018; Dal Bello et al., 2015). Among the few studies reported to improve the fatty acids (FAs) profile

* Corresponding author.
E-mail address: mpintado@porto.ucp.pt (M. Pintado).

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of yoghurts, vegetable oils and fish oils were usually used (Baba et al., 2018; Dal Bello et al., 2015; Van Nieuwenhove et al., 2019).

More recently, sustainability concerns have stimulated the food industry to develop added-value food ingredients from their by-products and use them to fortify yoghurts. Some examples are the incorporation of pomegranate peel powder (Kennas et al., 2020) to boost the antioxidant activity (AOX) of yoghurt, as well as the addition to yoghurt of hazelnut skins (Bertolino et al., 2015) and wine grape pomace powder (Tseng and Zhao, 2013) as a simultaneous source of DF and phenolics. More recently, pomegranate seeds obtained from the juice industry were incorporated into yoghurt for its enrichment in conjugated linolenic acid and antioxidant compounds (Van Nieuwenhove et al., 2019).

Olive pomace (OP) powders from the olive oil (OO) industry can be an attractive source of the same bioactive compounds reported above. OP is a semi-solid by-product obtained from the widely implemented 2-phase, being the most abundant and relevant by-product of the modern OO industry (Dermeche et al., 2013; Nunes et al., 2018). This by-product is a combination of olive husk and pulp, crushed olive stone and olive mill wastewater (moisture content of 65%) and it is associated with severe environmental problems and waste management costs (Moreno-Maroto et al., 2019). According to different authors, 1 ha of olive tree originates about 2500 kg of olives (Rodrigues et al., 2015) and approximately 40–70 kg of OP per 100 kg of olives are produced (AGAPA, 2015; Nunes et al., 2016; Romero-García et al., 2014; Ruiz et al., 2017). Nowadays, OP is mainly applied in energy sector (electricity generation or cogeneration, and thermal uses). Other typical applications are the direct incorporation of OP into the soil, composting and finally, as animal feed (AGAPA, 2015). However, these traditional OP treatments waste a significant amount of high value-added bioactive compounds as DF, MUFAs, PUFAs and phenolic compounds, associated not only to several health benefits but also to potential economic incomings to OO producers (Dermeche et al., 2013; Nunes et al., 2018). So, it is preeminent the adoption of valorisation approaches as the development of food ingredients to increase the OP value and consequently enhance the economic and environmental sustainability of OO sector.

In our previous works, **we have developed two biologically safe food powdered ingredients: the liquid-enriched olive pomace** powder (LOPP) and the pulp-enriched olive pomace powder (POPP) (Ribeiro et al., 2020b). The production of powdered ingredients has been proposed as a more feasible and low environmental impact approach in comparison to the traditional (involve the use of organic solvents) and emergent technologies (possess higher operational costs) with the advantage of retaining all the bioactive compounds of food by-products without any extraction step (García-Lomillo et al., 2014). Indeed, a "whole by-product valorisation" could be attained producing these multifunctional powdered ingredients (Crizel et al., 2016; Gouw et al., 2017; Saura-Calixto, 1998).

POPP was characterised to be a potential antioxidant dietary fibre (ADF) source that can deliver the physiological effects of both DF and antioxidants (Ribeiro et al., 2020b). Besides, POPP also contains significant MUFA (mainly oleic acid corresponding to 70% of POPP total FAs) and PUFA amounts (mainly linoleic acid which corresponds to 6% of POPP total FAs) (**Supplementary material 1**). The liquid-enriched olive pomace powder (LOPP) exhibits high hydroxytyrosol (HYD) and derivatives levels (Ribeiro et al., 2020a). HYD and its derivatives are well-known antioxidant compounds. Indeed, a daily intake of 5 mg of HYD and derivatives protects LDL particles from oxidative damage, according to the health claim approved by the EFSA (until now only allowed in OO) (EFSA, 2011). The significant content of LOPP and POPP in antioxidants could also be an opportunity to protect other UFAs sources added to yoghurt. UFAs are easily prone to oxidation, and its incorporation in food with AOX such as those containing phenolics may have an adjuvant effect against lipid oxidation (Román et al., 2019). Besides that, both OP powders demonstrated to be biologically safe and demonstrated adequate functional properties for food applications (Ribeiro et al., 2020b).

OP has been incorporated in food products, principally in the formulation of bakery products as biscuits and bread (Conterno et al., 2019; Di Nunzio et al., 2020). Regarding yoghurt formulations, other olive-derived powders from olive green (Cho et al., 2017) or extracts from the olive leaf (Cho et al., 2020; Peker and Arslan, 2017; Zoidou et al., 2017), olive-mill wastewater (phenolic concentrate) (Servili et al., 2011) and three-phase oil extraction process (Aliakbarian et al., 2015) have been tested in the preparation of functional milk beverages or yoghurts without interfering with the fermentation process and probiotic counts (Aliakbarian et al., 2015; Servili et al., 2011). The yoghurt matrix can be a great carrier of phenolic compounds. Proteins or large peptides present in yoghurts have been reported to have the capacity to maintain phenolics integrity during digestion (Helal and Tagliazucchi, 2018). Lipids were also described as protectors of phenolics, improving their stability during digestion (Jakobek, 2015). So, yoghurts' components could increase phenolics protection and bioaccessibility (Helal and Tagliazucchi, 2018). The bioaccessibility definition comprises the release of compounds from food matrices and their stability under the gastrointestinal condition (Helal and Tagliazucchi, 2018). However, to the author's knowledge until now, ingredients obtained from OP have never been applied to yoghurt formulations to improve its DF simultaneously, UFAs and phenolics compound content. Yoghurts fortified with OP powders are not only an excellent way to improve the daily intake of DF, phenolic compounds and UFAs but also an opportunity to dairy industry achieve new "sustainable" products - which is a new and growing food category at the same time that OP was valorised (Coderoni and Perito, 2020). This study brings new insights to help spread the circular bioeconomy concept in the whole food sector.

In this context, the main objective of the present study was to evaluate the potential of OP powders to enhance the nutritional and functional value of yoghurt as a source of DF, UFAs and phenolic compounds, and also to evaluate the bioaccessibility of phenolics and the AOX during in vitro simulation of gastrointestinal digestion (SGD). The potential interaction of phenolics-lipids was also analysed by the simultaneous incorporation of OO and OP powders.

2. Materials and methods

2.1. Chemicals and reagents

ABTS diammonium salt (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid), methanol, potassium sorbate and sodium carbonate were purchased from Sigma-Aldrich (Sintra, Portugal). Folin-Ciocalteu's reagent and potassium persulfate were purchased from Merck (Algés, Portugal). Standards of Trolox, gallic acid, p-coumaric acid, vanillin, protocatechuic acid, caffeic acid and quercetin were obtained from Sigma-Aldrich (Sintra, Portugal), whereas hydroxytyrosol, tyrosol, luteolin were purchased from Extrasynthese (Lyon, France).

2.2. Preparation of olive pomace powders

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

OP was fractionated by centrifugation (10,000×*g* for 10 min). The liquid fraction was freeze-dried (Telstar Lyo Quest HT 40) with 2% of mannitol (as a cryoprotectant and to prevent aggregation), and the powder obtained was denominated liquid-enriched olive pomace powder (LOPP). The 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 (potential physical hazard) were removed to obtain a food-grade ingredient denominated as pulp-enriched olive pomace powder (POPP).

2.3. Fortification of yoghurts with the OP powders

Yoghurts were prepared using homogenised (180 bar), pasteurised, and whole milk (supplemented with 3% milk powder (w/v)). The starter culture (fresh yoghurt) was added at 2% to the milk after cooling down to 44 °C. The mixture was packed and then fermented at 42 °C (oven) until the final pH of 4.6 (about 4.5 h). Six yoghurt formulations were obtained, including a control yoghurt (Y-control) and a yoghurt supplemented with OO (Y-OO), both without OP powders. Supplementation of yoghurts with OO was achieved by the addition of 5% of OO (w/v) before the homogenisation step. LOPP and POPP were incorporated before pasteurisation (90 °C) in order to obtain OP-fortified yoghurts without OO (Y-LOPP and Y-POPP) and with OO (Y-LOPP-OO and Y-POPP-OO). LOPP was added at 1% to provide the amount of HYD (5 mg) that would be needed to protect LDL particles from oxidative damage, according to the health claim (EFSA, 2011). The amount of LOPP was added in excess due to possible losses unintentionally caused by the thermal and mechanical procedures during yoghurt preparation. On the other hand, the Y-POPP (2% POPP) was formulated to fulfil the condition of being a "source of fibre" (>1.5 g of fibre per 100 kcal) (European Union, 2006). Supplementation with 5% of OO was used in OP-fortified yoghurts formulation with the aim that at least 45% of FAs present in the yoghurt derive from MUFAs. A flow diagram of the development of OP powders and the fortified yoghurts is present in Fig. 1.

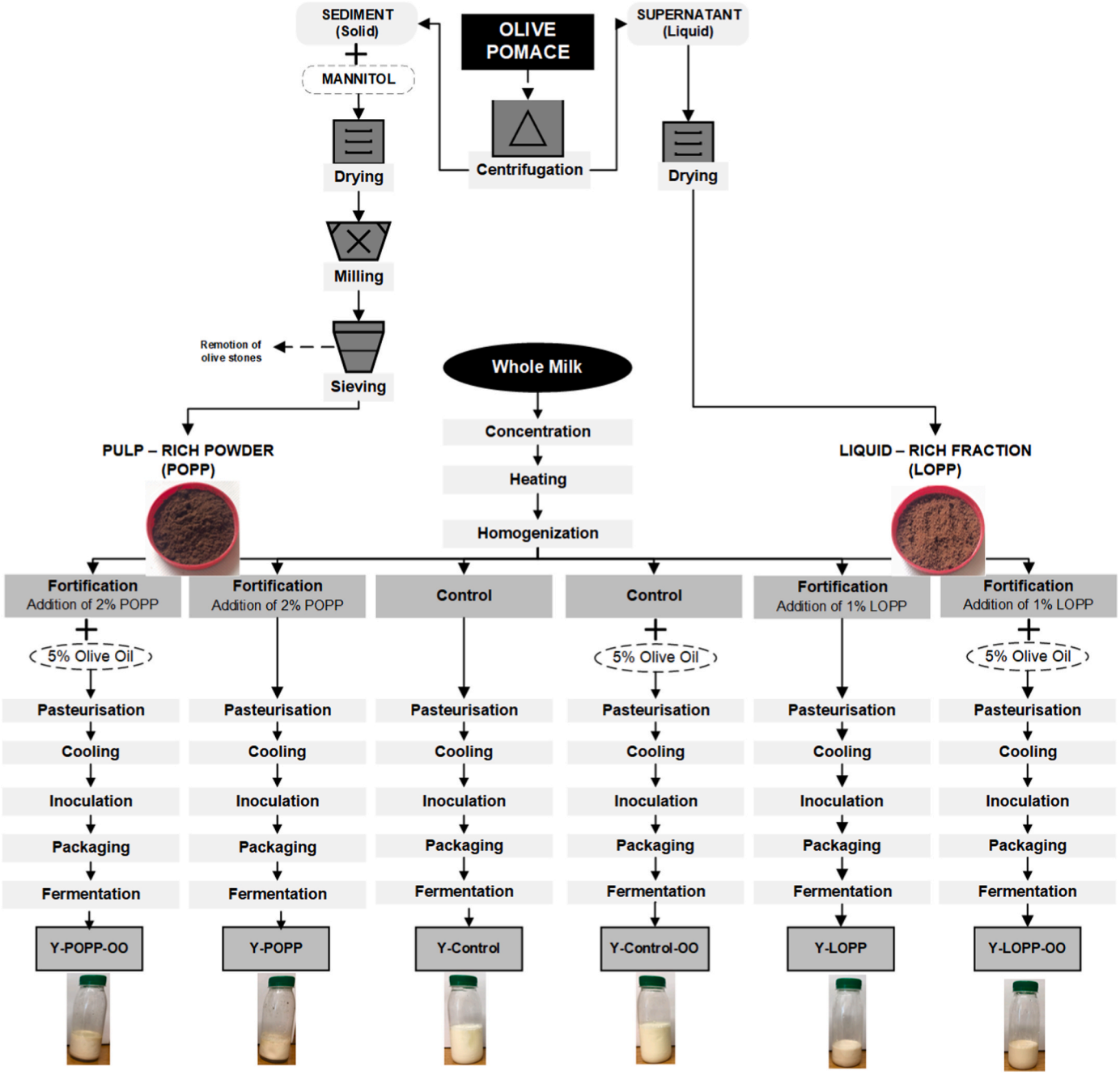


Fig. 1. Schematic flow diagram of the development of olive pomace powders (LOPP and POPP), fortified yoghurts with olive pomace powders (Y-LOPP and Y-POPP), olive pomace powders and olive oil (Y-LOPP-OO and Y-POPP-OO) and their controls (Y-Control and Y-Control-OO).

2.4. Chemical characterisation of yoghurts

2.4.1. Proximate composition

The crude protein content was determined using the Kjeldahl method, with a conversion factor of 6.25. The lipid content was obtained using an automated Soxhlet Soxtec™ 8000 (Foss, Spain) for 4 h using n-hexane as solvent. The ash content was determined in a muffle furnace (AOAC No. 942.05) according to the AOAC (1990). DF (TDF, SDF and IDF) was measured using the Megazyme Total Dietary analysis kit according to the enzymatic gravimetric method (991.43; AOAC (1990)), with slight modification in obtaining process of SDF as described previously by Ribeiro et al. (2020). SDF was obtained by dialysis (dialysis tube with 3.5 kDa) to avoid the error caused by ethanol precipitation of SDF. Afterwards, SDF was recovered by dialysate freeze-drying. All measurements were done in triplicate and expressed as g/100 g dry weight (DW).

2.4.2. Analyses of fatty acids and related health lipid indices

The yoghurts' FA profiles were obtained and analysed following the methodology of Pimentel et al. (2015) with some modifications as described previously by Ribeiro et al. (2020).

Nutritional quality indices of all yoghurts' formulations 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 et al., 2019).

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

$$AI = \frac{[C12:0 + 4 \times C14:0 + C16:0]}{[\sum MUFA + \sum PUFA]} \quad \text{Equation (4)}$$

$$SI = \frac{[C14:0 + C16:0 + C18:0]}{[\sum MUFA + \sum PUFA]} \quad \text{Equation (5)}$$

2.4.3. Phenolic compounds and antioxidant activity

Phenolic extracts from yoghurts' formulations were obtained according to Oliveira and Pintado (2015), with some modifications. This procedure was adopted in order to reduce the interferences from peptides. Each yoghurt formulation (in triplicate) was homogenised with 30 mL of methanol acidified with formic acid (9:1 v/v), using an orbital shaker at 250 RPM, for 1 h. The homogenised sample was centrifuged at 4000×*g*, at 4 °C for 10 min, and the supernatant kept at −20 °C overnight, to allow for protein precipitation. Then the slurry was centrifuged again to remove soluble proteins. The extract was evaporated to dryness in a speed-vacuum evaporator at 30 °C and the residue dissolved in 2 mL of methanol for further analysis.

The total phenolic content (TPC) of extracts was determined according to the Folin-Ciocalteu method. Results were expressed as mg gallic acid equivalents (GAE)/100 g DW.

The HPLC analysis was performed using a Waters e2695 separation module system interfaced with a Photodiode array UV/Vis detector (PDA 190–600 nm) as described by Ribeiro et al. (2020).

The AOX of yoghurts extracts was achieved according to the methods of DPPH, ABTS and ORAC (Costa et al., 2019; Ribeiro et al., 2020a) using a multidetection plate reader (Synergy H1, Vermont, USA). The radical stock solutions were freshly prepared. All analyses were performed in triplicate and expressed in μM of Trolox-equivalents (TE)/g DW.

2.5. In vitro digestion

The in vitro simulation of gastrointestinal digestion (SGD) was performed according to the method described previously by Ribeiro et al. (2020), using the dialyses process to simulate the intestinal and blood absorption. At the end of the incubation process, the solution left outside the dialysis tubing was taken as the OUT sample representing material that remained in the gastrointestinal tract (colon-available), and the solution that managed to diffuse into the dialysis tubing was taken as the IN sample (serum-available).

To screen the release of individual phenolics from yoghurt matrices (100 g) at different stages of digestion, samples of yoghurt were collected from the mouth (ca. 20 mL), gastric digesta (ca. 20 mL), intestinal digesta (ca. 20 mL) and used to make extracts to further phenolics analysis. Three replicas from the GI system were made.

2.5.1. Recovery and bioaccessibility index of phenolic compounds

The results of each extract determination (on the sample, after mouth, gastric and intestinal digestion) were reported in 100 g of DW of yoghurt.

Recovery index (RI%) and bioaccessibility index (BI%) were studied to evaluate the effect of the yoghurt composition on the digestion of its phenolic compounds (Lucas-Gonzalez et al., 2016).

The percentage of recovery (RI%) allows the determination of the amount of phenolic compounds on the food sample present in the digested sample after oral, gastric and intestinal digestion, according to:

$$\text{Recovery index } (\%) = \left(\frac{PC_{DF}}{PC_{TF}}\right) \times 100 \quad \text{Equation (1)}$$

where: PC_{DF} is the phenolic content (mg) in the digested, and PC_{TF} is the phenolic content (mg) quantified in the test matrix.

The bioaccessibility index is defined as the percentage of the phenolic compound that is solubilised after intestinal dialysis step. Thus, this index defines the proportion of the phenolic compound that could become available for absorption into the systematic circulation:

$$\text{Bioaccessibility index } (\%) = \left(\frac{PC_s}{PC_{DFE}}\right) \times 100 \quad \text{Equation (2)}$$

where: PC_S is the phenolic content (mg) in the digested sample after the dialysis step (IN) and PC_{DFE} is the total phenolic content (mg) in the digested sample after the dialysis step (IN + OUT).

2.5.2. Antioxidant activity: ABTS and DPPH

The AOX of yoghurts during the SGD was achieved according to the methods of DPPH and ABTS as reported above.

2.6. Statistical analyses

Software R was used to carry out statistical analyses. All experiments were carried out in triplicates, and data were reported as mean ± standard deviation. The Shapiro - Wilk test tested the normality of data distribution. The differences of mean values were analysed by one-way analysis of variance (ANOVA). Tukey's post hoc test was used for comparisons of means; differences were considered significant at $p < 0.05$.

Principal Component Analysis (PCA) and Discriminant Analysis (PLS-DA)) were applied to evaluate the nutrients and bioactives patterns of OP powders-fortified yoghurts using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/) on data after autoscaling (mean-centred and divided by the standard deviation of each variable).

3. Results and discussion

3.1. Chemical characterisation of yoghurts

Table 1 shows the proximate and FAs composition of the yoghurts. When comparing with the control (Y-control), moisture decreased in all fortified yoghurts, but the differences observed were not statistically significant ($p > 0.05$). Regarding ash content, yoghurts fortified with

Table 1
Protein, dietary fibre and fatty acid composition of fortified yoghurts and control.

	Yoghurt formulations without olive oil			Yoghurt formulations with olive oil		
	Control	LOPP fortified	POPP fortified	Control	LOPP fortified	POPP fortified
Moisture (g/100 g WW)	83.39 ± 1.21^{a}	82.89 ± 0.30^{a}	83.30 ± 0.34^{a}	81.23 ± 1.84^{a}	81.09 ± 2.89^{a}	82.13 ± 1.93^{a}
Ash (g/100 g DW)	5.69 ± 0.01 ^{ab}	5.84 ± 010^{a}	5.51 ± 0.24 ^{abc}	4.87 ± 0.23 ^{bc}	6.03 ± 0.16^{a}	4.55 ± 0.09^{c}
Protein (g/100 g DW)	28.13 ± 0.83^{a}	26.38 ± 0.92^{a}	27.40 ± 0.49^{a}	23.43 ± 1.52^{a}	24.58 ± 0.35^{a}	24.17 ± 0.89^{a}
Dietary fibre (g/100 g DW)						
TDF	ND	3.62 ± 0.26^{b}	6.42 ± 0.03^{a}	ND	2.75 ± 0.50^{b}	7.57 ± 0.19^{a}
IDF	ND	2.24 ± 0.07^{b}	5.12 ± 0.12^{a}	ND	0.93 ± 0.19^{c}	5.76 ± 0.22^{a}
SDF	ND	1.38 ± 0.20^{a}	1.31 ± 0.09^{a}	ND	1.82 ± 0.48^{a}	1.81 ± 0.22^{a}
Fat (g/100 g DW)	22.47 ± 0.61^{c}	19.87 ± 0.16^{d}	21.23 ± 0.26 ^{cd}	34.43 ± 0.53^{a}	32.28 ± 0.27^{b}	32.12 ± 0.37^{b}
Fatty acids (mg/g DW)						
C6:0	1.37 ± 0.03^{a}	1.17 ± 0.14 ^{abc}	1.18 ± 0.06 ^{abc}	1.27 ± 0.02 ^{ab}	0.92 ± 0.16 ^{bc}	0.89 ± 0.03^{c}
C8:0	1.30 ± 0.02^{a}	1.12 ± 0.11 ^{abc}	1.12 ± 0.05 ^{abc}	1.20 ± 0.02 ^{ab}	0.88 ± 0.18 ^{bc}	0.83 ± 0.02^{c}
C9:0	42.99 ± 3.25^{a}	34.61 ± 6.57 ^{ab}	36.96 ± 3.76 ^{ab}	39.37 ± 2.08 ^{ab}	25.41 ± 5.13^{b}	36.07 ± 2.35 ^{ab}
C10:0	3.96 ± 0.06^{a}	3.52 ± 0.22 ^{abc}	3.48 ± 0.07 ^{abc}	3.65 ± 0.11 ^{ab}	2.79 ± 0.64 ^{bc}	2.52 ± 0.09^{c}
C12:0	5.97 ± 0.08^{a}	5.47 ± 0.16 ^{ab}	5.36 ± 0.03 ^{ab}	5.54 ± 0.23 ^{ab}	4.36 ± 1.15 ^{ab}	3.82 ± 0.14^{b}
C14:0	23.67 ± 0.32^{a}	22.33 ± 0.10 ^{ab}	21.82 ± 0.43 ^{ab}	22.21 ± 1.13 ^{ab}	17.97 ± 2.70 ^{bc}	15.31 ± 0.59^{c}
C15:0	2.45 ± 0.03^{a}	2.35 ± 0.04^{a}	2.28 ± 0.07^{a}	2.34 ± 0.11^{a}	1.91 ± 0.33 ^{ab}	1.61 ± 0.05^{b}
C16:0	81.27 ± 1.05^{a}	78.58 ± 1.90^{a}	79.23 ± 2.85^{a}	93.74 ± 8.94^{a}	88.98 ± 14.97^{a}	78.30 ± 3.23^{a}
C17:0	1.31 ± 0.02^{a}	1.32 ± 0.04^{a}	1.32 ± 0.06^{a}	1.47 ± 0.06^{a}	1.23 ± 0.25^{a}	1.14 ± 0.04^{a}
C18:0	26.31 ± 0.41^{a}	25.79 ± 1.08^{a}	25.68 ± 1.16^{a}	28.15 ± 0.10^{a}	23.36 ± 5.60^{a}	20.92 ± 0.61^{a}
C10:1 c2	0.33 ± 0.00^{a}	0.30 ± 0.03 ^{abc}	0.29 ± 0.01 ^{abc}	0.31 ± 0.01 ^{ab}	0.23 ± 0.05 ^{bc}	0.21 ± 0.07 ^{bc}
C14:1 c9	1.17 ± 0.01^{a}	1.12 ± 0.01 ^{ab}	1.08 ± 0.03 ^{ab}	1.10 ± 0.07 ^{ab}	0.93 ± 0.12 ^{bc}	0.76 ± 0.04^{c}
C15:1 c10	0.67 ± 0.01^{a}	0.66 ± 0.01^{a}	0.64 ± 0.02^{a}	0.65 ± 0.05^{a}	0.52 ± 0.08 ^{ab}	0.44 ± 0.02^{b}
C16:1 t9	0.17 ± 0.01^{a}	0.17 ± 0.01^{a}	0.20 ± 0.05^{a}	0.22 ± 0.05^{a}	0.15 ± 0.02^{a}	0.11 ± 0.01^{a}
C16:1 c7	0.53 ± 0.02^{a}	0.51 ± 0.01^{a}	0.51 ± 0.01^{a}	0.60 ± 0.06^{a}	0.56 ± 0.04^{a}	0.50 ± 0.01^{a}
C16:1 c9	3.82 ± 0.06^{a}	3.64 ± 0.02^{a}	3.86 ± 0.08^{a}	5.55 ± 1.41^{a}	5.81 ± 0.62^{a}	5.37 ± 0.21^{a}
C17:1 c10	0.53 ± 0.00 ^{ab}	0.50 ± 0.04^{b}	0.57 ± 0.07 ^{ab}	0.85 ± 0.17 ^{ab}	0.87 ± 0.12^{a}	0.85 ± 0.04 ^{ab}
C18:1 t11	4.54 ± 0.01^{a}	4.25 ± 0.21^{a}	4.25 ± 0.15^{a}	4.89 ± 0.77^{a}	3.31 ± 0.64 ^{ab}	2.60 ± 0.04^{b}
C18:1 c9	54.80 ± 0.77^{b}	54.36 ± 0.72^{b}	70.01 ± 4.67^{b}	87.71 ± 6.73^{b}	148.23 ± 30.45^{a}	154.09 ± 6.46^{a}
C18:1 c11	2.01 ± 0.04^{b}	1.99 ± 0.02^{b}	2.65 ± 0.08^{b}	8.27 ± 0.40^{a}	7.46 ± 0.64^{a}	7.09 ± 0.27^{a}
C18:1 c12	0.85 ± 0.04^{a}	0.85 ± 0.05^{a}	0.80 ± 0.04^{a}	0.84 ± 0.05^{a}	0.60 ± 0.06^{b}	0.53 ± 0.04^{b}
C18:1 c13	0.27 ± 0.02^{a}	0.28 ± 0.02^{a}	0.26 ± 0.01^{a}	0.29 ± 0.00^{a}	0.22 ± 0.05^{a}	0.20 ± 0.00^{a}
C18:1 c14+t16	0.74 ± 0.06^{a}	0.76 ± 0.02^{a}	0.70 ± 0.04^{a}	0.76 ± 0.05^{a}	0.51 ± 0.07^{b}	0.49 ± 0.02^{b}
C20:1 c9	0.19 ± 0.02^{c}	0.20 ± 0.01^{c}	0.27 ± 0.02 ^{bc}	0.55 ± 0.14 ^{ab}	0.60 ± 0.14^{a}	0.62 ± 0.04^{a}
C18:2 t9 t12	0.79 ± 0.04^{a}	0.77 ± 0.04^{a}	0.73 ± 0.04 ^{ab}	0.78 ± 0.04^{a}	0.59 ± 0.03 ^{bc}	0.50 ± 0.02^{c}
C18:2 c9 t12	0.35 ± 0.01 ^{ab}	0.30 ± 0.10 ^{abc}	0.34 ± 0.06 ^{abc}	0.42 ± 0.02^{a}	0.20 ± 0.05 ^{bc}	0.14 ± 0.02^{c}
C18:2 c9 c12	4.32 ± 0.08^{c}	4.24 ± 0.09^{c}	5.59 ± 0.18 ^{bc}	9.72 ± 1.29^{a}	9.18 ± 2.22 ^{ab}	10.44 ± 0.27^{a}
C18:2 c9 t11	1.44 ± 0.02 ^{ab}	1.39 ± 0.04 ^{ab}	1.43 ± 0.03 ^{ab}	1.85 ± 0.30^{a}	1.41 ± 0.40 ^{ab}	1.03 ± 0.08^{b}
C18:3 t9 t12 c15	0.37 ± 0.03^{a}	0.39 ± 0.05^{a}	0.40 ± 0.05^{a}	0.45 ± 0.01^{a}	0.34 ± 0.08^{a}	0.27 ± 0.04^{a}
α C18:3 c9 c12 c15	0.40 ± 0.01^{c}	0.41 ± 0.02^{c}	0.56 ± 0.00 ^{bc}	1.02 ± 0.22 ^{ab}	1.11 ± 0.28 ^{ab}	1.27 ± 0.04^{a}
C20:4 c5 c8 c11 c14	0.28 ± 0.01^{a}	0.26 ± 0.01^{a}	0.26 ± 0.01^{a}	0.27 ± 0.01^{a}	0.22 ± 0.06^{a}	0.18 ± 0.02^{a}
∑*SFA*	190.57 ± 5.21^{a}	176.27 ± 4.38^{a}	178.44 ± 3.34^{a}	198.96 ± 9.57^{a}	167.81 ± 27.36^{a}	154.42 ± 12.52^{a}
∑*UFA*	78.57 ± 1.11^{c}	77.33 ± 0.85^{c}	95.37 ± 5.34^{c}	127.09 ± 11.02 ^{bc}	183.05 ± 35.38 ^{ab}	187.71 ± 7.28^{a}
∑*MUFA*	70.63 ± 1.00^{b}	69.58 ± 0.78^{b}	86.09 ± 5.01^{b}	112.58 ± 9.31^{b}	170.01 ± 32.74^{a}	173.87 ± 7.06^{a}
∑*PUFA*	7.94 ± 0.11^{c}	7.76 ± 0.10^{c}	9.28 ± 0.32 ^{bc}	14.51 ± 1.71^{a}	13.04 ± 2.67 ^{ab}	13.84 ± 0.30 ^{ab}
∑*Omega-3* (ω-3)	0.40 ± 0.01^{c}	0.41 ± 0.02^{c}	0.56 ± 0.00 ^{bc}	1.02 ± 0.22 ^{ab}	1.11 ± 0.28 ^{ab}	1.27 ± 0.04^{a}
∑*Omega-6* (ω-6)	4.60 ± 0.06^{c}	4.50 ± 0.10^{c}	5.83 ± 0.18 ^{bc}	9.99 ± 1.28^{a}	9.39 ± 2.25 ^{ab}	10.63 ± 0.28^{a}
Omega-6/Omega-3 ratio	11.56 ± 0.11^{a}	10.87 ± 0.32^{a}	10.41 ± 0.44 ^{ab}	9.93 ± 0.90 ^{ab}	8.53 ± 0.71^{b}	8.39 ± 0.19^{b}
Thrombogenic index (TI)	3.37 ± 0.00^{a}	3.30 ± 0.06^{a}	2.66 ± 0.13^{b}	2.24 ± 0.09^{b}	1.41 ± 0.17^{c}	1.12 ± 0.13^{c}
Atherogenic index (AI)	2.32 ± 0.00^{a}	2.24 ± 0.00^{a}	1.81 ± 0.09^{b}	1.49 ± 0.12^{c}	0.91 ± 0.09^{d}	0.76 ± 0.00^{d}
Saturation index (SI)	1.67 ± 0.00^{a}	1.64 ± 0.02^{a}	1.33 ± 0.07^{b}	1.14 ± 0.06^{b}	0.72 ± 0.08^{c}	0.57 ± 0.07^{c}

ND-non-detected; SFA - Saturated fatty acids; UFA - unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids. C6:0 – Caproic acid; C8:0 – Caprylic acid; C9:0 – Pelargonic acid; C10:0 – Capric acid; C12:0 – Lauric acid; C14:0 – Myristic acid; C15:0 – Pentadecylic acid; C16:0 – Palmitic acid; C17:0 – Margaric acid, C18:0 – Estearic acid; C10:1 c2 – Decenoic acid; C14:1 c9 – Myristoleic acid; C15:1 c10 – Pentadecanoic acid; C16:1 t9 - trans-palmitoleic acid; C16:1 c7 – cis-7-hexadecenoic acid; C16:1 c9 – Palmitoleic acid; C17:1 c10 - cis-10-heptadecenoic acid; C18:1 t11 – trans-11-octadecenoic acid; C18:1 c9 – Oleic acid; C18:1 c11 – cis-Vaccenic acid; C18:1 c12 – cis-12-Oleic acid; C18:1 c13 – cis-13-Oleic acid; C18:1 c14+t16 – c14+t16-octadecenoic; C20:1 c9 – cis-Eicosanoid acid; C18:2 t9 t12 – trans-9-trans-12-Octadecadienoic; C18:2 c9 t12 – cis-9-trans-12-Octadecadienoic; C18:2 t9 c12 – trans-9-cis-12-Octadecadienoic; C18:2 c9c12 – Linoleic acid; C18:2 c9 t11 – cis-9-trans-11-Octadecadienoic; C18:3 t9 t12 c15 – trans-9-trans-12-cis-15-octadecatrienoic; *α* C18:3 c9c12c15 – *α*-Linolenic acid C20:4 c5 c8 c11 c14 - Arachidonic acid. Results are the means of three determinations ± standard deviation. Values with different letters in the same line are significantly different, as determined by one-way ANOVA test ($p < 0.05$).

LOPP exhibited a statistically significant higher amount of ash ($p < 0.05$) than the other yoghurt formulations. On the other hand, the addition of POPP decreased the ash content in comparison with the control (Y-control). In contrast, the addition of OO and OP powders did not affect the composition of yoghurts negatively as a rich source of protein. The yoghurts' protein content was maintained between 23 and 28% (DW) without significant differences ($p > 0.05$). Total fat content was significantly higher in yoghurt formulations with OO (32–34% DW) than in the other formulations without OO (20–22% DW). LOPP and POPP addition had a similar decreasing effect on the total fat content of yoghurt, statistically significant ($p < 0.05$), in yoghurt formulations with OO. Similar results on moisture, ash, protein and lipids content were reported in yoghurts fortified with hazelnut skins as a source of fibre and phenolics (Bertolino et al., 2015) and in yoghurts fortified with omega-3 (ω-3) FAs from vegetable sources (Dal Bello et al., 2015).

The addition of OP powders was associated with an increase in TDF levels. The higher increase was detected in the POPP-fortified yoghurts, which exhibited TDF amounts of 6.42 ± 0.03 g/100 g DW in Y-POPP and 7.57 ± 0.19 g/100 g DW in Y-POPP-OO, with no significant differences between both ($p > 0.05$). Similar TDF increase in yoghurt due to added

Table 2
Amount of individual phenolic compounds determined by HPLC (mg/100 g DW) in OP powders-fortified yoghurts supernatants.

mg/100 g DW	Hydroxytyrosol glucoside				Hydroxytyrosol				Tyrosol glucoside				Tyrosol
	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP
Phenolic amount	0.91 ± 0.11^{b}	2.33 ± 0.24^{a}	0.91 ± 0.06^{b}	nd	12.72 ± 0.98^{b}	16.46 ± 2.65^{a}	1.47 ± 0.33^{d}	5.47 ± 0.53^{c}	4.90 ± 0.33^{a}	4.10 ± 0.88^{a}	0.80 ± 0.05^{b}	1.37 ± 0.30^{b}	3.72 ± 0.15^{a}
Theoretical amount expected (TAE) *1	10.79	11.01	3.17	3.37	68.97	70.36	4.94	5.26	25.69	26.21	6.51	6.94	16.17
Maximum theoretical amount expected (MTAE)*2	10.79	11.01	3.17	3.37	83.62	85.30	42.82	45.63	25.69	26.21	6.51	6.94	16.17
Recovery in yoghurt formulation based in TAE (%)	8.43	21.16	28.74	0.00	18.44	23.39	29.80	103.91	19.07	15.64	12.28	19.74	23.01
Recovery in yoghurt formulation based in MTAE (%)	8.43	21.16	28.74	0.00	15.21	19.30	3.44	11.99	19.07	15.64	12.28	19.74	23.01

nd – not detected; nq – not quantifiable; na – not applicable. Results are the means of three determinations ± standard deviation. Values with different letters are significantly different, as determined by one-way ANOVA test ($p < 0.05$). *1 Based on the amount of free compounds quantified in OP powders. *2 Based on the amount of free and bound phenolic compounds quantified in OP powders.

fibre was achieved by Tseng & Zhao (2013) and Bertolino et al. (2015) using wine grape pomace and hazelnut skins, respectively. Regarding SDF and IDF content, the highest IDF concentration was observed in Y-POPP and Y-POPP-OO, as expected. However, the SDF content was similar for all yoghurts fortified with the OP powders as a result of the identical SDF content of LOPP and POPP ($p > 0.05$).

3.2. Fatty acid composition

The FAs profile in yoghurts with different OP powders, supplemented with 5% OO or not, is shown in Table 1. Addition of OP powders in yoghurt samples showed to have a lower effect on the amount of SFAs. In all yoghurt formulations, the most abundant SFA was palmitic acid (C16:0) as reported before in several yoghurt studies (Ardabilchi Marand et al., 2020; Baba et al., 2018; Van Nieuwenhove et al., 2019). LOPP, as a poor FAs source (**Supplementary material 1**), did not affect the FA content of yoghurt, but POPP seemed to have enhancer effect in UFAs. However, when LOPP or POPP were incorporated together with OO, a significant increase in UFAs occurred. Y-LOPP-OO and Y-POPP-OO showed a significantly higher UFAs content than the Y-control and Y-OO ($p < 0.05$). LOPP and POPP appeared to protect UFAs, principally the MUFAs. Y-LOPP-OO and Y-POPP-OO showed the most significant MUFA amounts. The OP powderś richness in phenolics seemed to enhance OO stability. Similar lipid protector effect was reported when a cocoa bean husk phenolic extract was added to extra virgin olive jam (Hernández-Hernández et al., 2019).

Oleic acid (C18:1 c9) was the most abundant MUFA in all yoghurt formulations, being significantly higher in Y-LOPP-OO and Y-POPP-OO ($p < 0.05$). PUFAs were also positively affected by the simultaneous addition of OO and OP powders ($p < 0.05$) when compared to Y-control and Y-OO. However, the POPP incorporation without OO (Y-POPP) also significantly increased the PUFAs amount when comparing with the Y-control. The addition of oleic acid, PUFAs or combinations of both to dairy products has been used to produce healthier products (Lopez--Huertas, 2010).

A normal balance between omega-6 (ω-6) and omega-3 (ω-3) in the range of 4:1 to 10:1 is also crucial in order to obtain healthier products (Ardabilchi Marand et al., 2020). ω-3 and ω-6 are essential FAs not synthesised by mammals and thus must be obtained from the diet. They include the ω-3 linoleic acid (LA, C18:2 c9 c12), ω-6 α - linoleic acid (ALA, α C18:3 c9 c12 c15) and ω-6 arachidonic acid (ARA, C20:4 c5 c8 c11 c14) (Román et al., 2019). After POPP incorporation (Y-POPP) the total ω-3 and ω-6 amount increased significantly ($p < 0.05$) when comparing with the Y-control. Y-LOPP-OO and Y-POPP-OO also exhibited significantly higher content of total ω-3 and ω-6 than both Y-control and Y-OO ($p < 0.05$). For that reason, all fortified yoghurts showed significant lower ω-6/ω-3 ratio than the controls, Y-LOPP-OO (8.53 ± 0.71), Y-POPP-OO (8.39 ± 0.19) against Y-OO control (9.93 ± 0.90) and Y-POPP (10.41 ± 0.44) and Y-LOPP (10.87 ± 0.32) against Y-control (11.56 ± 0.11).

The recommended ω-6/ω-3 ratio 4:1 to 10:1 ratio was achieved in both fortified yoghurts with OO and in Y-POPP. OP powders allowed to reduce ω-6/ω-3 ratio ca. 10% in Y-POPP and 26–27% in OO formulations (Y-LOPP-OO and Y-POPP-OO). In previous studies the addition of oil (Baba et al., 2018) and powder (Ardabilchi Marand et al., 2020) from flaxseed, which is an oilseed known by its low ω-6/ω-3 ratio, reduced in 29% and 89% the ω-6/ω-3 ratio of yoghurt samples, respectively. However, a ratio lower than 1:1 was obtained, which could compromise ω-6 metabolism (Simopoulos, 2002). Thus, the addition of single POPP and both OP powders with OO increased MUFA, and PUFA content yoghurts also improved the ω-6/ω-3 ratio.

Nutritional quality indices regarding the FA profile of yoghurt samples were calculated, namely the atherogenic (AI), thrombogenic (TI) and saturation indices (SI) (Table 1). TI and AI measure the influence of the different FAs ingested on coronary heart disease (de Alba et al., 2019). TI values relate to the tendency of forming clots in the blood vessels, defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenicity acids (MUFAs, ω-6 PUFAs and ω-3 PUFAs). AI correlates the risk of atherosclerosis, i.e. the increase of the level of blood cholesterol with the increase of the SFAs (C12:0, C14:0 and C16:0) or the decrease of the ∑MUFA, and ∑PUFA. The UFAs C12:0, C14:0 and C16:0 are considered pro-atherogenic and MUFAs and PUFAs, antiatherogenic. Low values for AI and TI are recommended (Ardabilchi Marand et al., 2020). In the present study, Y-POPP, Y-LOPP-OO and Y-POPP-OO exhibited significantly lower values of TI and AI than the Y-control ($p < 0.05$). TI and AI were reduced around 20% in Y-POPP, but the simultaneous addition of OO with OP powders (Y-LOPP-OO and Y-POPP-OO) allowed the reducing of TI and AI in 60–67%. Another good indicator of the nutritional value of dietary fat is the saturation index (SI). 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 C14:0, C16:0 and C18:0 compared to UFAs would be considered healthier foods (de Alba et al., 2019). Y-POPP, Y-LOPP-OO and Y-POPP-OO presented significantly lower SI values than the Y-control ($p < 0.05$). The reduction of TI, AI and SI by the POPP addition and by

Tyrosol			Caffeic acid				p-coumaric acid				Luteolin			
Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO
2.34 ± 0.62^{b}	1.00 ± 0.10^{c}	nd	0.48 ± 0.05^{a}	0.06 ± 0.01^{c}	0.35 ± 0.08^{b}	0.08 ± 0.02^{c}	0.30 ± 0.04^{a}	nd	0.32 ± 0.08^{a}	0.04 ± 0.01^{b}	nd	nd	1.82 ± 0.24^{a}	0.59 ± 0.13^{b}
16.49	6.73	7.18	2.59	2.64	1.35	1.44	1.18	1.20	2.13	2.27	0.00	0.00	5.14	5.48
16.49	6.73	7.18	10.95	11.17	20.62	21.97	0.00	0.00	7.15	7.62	0.00	0.00	7.15	7.62
14.19	14.85	0.00	18.56	2.27	25.83	5.54	25.44	0.00	15.00	1.76	0.00	0.00	35.38	10.76
14.19	14.85	0.00	4.38	0.54	1.70	0.36	7.68	0.00	4.54	0.53	0.00	0.00	25.45	7.74

simultaneous fortification with OO and OP powders could be used as an innovative strategy to increase the health appeal of high-fat yoghurts.

3.3. Phenolic compounds and antioxidant activity

OP powders-fortified yoghurts exhibited significant higher TPC content than the plain yoghurt (Y-control) ($p < 0.05$) (Fig. 1). Y-control exhibited TPC probably due to the presence of compounds in milk such as low molecular weight antioxidants, lactose, free amino acids, peptides, proteins or reducing compounds, which respond to the Folin–Ciocalteau photometric measurement (Chouchouli et al., 2013; Oliveira and Pintado, 2015).

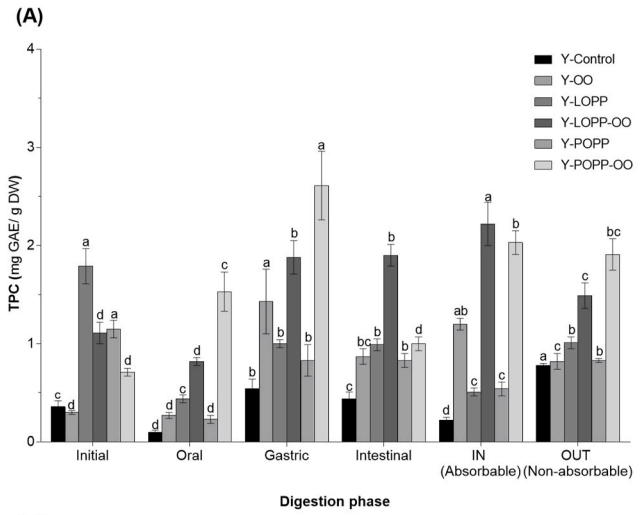
The addition of LOPP powder to yoghurts increased more significantly ($p < 0.05$) TPC values than POPP powder, following the trend observed in powders composition (**Supplementary material 1**). From all the formulations, the higher TPC was exhibited by Y-LOPP (179.38 ± 18.05 mg GAE/100 g DW), which resulted in a value of 143.42 mg GAE/100 g DW when corrected with the contribution from control yoghurt (35.96 ± 5.94 mg GAE/100 g DW). The Y-POPP formulation, besides being a source of DF, also contains a significant TPC content (114.74 mg ± 9.27 GAE/100 g DW), which is three times higher than the Y-control. On the other hand, the yoghurts formulated with 5% of OO and OP powders showed the lowest TPC values (half of the TPC values assessed to Y-LOPP and Y-POPP). These lower TPC values assessed in Y-LOPP-OO (110.81 ± 11.44 mg GAE/100 g DW) and Y-POPP-OO (71.07 ± 3.82 mg GAE/100 g DW) could be a consequence of the interactions between the higher amount of MUFAs/PUFAs and phenolics during yoghurt production. Phenolic compounds can protect lipids from lipid peroxidation reacting with the hydrophilic radicals and are eventually lost on preventing UFAs' oxidation, explaining the lower amount of TPC in yoghurts formulated with 5% of OO (Gorelik et al., 2013; Jakobek, 2015).

The most representative phenolic compounds were identified and quantified using HPLC in the methanolic extracts of OP-fortified yoghurts (Y-LOPP, Y-POPP, Y-LOPP-OO and Y-POPP-OO) (Table 2). No phenolic compounds were found in the control yoghurt (Y-control), nor in the yoghurt fortified with OO (Y-OO). The most typical phenolic compounds in the OP-fortified yoghurts were HYD and derivatives, in agreement with OP powders composition (**Supplementary material**).

Table 3
Antioxidant activity measured by ABTS, DPPH and ORAC (μM TE/g DW) and potential nutrition and health claims of fortified yoghurts.

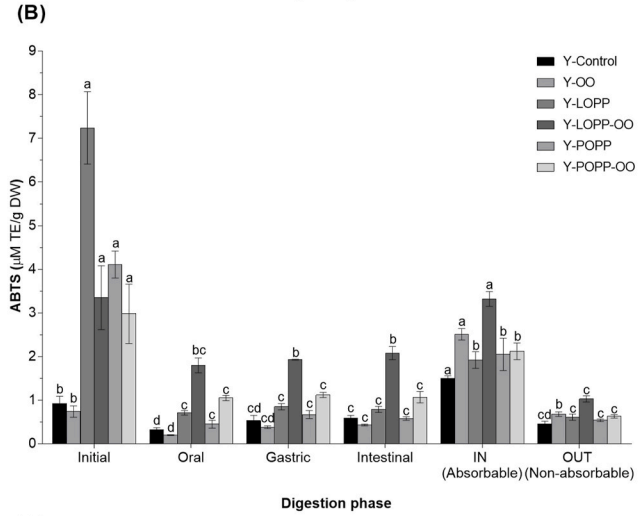
	Yoghurt formulations without olive oil			Yoghurt formulations with olive oil		
	Control	LOPP fortified	POPP fortified	Control	LOPP fortified	POPP fortified
Antioxidant activity						
ABTS	0.92 ± 0.17^{d}	7.24 ± 0.83^{a}	4.11 ± 0.31^{b}	0.74 ± 0.13^{d}	3.35 ± 0.73 ^{bc}	2.98 ± 0.68^{c}
DPPH	0.16 ± 0.04^{c}	2.88 ± 0.22^{a}	1.82 ± 0.11^{b}	0.45 ± 0.06^{c}	3.13 ± 0.71^{a}	2.79 ± 0.72^{a}
ORAC	6.68 ± 1.03^{d}	31.06 ± 6.42 ^{ab}	27.04 ± 5.82^{b}	17.79 ± 2.50^{c}	36.47 ± 4.62^{a}	26.40 ± 2.61^{b}
Total dietary fibre						
g/100 WW	ND	0.61 ± 0.04^{b}	1.07 ± 0.01^{a}	ND	0.52 ± 0.09^{b}	1.35 ± 0.03^{a}
g/100 kcal *1	ND	1.01 ± 0.07^{b}	1.76 ± 0.01^{a}	ND	0.84 ± 0.15^{b}	2.22 ± 0.06^{a}
Hydroxytyrosol and derivatives						
mg/100 g WW	ND	3.83 ± 0.20^{b}	0.70 ± 0.07^{c}	ND	4.79 ± 0.48^{a}	1.23 ± 0.13^{c}
mg/120 g WW *2	ND	4.60 ± 0.24^{b}	0.83 ± 0.08^{c}	ND	5.75 ± 0.58^{a}	1.48 ± 0.15^{c}
Potential claims		*Consumption of olive oil polyphenols contributes to the protection of blood lipids from oxidative damage*	Source of fibre		*Consumption of olive oil polyphenols contributes to the protection of blood lipids from oxidative damage*	*Source of fibre*

ND-non-detected; *1 A yoghurt plain - whole milk contains 61 kcal/100 g *2 A regular dose of solid yoghurt is 120 g. Results are the means of three determinations ± standard deviation. Values with different letters in the same line are significantly different, as determined by one-way ANOVA test ($p < 0.05$).



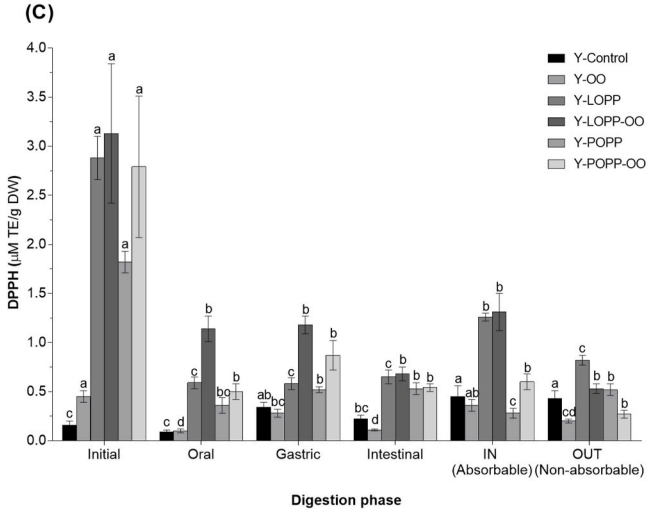
Recovery index (RI %) and bioaccessibility index (%) of OP powders-fortified yoghurts throughout SGD

Total phenolic compounds			Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO
Without yoghurt correction	RI (%)	*Oral*	24.70	74.08	20.42	215.15
		Gastric	55.48	169.45	60.08	357.02
		Intestinal	54.98	171.10	72.72	102.77
		IN	28.94	200.68	47.05	286.07
		OUT	28.49	134.89	72.58	214.73
	BI (%)		33.49	59.06	39.21	61.60
With yoghurt correction	RI (%)	*Oral*	24.82	67.80	16.47	306.17
		Gastric	27.07	54.26	36.15	309.78
		Intestinal	37.96	127.83	49.83	39.47
		IN	20.08	125.97	40.23	201.63
		OUT	16.09	82.90	6.99	263.86
	BI (%)		33.57	59.15	86.51	44.32



Recovery index (RI %) and remained activity (%) of OP powders-fortified yoghurts throughout SGD

Total phenolic compounds			Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO
Without yoghurt correction	RI (%)	*Oral*	9.84	53.63	10.87	35.34
		Gastric	11.79	57.59	16.26	37.35
		Intestinal	10.87	61.99	14.20	35.82
		IN	26.58	99.00	49.81	71.27
		OUT	8.46	30.82	13.05	21.21
	Remained activity (%)		75.87	59.06	78.80	77.02
With yoghurt correction	RI (%)	*Oral*	6.06	61.27	5.06	38.25
		Gastric	5.11	59.37	5.66	33.32
		Intestinal	3.39	63.01	0.00	28.43
		IN	7.63	30.87	20.90	0.00
		OUT	2.59	13.57	2.14	0.00
	Remained activity (%)		70.79	69.38	83.02	0.00



Recovery index (RI %) and remained activity (%) of OP powders-fortified yoghurts throughout SGD

Total phenolic compounds			Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO
Without yoghurt correction	RI (%)	*Oral*	20.58	36.54	19.94	18.01
		Gastric	20.00	37.77	28.72	31.16
		Intestinal	22.71	21.82	29.06	19.30
		IN	43.67	41.89	15.38	20.72
		OUT	28.61	16.95	28.78	9.61
	Remained activity (%)		60.44	70.98	36.74	74.22
With yoghurt correction	RI (%)	*Oral*	18.19	38.90	15.96	17.16
		Gastric	8.65	33.80	10.97	25.36
		Intestinal	15.99	21.35	18.67	18.28
		IN	29.62	35.39	0.00	11.21
		OUT	14.45	10.45	7.61	2.77
	Remained activity (%)		65.32	71.54	0.00	78.13

Fig. 2. Effect of in vitro gastrointestinal digestion on OP fortified-yoghurts total phenolics and 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 activity measured by ABTS (μM TE/g DW). (C) Antioxidant activity measured by DPPH (μM TE/g DW). Results are the means of three determinations ± standard deviation. Values with different letters above are significantly different, as determined by one-way ANOVA test ($p < 0.05$). Note: Without yoghurt correction - The amount of phenolic compounds or antioxidant activity in OP-fortified yoghurt was not corrected for the contribution of control yoghurt (Y-Control) and control yoghurt with olive oil (Y-OO; With yoghurt correction - The amount of phenolic compounds or antioxidant activity in OP-fortified yoghurt was corrected for the contribution of control yoghurt (Y-Control) to Y-LOPP and Y-POPP, and the contribution of control yoghurt with olive oil (Y-OO) to Y-LOPP-OO and Y-POPP-OO.

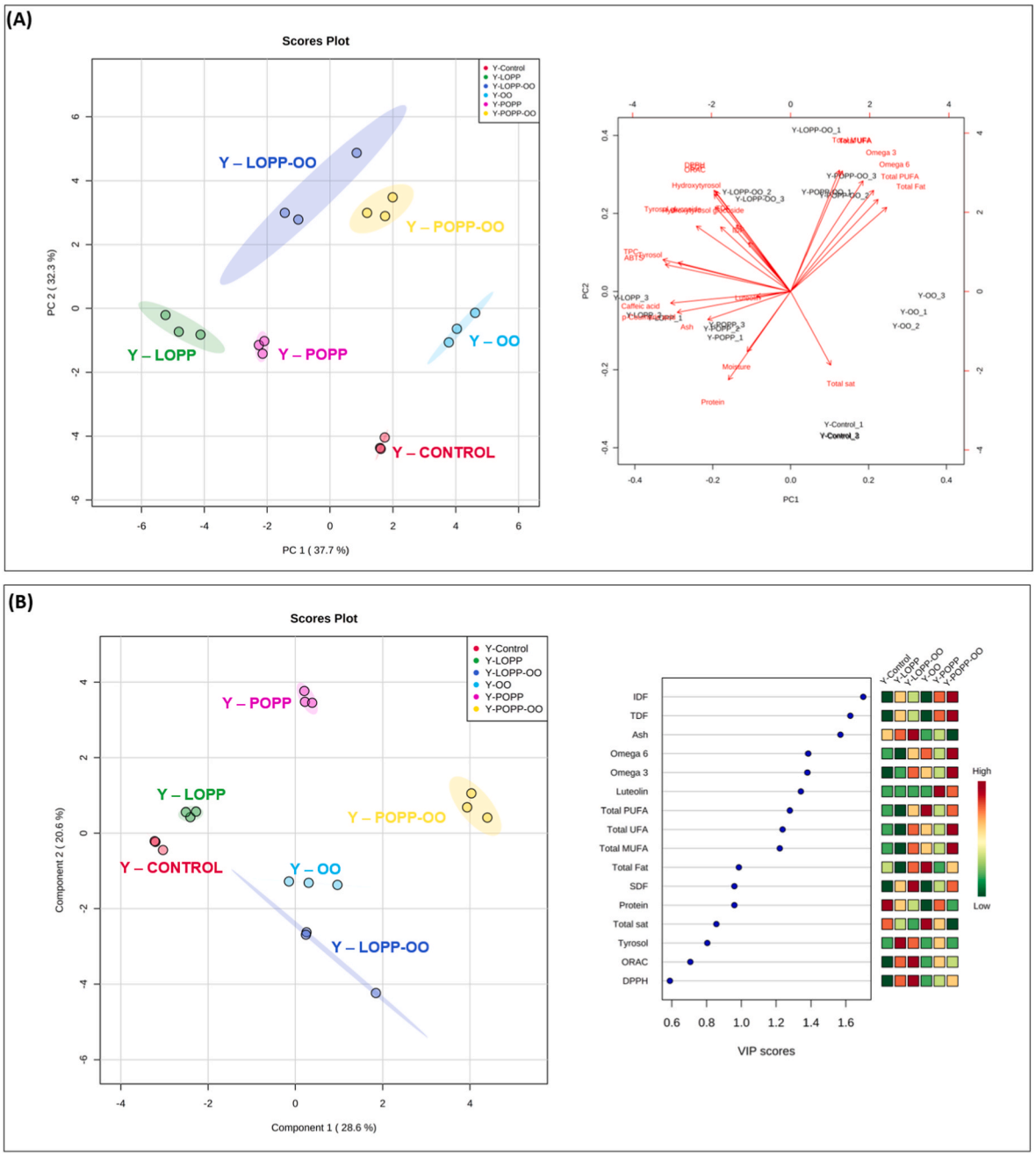


Fig. 3. PCA and PLS-DA of chemical and antioxidant properties characterisation of yoghurt formulations. (A) Scree plot of the principal component analysis and scores plot of chemical compounds and bioactivities identified in yoghurts formulations. (B) Partial Least Squares - Discriminant Analysis (PLS-DA) and VIP (Variable Importance in Projection) for component 1 of chemical compounds and bioactivities identified in yoghurts formulations following the PLS-DA model. VIP allowed to measure the variable's importance in the PLS-DA model. Green and red tiles, respectively, indicate a lower or higher intensity of chemical compounds and bioactivities amount in the mean of all yoghurt samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The results obtained by HPLC were also in line with TPC results. LOPP fortified yoghurts showed higher TPC plus higher HYD content than the formulations where POPP was added.

Other phenolics as luteolin, caffeic and p-coumaric acid were also detected in OP powders (**Supplementary material**). In the case of yoghurts, luteolin was only detected in Y-POPP (1.82 ± 0.24 mg/100 g DW) and Y-POPP-OO (0.59 ± 0.16 mg/100 g DW).

Comparing the formulations with the same OP powder without and with OO, it was evident that OO reduced TPC values in the yoghurts (Y-LOPP-OO and Y-POPP-OO), which could be related to the detection of significantly lesser amounts of luteolin, caffeic and p-coumaric acids ($p < 0.05$) in these yoghurt formulations. However, OO addition did not affect the content in HYD and derivatives negatively; on the contrary, Y-LOPP-OO showed a statistically significant higher amount of HYD and HYD glucoside than Y-LOPP ($p < 0.05$).

Even though OP fortified-yoghurts formulations exhibited considerable amounts of individual phenolics and TPC, only a part of added phenolics by OP powder addition remained in the final products (**Supplementary material**). A higher loss of total phenolics content occurred in Y-LOPP-OO (about 54%) rather than in Y-LOPP (about 13%). This higher loss was linked mainly to the lower recovery of caffeic and p-coumaric acid in Y-LOPP-OO formulation than in Y-LOPP (Table 2).

Table 4
Amount of Individual phenolic compounds determined by HPLC (mg/100 g DW) in OP powders-fortified yoghurts throughout simulated gastrointestinal digestion (SGD).

SGD phase	Hydroxytyrosol glucoside				Hydroxytyrosol				Tyrosol glucoside				Tyrosol
	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP
Initial	0.91 ± 0.11^{c}	2.33 ± 0.24^{b}	0.91 ± 0.06^{c}	nd	12.72 ± 0.98^{a}	16.46 ± 2.65^{a}	1.47 ± 0.33^{a}	5.47 ± 0.53^{a}	4.90 ± 0.33^{a}	4.10 ± 0.88^{a}	0.80 ± 0.05^{b}	1.37 ± 0.30^{b}	3.72 ± 0.15^{a}
Oral	0.15 ± 0.04^{d}	0.57 ± 0.12^{c}	0.15 ± 0.04^{b}	0.22 ± 0.11^{d}	2.02 ± 0.10^{b}	5.35 ± 1.25 ^{bc}	0.23 ± 0.06^{b}	1.11 ± 0.22^{b}	0.88 ± 0.05 ^{bc}	1.23 ± 0.18^{b}	0.53 ± 0.16^{a}	0.97 ± 0.10^{a}	0.56 ± 0.03^{b}
RI (%)	**17.48**	**24.26**	**16.10**	**na**	**15.84**	**32.49**	**15.38**	**20.25**	**17.98**	**30.05**	**70.44**	**23.58**	**15.17**
Gastric	nd	0.57 ± 0.02^{c}	nd	0.38 ± 0.18 ^{cd}	1.89 ± 0.15^{b}	5.35 ± 0.25^{b}	0.29 ± 0.02^{b}	1.11 ± 0.03^{b}	0.82 ± 0.09 ^{bc}	1.21 ± 0.12^{b}	0.70 ± 0.08^{a}	0.99 ± 0.10^{a}	nd
RI (%)	**0.00**	**24.49**	**0.00**	**na**	**14.85**	**36.06**	**19.58**	**20.22**	**16.78**	**29.56**	**87.15**	**24.14**	**0.00**
Intestinal	2.01 ± 0.17^{b}	5.16 ± 0.40^{a}	2.42 ± 0.18^{b}	4.28 ± 0.24^{a}	1.53 ± 0.08^{b}	2.47 ± 0.22 ^{cd}	0.14 ± 0.02^{b}	0.53 ± 0.05 ^{bc}	0.93 ± 0.11 ^{bc}	0.73 ± 0.09^{b}	0.50 ± 0.17^{a}	nd	nd
RI (%)	**221.28**	**221.32**	**266.04**	**na**	**12.06**	**15.00**	**9.34**	**9.75**	**19.03**	**17.90**	**62.83**	**0.00**	**0.00**
IN	0.78 ± 0.23^{c}	nd	1.10 ± 0.10^{c}	2.02 ± 0.09^{b}	0.43 ± 0.08^{c}	6.75 ± 0.35^{b}	nd	0.89 ± 0.04 ^{bc}	0.48 ± 0.00^{c}	nd	nd	nd	nd
RI (%)	**80.22**	**0.00**	**120.67**	**na**	**3.35**	**40.99**	**0.00**	**16.13**	**9.69**	**0.00**	**0.00**	**0.00**	**0.00**
OUT	3.13 ± 0.02^{a}	0.72 ± 0.10^{c}	2.93 ± 0.11^{a}	0.62 ± 0.10^{c}	1.37 ± 0.12 ^{bc}	0.61 ± 0.05^{d}	0.56 ± 0.00^{b}	0.15 ± 0.03^{c}	1.08 ± 0.25^{b}	0.23 ± 0.02^{b}	nd	nd	nd
RI (%)	**343.85**	**31.11**	**322.08**	**na**	**10.77**	**3.72**	**37.80**	**2.88**	**22.02**	**5.53**	**0.00**	**0.00**	**0.00**
BI (%)	**18.79**	**0.00**	**26.98**	**76.67**	**22.95**	**91.66**	**0.00**	**0.42**	**31.18**	**0.00**	**0.00**	**0.00**	**0.00**

nd – not detected; nq – not quantifiable; na – not applicable. Results are the means of three determinations ± standard deviation. Values with different letters are significantly different, as determined by one-way ANOVA test ($p < 0.05$).
Note: The initial amount before digestion (BC_{TF}) and the amounts detected in the digested sample for each digestion step (BC_{DF}) expressed in this table were used to calculate the Recovery Index (RI %) for each phenolic compound. On the other hand, to calculate the Bioaccessibility Index (BI %) of each phenolic compound, the BC_S which is the amount detected in the digested sample after the duodenal dialysis step (IN) and BC_{DFE} content which is the sum of the amounts after the duodenal step (IN + OUT) detected in this figure were used.

However, it is vital to stand out that the OP powders were added to homogenised milk, followed by pasteurisation, inoculation with yoghurt culture and fermentation, which could explain the loss of phenolic compounds. Chouchouli et al. (2013) verified equivalent losses when supplemented yoghurts with grape seed extract; indeed, in this study, control and fortified yoghurt revealed similar TPC and individual phenolics amount. As observed in other studies on yoghurt fortification (Helal and Tagliazucchi, 2018), Chouchouli et al., (2013) designed the study with the direct fortification of plain yoghurts, producing stirred fortified yoghurts. The main reasons to justify the reduction or absence of phenolics in fortified yoghurts was linked to phenolics-protein interactions which were enhanced by the heating/pasteurisation of the yoghurt mixture before inoculation (Chouchouli et al., 2013). The acidification of milk during yoghurt production, which results in gel formation (charge neutralisation of the protein particles in milk), decreases the binding capacity of milk proteins, and a fraction of added phenolics were unbound to be detected (Najgebauer-Lejko et al., 2011).

On the other hand, in comparison to Y-LOPP-OO, an increase (approx. 39%) and decrease (approx. 23%) of TPC were verified in Y-POPP and Y-POPP-OO, respectively. The protection of bound phenolics by POPP fibre could explain these higher TPC values during the yoghurt production **(Supplementary material**). Indeed, the RI (%) of almost all individual phenolics in POPP-fortified formulations was higher than in LOPP-fortified formulations (Table 2).

The AOX of plain yoghurt and OP-fortified yoghurts are shown in Table 3. Fortified yoghurts exhibited significantly higher radical scavenging activity than the plain yoghurt (Y-control) both in ABTS and in DPPH assays ($p < 0.05$). The free radicals quenching activity by hydrogen donation measured by ORAC was also higher in OP-fortified yoghurts than in Y-control ($p < 0.05$). The AOX observed in Y-control and Y-OO is mainly due to the formation of bioactive peptides with AOX because of the proteolytic activity of the starter lactobacilli used in yoghurt production (Helal and Tagliazucchi, 2018). Following the higher amount of AOX of LOPP compared to POPP (Supplementary material), LOPP-fortified yoghurts revealed statistically significant superior values of AOX than POPP-fortified yoghurts ($p < 0.05$) for all the methodologies tested. Nevertheless, the AOX retained in fortified yoghurts using ABTS methodology from the OP powders was similar to Y-LOPP (86%) and Y-POPP (85%), probably due to the role of fibre as a protector of phenolics (Jakobek and Matić, 2019). A lower ABTS retention was verified in OP-fortified yoghurts when OO was incorporated. The Y-POPP-OO still contained 61% of the expected ABTS from the supplementation with 2% of POPP, but Y-LOPP-OO only retained 37% of the ABTS value assessed previously to LOPP (1%). The higher loss of TPC and OP phenolics reported above in Y-LOPP-OO, and Y-POPP-OO formulations was probably linked to the higher AOX losses measured by ABTS in these yoghurts' samples. However, DPPH showed lower values than ABTS and higher AOX losses when LOPP (24%) was incorporated in yoghurt when compared with the incorporation of POPP (33–50%). On the other hand, the yoghurt formulations with and without OO showed similar DPPH retention values from LOPP and POPP. For example, Y-LOPP and Y-LOPP-OO showed similar retention values of DPPH from LOPP (around 24%). The addition of OO did not affect so negatively the DPPH values as when observed for ABTS, possibly due to the higher efficiency of DPPH in measuring the AOX of less polar compounds (Sadeer et al., 2020; Schaich et al., 2015). This superior DPPH's capacity to measure polar compounds could also explain the higher retention of DPPH from POPP addition with (33%) and without OO (50%). At least ORAC values showed similar behaviour to ABTS, with higher AOX losses in formulations with (50–90%) than without OO (37–76%). ORAC assay is based on the reaction of water and lipid-soluble substances with peroxyl free radical from ROS generator AAPH ((2,2′-azobis(2-methylpropionamidine) dihydrochloride)). The higher reactivity of AAPH with soluble compounds explained the higher ORAC recovery attained after incorporation of LOPP (49–62%) than POPP (10–24%) since LOPP is a water-soluble ingredient.

In conclusion, all methodologies used showed AOX losses when compared to the expected values to added OP powders. Similar results where the AOX of yoghurts was reduced due to the phenolic-protein interaction were previously reported to strawberry-fortified yoghurt

Tyrosol			Caffeic acid				p-coumaric acid				Luteolin			
Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO	Y-LOPP	Y-LOPP-OO	Y-POPP	Y-POPP-OO
2.34 ± 0.62^{a}	1.00 ± 0.10^{a}	nd	0.48 ± 0.05^{a}	0.06 ± 0.01^{c}	0.35 ± 0.08^{a}	0.08 ± 0.02 ^{bc}	0.30 ± 0.04^{a}	nd	0.32 ± 0.08^{a}	0.04 ± 0.01^{b}	nd	nd	1.82 ± 0.24^{a}	0.59 ± 0.13^{a}
1.88 ± 0.38^{a}	0.31 ± 0.09^{c}	nd	0.08 ± 0.00^{b}	nd	0.04 ± 0.02^{b}	0.13 ± 0.03^{a}	0.05 ± 0.01^{b}	nd	0.03 ± 0.01^{b}	0.12 ± 0.02^{a}	nd	nd	0.32 ± 0.04^{b}	0.41 ± 0.06 ^{ab}
80.17	**32.91**	**na**	**15.98**	**0.00**	**11.66**	**168.16**	**13.08**	**0.00**	**8.09**	**293.11**	**na**	**na**	**17.70**	**69.24**
1.88 ± 0.18^{a}	nd	nd	0.07 ± 0.01^{b}	0.16 ± 0.01^{a}	0.04 ± 0.01^{b}	0.11 ± 0.02 ^{ab}	nd	0.11 ± 0.01	0.05 ± 0.01^{b}	0.12 ± 0.02^{a}	nd	nd	0.30 ± 0.05^{b}	0.42 ± 0.09 ^{ab}
80.47	**0.00**	**na**	**14.56**	**270.89**	**11.97**	**141.87**	**14.21**	**na**	**14.40**	**293.56**	**na**	**na**	**16.43**	**71.42**
nd	nd	nd	0.04 ± 0.00^{b}	0.09 ± 0.01^{b}	0.02 ± 0.00^{b}	0.06 ± 0.00^{c}	0.02 ± 0.00^{b}	nd	0.02 ± 0.00^{b}	0.06 ± 0.00^{b}	nd	nd	0.22 ± 0.01^{b}	0.22 ± 0.01 ^{bc}
0.00	**0.00**	**na**	**7.60**	**19.18**	**6.11**	**75.84**	**0.00**	**0.00**	**5.54**	**147.78**	**na**	**na**	**12.13**	**37.94**
nd	0.62 ± 0.06^{b}	nd	nd	nd	nd	nq	nd	nd	nd	nq	nd	nd	nd	nd
0.00	**62.36**	**na**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**na**	**na**	**0.00**	**0.00**
nd	nd	nd	0.04 ± 0.01^{b}	nd	0.03 ± 0.00^{b}	nq	0.02 ± 0.01 ^{bc}	nd	0.03 ± 0.00^{b}	nq	nd	nd	0.23 ± 0.00^{b}	0.15 ± 0.00^{c}
0.00	**0.00**	**na**	**8.66**	**0.00**	**8.26**	**0.00**	**9.41**	**0.00**	**8.39**	**0.00**	**na**	**na**	**12.57**	**24.96**
0.00	**100.00**	**na**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**na**	**na**	**0.00**	**0.00**

(Oliveira and Pintado, 2015). However, it is necessary to mention that only AOX of free or unbounded phenolics was quantified in the supernatant of yoghurt samples. The action of the digestive enzymes could liberate the phenolics bounded to proteins and also to DF and thus become available to being absorbed by the human intestine and exert its AOX potential (Jakobek, 2015).

3.4. Nutritional and antioxidant properties of the yoghurt formulations obtained by addition of OP powders and olive oil

Regarding nutritional claims, both Y-POPP and Y-POPP-OO (2% POPP) fulfilled the condition of being a "source of fibre" (>1.5 g of fibre per 100 kcal) (European Union, 2006) (Table 2). Y-POPP-OO (2.22 ± 0.06 g/100 kcal) and Y-POPP (1.76 ± 0.01) exhibited a TDF amount of about 2.00 g/100 kcal. On the other hand, in the case of LOPP, to achieve the fibre content required to bear the claim "source of fibre", it would be necessary to ensure an amount of at least 3%.

Additionally, the objective of obtaining a yoghurt with a healthier FA profile, i.e. a ratio between SFAs and UFAs more equilibrated, was also attained by the addition of POPP (34% UFAs which 31% are MUFAs) and both OP powders together with OO (LOPP: 47% UFAs/MUFAs; POPP: 54% UFAs which 51% are MUFAs). The MUFA and PUFA content was increased in Y-POPP, Y-LOPP-OO and Y-POPP-OO. Besides that, these yoghurt formulations exhibited ω-6/ω-3 ratios improved to a healthier range (≤than 10).

The aim of achieving a yoghurt rich in HYD and derivatives was attained in the two formulations with LOPP. Both formulations with LOPP could provide the amount of HYD and derivatives (5 mg) in a regular dose of standard yoghurt (120 g) that would be needed to protect LDL particles from oxidative damage, according to the health claim approved by the EFSA only to OO until now (Table 2). Indeed, LOPP-OO (5.75 ± 0.58 mg/120 g WW yoghurt) exhibited a higher amount than Y-LOPP (5.44 ± 0.33 mg/120 g WW yoghurt). Despite the higher recovery of phenolics from POPP than LOPP due to the liberation of the bound phenolics present in DF of POPP during yoghurt fermentation, the yoghurts fortified with POPP supply only about 1 mg of HYD and derivatives in a regular dose of a standard yoghurt (120 g). This is explained by the fact that POPP has a lower amount of HYD and derivatives. POPP and POPP-OO contained 0.83 ± 0.33 mg/120 g WW yoghurt and 1.48 ± 0.15 mg/120 g WW yoghurt, respectively.

The positive effect of OO addition, together with OP powders, was supported by the chemical and bioactives analyses. PCA and PLS-DA were applied to reduce the multidimensional structure of the data and provided a two-dimensional map to understand the nutritional and antioxidant variance of yoghurt formulations after the addition of OP powders and OO. The scree plot of PCA analysis and scores plot of the yoghurt formulations are presented in Fig. 2 (A). The scree plot indicates that the first two principal components account for 70% of the total variance (PC1 = 37.7% and PC2 = 32.3%). PCA revealed separate clusters for each yoghurt formulation. Clusters of control yoghurts (Y-control and Y-OO) were close to each other in quadrant II separated from OP fortified – yoghurts. According to the scores plot, the main difference between control and OP fortified yoghurts was the content in total UFAs. The OP - fortified yoghurts without OO (Y-LOPP and Y-POPP) were in quadrant III and IV. On the other hand, OP fortified yoghurts with OO (Y-LOPP-OO and Y-POPP-OO) were positioned in quadrant I. The main difference between OP-fortified yoghurts with and without OO was the UFAs content (UFA, MUFA, PUFA, ω-6 and ω3).

PLS-DA of the chemical components and bioactivities also revealed separate clusters for each yoghurt formulation as evidenced in Fig. 2 (B). PLS-DA maximises the covariance between X (data) and Y (group). Variable importance in projection (VIP) was obtained (Fig. 2 (B)) in order to understand better, the differences observed between the different clusters of OP powders – yoghurt formulations. Markers assigned a VIP score >0.6 were counted as the 16 most significant compounds, which define the differences in terms of nutritional and bioactive properties of yoghurt formulations in component 1 and component 2. IDF, TDF, ash, luteolin and UFA content (ω-3, ω-6, total UFA, total MUFA, total PUFA) were the most significant variants (VIP > 1.2) associated to both components. These relevant variants explain the separation of yoghurts fortified with POPP (Y-POPP and Y-POPP-OO) from the other yoghurt formulations.

After analyses of PCA and PLS-DA, yoghurt formulations with POPP (Y-POPP and Y-POPP-OO) were substantially different from the other formulations due to their content in TDF, IDF and luteolin. Yoghurts with LOPP (L-POPP and Y-LOPP-OO) distinguished from the other yoghurt formulations by their content in HYD and its derivatives and AOX. At last, the UFA content was the main reason for the main

differences observed between OP-fortified yoghurts with controls (Y-control and Y-OO).

It is also important to refer that OP-fortified yoghurts could be considered an excellent example of the newly emerging food category – "sustainable food products". "Sustainable food products" are new value-added foods with higher nutritional properties formulated using ingredients developed from by-products generated during the manufacturing of other foods. Nowadays, the preferences of consumers for this new emerging food category it is rising, not only for its environmentally sustainable character but also by health concerns related to consumers' preferences for natural food products (Coderoni and Perito, 2020).

In the future, sensorial analyses of the developed yoghurts should be performed to validate if the levels of LOPP (1%), POPP (2%) and OO (5%) used to achieve the nutritional and health claims influence the sensory properties of the yoghurts negatively. Some studies reported adverse effects as very bitter and spicy taste after 10% (w/w) OP incorporation into bread and spaghetti. However, no significative sensorial negative effects were reported when olive mill wastewater was used to replace the water in the bread and spaghetti formulations (Cedola et al., 2020). On the other hand, Di Nunzio et al. (2020) established a limit of organoleptic acceptance in a consumer preference test using 2.5 or 4% of OP into biscuits and bread. To be noted, in our study, lower LOPP and POPP concentrations for the enrichment of yoghurt were chosen to guarantee a most promising organoleptic acceptance in a future consumer preference test. Moreover, the incorporation of sweeteners and other bitterness masking ingredients showed to be a viable option to reduce the potential negative sensorial impact of olive phenolics into fruit smoothies (Kranz et al., 2010). This option could be explored in the future to improve potential negative effects on organoleptic attributes of OP-fortified yoghurts.

3.5. *Evolution of phenolic compounds and antioxidant activity throughout the gastrointestinal tract*

The alterations in TPC in the yoghurt samples during the SGD are shown in Fig. 3. Regarding TPC content, all yoghurt formulations were significantly affected by SGD ($p < 0.05$). The OP-yoghurt formulations showed the highest TPC values in all SGD phases when compared to its yoghurt controls (Y-control and Y-OO). The TPC values measured in OP-fortified yoghurts during SGD can be explained by the presence of individual phenolic compounds, as reported in Table 4. However, both control yoghurts (Y-control and Y-OO) also showed substantial TPC amount in all SGD phases, principally the Y-OO formulation. Since no phenolic compounds were detected in Y-control and Y-OO by HPLC after and before SGD, its TPC values possibly reflect phenolic compounds related to milk protein breakdown. For example, the amino acid tyrosine has a phenolic side chain suggested the rise in the TPC reading (Amirdivani and Baba, 2011).

Between all digestion phases, oral steps affected more negatively TPC content for all OP fortified-yoghurt formulations except for the Y-POPP-OO. In the mouth step, the recovery indexes (RI%) after control yoghurt correction varied from 16.47% (Y-POPP) < 24.82% (Y-POPP) < 67.80% (Y-LOPP-OO) < 306.17% (Y-POPP-OO). During the gastric step, TPC values increased and then in the intestine increased or were maintained to all OP-fortified yoghurts, except for Y-POPP-OO. The TPC values increased during gastric digestion in yoghurts fortified with phenolics. Previous studies explained that this increase could be mainly attributed to the acidic pH and enzymatic activity during the gastric phase, which can induce the hydrolysis of some phenolic compounds bound to proteins, or even to lipids, of the yoghurt matrix (Helal and Tagliazucchi, 2018; Oliveira and Pintado, 2015). The free form of phenolics after stomach normally turns these compounds more sensitive to degradation in the intestine due to the intestinal alkaline conditions (Oliveira and Pintado, 2015). However, the negative effect of the alkaline pH of the intestine was only verified in Y-POPP-OO.

During Y-POPP-OO digestion, high TPC values were reported in the mouth (RI = 306.17%) and stomach (RI = 309.78%) followed by a significant decrease in the intestine (RI = 39.47%). A higher liberation of caffeic and p-coumaric acids occurred during the oral and gastric phase, decreasing during the intestinal phase. HYD and tyrosol glucoside amount in Y-POPP-OO also decreased during the intestinal step. Another factor that could decrease TPC values in the intestine could be related to the higher fat content of Y-POPP-OO. Fat digestion takes place mainly in the duodenum where emulsions formed during mastication are exposed to several surface active-components and lipases carry out a process of lipolysis, i.e. a breakdown of lipids into smaller particles which can then be absorbed (Jakobek, 2015). Several studies supported the inhibition of the lipase activity and fat absorption process by phenolic compounds (Paz-Yépez et al., 2019). Lipid-phenolics interaction might also help in delivering phenolics into the lower parts of the gastrointestinal tract (Jakobek, 2015). An increase of TPC was observed during intestinal absorption phase to Y-POPP-OO.

On the other hand, OP-fortified yoghurt (Y-LOPP, Y-LOPP-OO, Y-POPP) exhibited an increase of TPC values after intestinal digestion, which could be related to the increase of HYD glucoside amount in all these yoghurt formulations. A similar increase was obtained in cinnamon-fortified yoghurts at the end of the intestinal phase (Helal and Tagliazucchi, 2018). The main reason for this increase could be associated with the hydrolysis of caseins during the intestinal phase, which allows the release of the bound phenolic compounds in the intestine. However, a decrease in TPC occurred in all these yoghurt formulations during intestinal absorption.

Comparing OP powders, at the end of intestinal digestion, Y-LOPP (0.54 ± 0.05 mg GAE/g DW) exhibited slightly higher TPC value than Y-POPP (0.39 ± 0.07 mg GAE/g DW) after yoghurt correction. Nevertheless, as a lower source of phenolic compounds, POPP exhibited a higher RI than LOPP after the intestinal step and during intestinal absorption. These higher releases of phenolic compounds could be justified by the higher liberation of the glucosidic form of phenolics and p-coumaric linked to DF, as reported in Table 4. The higher release of HYD glucoside, tyrosol glucoside and p-coumaric during intestinal absorption could be linked 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.

Despite the importance of the recovery in each digestion phase, phenolics will need to be released from their food matrix and reach the intestine in order to be bioavailable, so they can become absorbable (bioaccessible), meaning that they can be absorbed by intestinal cells and be metabolised. Between all OP-fortified yoghurt formulations, Y-POPP also showed the highest bioaccessibility index (BI%) of TPC (86.51%), but also the highest BI for tyrosol (100%). In comparison, POPP-OO showed higher TPC values in the absorbable fraction (IN) than Y-POPP, but a higher amount of phenolics were retained in non-absorbable (OUT) of Y-POPP-OO, which decreased its BI to 44.32%. Among LOPP-fortified yoghurts, OO incorporation seemed to increase the bioaccessibility of phenolics. Y-LOPP-OO showed not only higher BI values (59.15%) than Y-LOPP for TPC but also higher BI values for HYD. Y-LOPP-OO (91.66%) showed a BI four times higher for HYD than Y-LOPP (22.95%). This positive effect of OO in HYD absorption was reported before in a rat model study, where HYD absorption from a lipid-rich matrix (OO) was higher (≈25%) than that from an aqueous solution (Bohn, 2014) or low-fat yoghurt (Visioli et al., 2003). The metabolization of these bioaccessible olive phenolics could exert several biological properties and to have a potential role in the prevention of various inflammatory diseases. Recent studies with OP-enriched water extracts (Di Nunzio et al., 2018) and bakery-enriched products (Di Nunzio et al., 2020) demonstrated a significant anti-inflammatory effect, significantly reducing IL-8 secretion in Caco-2 cells. Futures studies

about the potential anti-inflammatory activity of OP-fortified yoghurts with OO need to be assessed.

Despite the low BI of Y-LOPP, not only for HYD (22.95%) and TPC (33.57%) but also for HYD glucoside (18.79%) and tyrosol glucoside (31.18%), a significant amount of phenolics were available in the non-absorbable fraction (OUT) to be metabolised by the microbiota. This may increase the amount of phenolics metabolites and their potential biological activities on the gut as promotors of the growth of healthy bacteria (Liu et al., 2019), as anti-inflammatory agents and as protectors of the Caco-2 intestinal mucosal cells against the cytostatic and cytotoxic effect of oxidised LDL (Bonechi et al., 2019). On the other hand, Y-POOP and Y-POPP-OO have also shown to contain a significant amount of phenolics in OUT fraction per 100 g DW, which includes HYD glucoside (Y-POPP: 2.93 ± 0.11 mg; Y-POPP-OO: 0.62 ± 0.10), HYD (Y-POPP: 0.56 ± 0.10 mg; Y-POPP-OO: 0.15 ± 0.03 mg) and luteolin (Y-POPP: 0.23 ± 0.00 mg; Y-POPP-OO: 0.15 ± 0.00 mg). HYD, as mentioned above, is a potent antioxidant agent with several health benefits and luteolin has been pointed out as a potent intestinal anti-inflammatory agent by different mechanisms using in vitro gut inflammation models (Mizuno and Nishitani, 2013). Recently the ingestion of OP-enriched biscuits showed not only to increase the metabolic output of the gut microbiota significantly but also, to boost the homovanillic acid and DOPAC levels involved in reducing oxidative LDL cholesterol (Conterno et al., 2019). Moreover, the administration of OP as feed supplemented showed potential to be used aimed at the production of meat or dairy products enriched with functional lipids through the modification of gut microbiota composition (Romani et al., 2019). These potential gut health benefits of LOPP and POPP-fortified yoghurts need to be explored in more detail in future studies.

Changes in radical scavenging activity were also evaluated during the in vitro digestion, and the data are presented in Fig. 3. The radical scavenging activity of OP-fortified yoghurts decreased after mouth phase in both the assays during digestion because of the loss of individual phenolic compounds reported in this digestion phase. After the oral phase, the AOX values of both methods were maintained until the intestinal phase, with no significant differences ($p < 0.05$). During intestinal absorption, a significant increase of AOX was observed for all OP-fortified yoghurts in the absorbable fraction (IN) ($p < 0.05$), even after yoghurt control correction, except for Y-POPP in DPPH method and Y-POPP-OO in ABTS method. These differences were also expressed in the percentage of the remained AOX attained in the absorbable fraction (IN). After SGD, according to ABTS method, Y-POPP revealed to have 83.03% of AOX that reach intestine accessible in the absorbable fraction, but Y-POPP-OO had no AOX accessible. However, when AOX was measured using DPPH, a counter behaviour was verified: Y-POPP did not have accessible AOX, and 78.13% of the AOX of Y-POPP-OO that reached intestine was accessible to be metabolised. The main reason for such distinct AOX values in the different methodologies applied is linked to the phenolic composition of the IN fraction. From the phenolics identified in yoghurts, Y-POPP revealed to contain HYD glucoside and tyrosol in fraction IN, and Y-POPP-OO contained only a small amount of HYD. The higher percentage of the remained ABTS in Y-POPP could be related to their affinity with more polar compounds like tyrosol and HYD glucoside.

On the other hand, the HYD that is bioaccessible in Y-POPP-OO was probably delivered by a fat fraction, which explains the higher AOX measured by DPPH. Another critical feature of tyrosol and its derivatives is its lower AOX in comparison to HYD (González et al., 2019). The AOX of tyrosol is only as hydroxyl radical scavenger or at most α-tocopherol regenerator (Bonechi et al., 2019). None of the mechanisms of action of tyrosol was individually evaluated by the AOX assays used. This lower AOX of tyrosol explained the reduced reactivity of the IN fraction of Y-POPP with DPPH since DPPH radicals are lesser reactive than ABTS radicals (Hsu et al., 2011). However, it is essential to highlight that the conversion of tyrosol into HYD reported in vivo in humans allowed expecting an AOX higher than the one reported (Boronat et al., 2019).

Regarding yoghurt formulations with LOPP, in Y-LOPP-OO (ABTS: 69.38%; DPPH: 71.54%) a higher percentage of AOX was attained in the absorbable fraction (IN) for both AOX methodologies than in Y-LOPP (ABTS: 70.79%; DPPH: 65.32%). This result was intrinsically linked to the higher BI verified for HYD in Y-LOPP-OO. The AOX variations throughout the SGD allowed understanding that phenolic compounds bioaccessibility has an essential role in AOX.

OP powder-fortified yoghurt showed substantial phenolic content with high BI after SGD and therefore may be used to deliver HYD and its derivatives into the human diet. Although the reduction of AOX verified after SGD, the AOX values attained for OP-fortified yoghurts permitted these yoghurt formulations to be considered as good candidates to create an antioxidant environment in the gastrointestinal tract. Some approaches, like the application of nanoemulsions or acidified milk gels, could be useful strategies for improving stability and AOX of OP powder phenolics after SGD (Villalva et al., 2020).

4. Conclusion

Olive pomace powders were successfully employed as functional ingredients into the fortification of yoghurt. By consuming a standard yoghurt (120 g) the Y–POPP formulation fulfilled the condition of being a “source of fibre”, and the Y-LOPP formulation provided the 5 mg of hydroxytyrosol and derivatives needed to protect LDL particles from oxidative damage. POPP also improved improved the quality of the fatty acid profile of yoghurts, increasing the amount of monounsaturated and polyunsaturated fatty acids. Olive oil addition together with olive pomace powders, enhanced its action as a source of unsaturated fatty acids and made hydroxytyrosol more stable after yoghurt fermentation, and also, more bioaccessible after in vitro digestion. Therefore, olive pomace powders can be considered a key source of dietary bioaccessible phenolics, fibre and unsaturated fatty acids. Taking into account the significative amount of olive pomace produced annually, the incorporation of its powders into dairy products could be a straightforward way to increase the economic and environmental sustainability of olive oil sector, but also to dairy sector offers to its consumers “sustainable food products” with the benefits of dietary fibre, unsaturated fatty acids and of the health claimed olive oil phenolics – hydroxytyrosol and derivatives. This study brings new insights to help spread the circular bioeconomy concept through the whole food sector.

The results obtained in this study should be compared with additional in vivo studies to correlate the bioaccessibility of bioactive compounds between in vivo and in vitro methodologies, but also to validate the health claimed benefits of hydroxytyrosol and derivatives in other food matrices than olive oil. Sensorial analyses of the developed yoghurts should also be performed.

CRediT author statement

Tânia Bragança Ribeiro: Conceptualization, Investigation, Formal analysis, Writing – original draft. Teresa Bonifácio – Lopes: Investigation. Pilar Morais: Conceptualization, Writing – review & editing. Arménio Miranda: Conceptualization, Investigation. João Nunes: Supervision, Writing – review & editing. António A. Vicente: Supervision, Writing – review & editing. Manuela Pintado: Resources, Validation, Supervision, Conceptualization, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

AGAPA, 2015. Evaluación de la producción y usos de los subproductos de las agroindustrias del olivar en Andalucía. Cons. Agric. Pesca y Desarro. https://doi.org/10.13140/RG.2.2.14921.39520. https://www.juntadeandalucia.es/agriculturapesca/observatorio/servlet/FrontController?ec=default&action=DownloadS&table=11030&element=1585171&field=DOCUMENTO.

Aliakbarian, B., Casale, M., Paini, M., Casazza, A.A., Lanteri, S., Perego, P., 2015. Production of a novel fermented milk fortified with natural antioxidants and its analysis by NIR spectroscopy. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 62, 376–383. https://doi.org/10.1016/j.lwt.2014.07.037.

Amirdivani, S., Baba, A.S., 2011. Changes in yogurt fermentation characteristics, and antioxidant potential and in vitro inhibition of angiotensin-1 converting enzyme upon the inclusion of peppermint, dill and basil. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 44, 1458–1464. https://doi.org/10.1016/j.lwt.2011.01.019.

AOAC, 1990. In: AOAC Official Methods of Analysis, 15th Ed, Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Washington Dc.

Baba, W.N., Jan, K., Punoo, H.A., Wani, T.A., Dar, M.M., Masoodi, F.A., 2018. Techno-functional properties of yoghurts fortified with walnut and flaxseed oil emulsions in guar gum. LWT 92, 242–249. https://doi.org/10.1016/j.lwt.2018.02.007.

Bertolino, M., Belviso, S., Dal Bello, B., Ghirardello, D., Giordano, M., Rolle, L., Gerbi, V., Zeppa, G., 2015. Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 63, 1145–1154. https://doi.org/10.1016/j.lwt.2015.03.113.

Bohn, T., 2014. Dietary factors affecting polyphenol bioavailability. Nutr. Rev. 72, 429–452. https://doi.org/10.1111/nure.12114.

Bonechi, C., Donati, A., Tamasi, G., Pardini, A., Rostom, H., Leone, G., Lamponi, S., Consumi, M., Magnani, A., Rossi, C., 2019. Chemical characterization of liposomes containing nutraceutical compounds: tyrosol, hydroxytyrosol and oleuropein. Biophys. Chem. 246, 25–34. https://doi.org/10.1016/j.bpc.2019.01.002.

Boronat, A., Mateus, J., Soldevila-Domenech, N., Guerra, M., Rodríguez-Morató, J., Varon, C., Muñoz, D., Barbosa, F., Morales, J.C., Gaedigk, A., Langohr, K., Covas, M., Pérez-Mañá, C., Fitó, M., Tyndale, R.F., de la Torre, R., 2019. Cardiovascular benefits of tyrosol and its endogenous conversion into hydroxytyrosol in humans. A randomized, controlled trial. Free Radic. Biol. Med. 143, 471–481. https://doi.org/10.1016/j.freeradbiomed.2019.08.032.

Cedola, A., Cardinali, A., D'Antuono, I., Conte, A., Del Nobile, M.A., 2020. Cereal foods fortified with by-products from the olive oil industry. Food Biosci 33, 100490. https://doi.org/10.1016/j.fbio.2019.100490.

Cho, W.Y., Yeon, S.J., Hong, G.E., Kim, J.H., Tsend-Ayush, C., Lee, C.H., 2017. Antioxidant activity and quality characteristics of yogurt added green olive powder during storage. Korean J. Food Sci. Anim. Resour. 37, 865–872. https://doi.org/10.5851/kosfa.2017.37.6.865.

Cho, W.-Y., Kim, D.-H., Lee, H.-J., Yeon, S.-J., Lee, C.-H., 2020. Quality characteristic and antioxidant activity of yogurt containing olive leaf hot water extract. CyTA - J. Food 18, 43–50. https://doi.org/10.1080/19476337.2019.1640797.

Chouchouli, V., Kalogeropoulos, N., Konteles, S.J., Karvela, E., Makris, D.P., Karathanos, V.T., 2013. Fortification of yoghurts with grape (Vitis vinifera) seed extracts. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 53, 522–529. https://doi.org/10.1016/j.lwt.2013.03.008.

Coderoni, S., Perito, M.A., 2020. Sustainable consumption in the circular economy. An analysis of consumers' purchase intentions for waste-to-value food. J. Clean. Prod. 252, 119870. https://doi.org/10.1016/j.jclepro.2019.119870.

Conterno, L., Martinelli, F., Tamburini, M., Fava, F., Mancini, A., Sordo, M., Pindo, M., Martens, S., Masuero, D., Vrhovsek, U., Dal Lago, C., Ferrario, G., Morandini, M., Tuohy, K., 2019. Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. Eur. J. Nutr. 58, 63–81. https://doi.org/10.1007/s00394-017-1572-2.

Costa, J.R., Amorim, M., Vilas-Boas, A., Tonon, R.V., Cabral, L.M.C., Pastrana, L., Pintado, M., 2019. Impact of in vitro gastrointestinal digestion on the chemical composition, bioactive properties, and cytotoxicity of Vitis vinifera L. cv. Syrah grape pomace extract. Food Funct 10, 1856–1869. https://doi.org/10.1039/C8FO02534G.

Crizel, T. de M., Hermes, V.S., de Oliveira Rios, A., Flôres, S.H., 2016. Evaluation of bioactive compounds, chemical and technological properties of fruits byproducts powder. J. Food Sci. Technol. 53, 4067–4075. https://doi.org/10.1007/s13197-016-2413-7.

Dal Bello, B., Torri, L., Piochi, M., Zeppa, G., 2015. Healthy yogurt fortified with n-3 fatty acids from vegetable sources. J. Dairy Sci. 98, 8375–8385. https://doi.org/10.3168/jds.2015-9688.

de Alba, M., Pérez-Andrés, J.M., Harrison, S.M., Brunton, N.P., Burgess, C.M., Tiwari, B. K., 2019. High pressure processing on microbial inactivation, quality parameters and nutritional quality indices of mackerel fillets. Innovat. Food Sci. Emerg. Technol. 55, 80–87. https://doi.org/10.1016/j.ifset.2019.05.010.

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., Michaud, P., 2013. Olive mill wastes: biochemical characterizations and valorization strategies. Process Biochem. 48, 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

Di Nunzio, M., Picone, G., Pasini, F., Caboni, M.F., Gianotti, A., Bordoni, A., Capozzi, F., 2018. Olive oil industry by-products. Effects of a polyphenol-rich extract on the metabolome and response to inflammation in cultured intestinal cell. Food Res. Int. 113, 392–400. https://doi.org/10.1016/j.foodres.2018.07.025.

Di Nunzio, M., Picone, G., Pasini, F., Chiarello, E., Caboni, M.F., Capozzi, F., Gianotti, A., Bordoni, A., 2020. Olive oil by-product as functional ingredient in bakery products. Influence of processing and evaluation of biological effects. Food Res. Int. 131, 108940. https://doi.org/10.1016/j.foodres.2019.108940.

EFSA, 2011. Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), mainte. EFSA J 9, 2033. https://doi.org/10.2903/j.efsa.2011.2033.

Eurpean Union, 2006. REGULATION (EC) No 1924/2006 of the EUROPEAN PARLIAMENT and of the COUNCIL of 20 December 2006 on Nutrition and Health Claims Made on Foods, vol. 404. Off. J. Eur. Union L.

García-Lomillo, J., González-SanJosé, M.L., Del Pino-García, R., Rivero-Pérez, M.D., Muñiz-Rodríguez, P., 2014. Antioxidant and antimicrobial properties of wine byproducts and their potential uses in the food industry. J. Agric. Food Chem. 62, 12595–12602. https://doi.org/10.1021/jf5042678.

González, E., Gómez-Caravaca, A.M., Giménez, B., Cebrián, R., Maqueda, M., Martínez-Férez, A., Segura-Carretero, A., Robert, P., 2019. Evolution of the phenolic compounds profile of olive leaf extract encapsulated by spray-drying during in vitro gastrointestinal digestion. Food Chem. 279, 40–48. https://doi.org/10.1016/j.foodchem.2018.11.127.

Gorelik, S., Kanner, J., Schurr, D., Kohen, R., 2013. A rational approach to prevent postprandial modification of LDL by dietary polyphenols. J. Funct. Foods 5, 163–169. https://doi.org/10.1016/j.jff.2012.09.008.

Gouw, V.P., Jung, J., Zhao, Y., 2017. Functional properties, bioactive compounds, and in vitro gastrointestinal digestion study of dried fruit pomace powders as functional food ingredients. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 80, 136–144. https://doi.org/10.1016/j.lwt.2017.02.015.

Hashemi Gahruie, H., Eskandari, M.H., Mesbahi, G., Hanifpour, M.A., 2015. Scientific and technical aspects of yogurt fortification: a review. Food Sci. Hum. Wellness 4, 1–8. https://doi.org/10.1016/j.fshw.2015.03.002.

Helal, A., Tagliazucchi, D., 2018. Impact of in-vitro gastro-pancreatic digestion on polyphenols and cinnamaldehyde bioaccessibility and antioxidant activity in stirred cinnamon-fortified yogurt. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 89, 164–170. https://doi.org/10.1016/j.lwt.2017.10.047.

Hernández-Hernández, C., Morales-Sillero, A., Fernández-Prior, M.Á., Fernández-Bolaños, J., Aguilera-Herrera, M. de la P., Rodríguez-Gutiérrez, G., 2019. Extra virgin olive oil jam enriched with cocoa bean husk extract rich in theobromine and phenols. LWT (Lebensm.-Wiss. & Technol.) 111, 278–283. https://doi.org/10.1016/j.lwt.2019.05.027.

Hsu, C.F., Peng, H., Basle, C., Travas-Sejdic, J., Kilmartin, P.A., 2011. ABTS•+ scavenging activity of polypyrrole, polyaniline and poly(3,4-ethylenedioxythiophene). Polym. Int. 60, 69–77. https://doi.org/10.1002/pi.2912.

Jakobek, L., 2015. Interactions of polyphenols with carbohydrates, lipids and proteins. Food Chem. 175, 556–567. https://doi.org/10.1016/j.foodchem.2014.12.013.

Jakobek, L., Matić, P., 2019. Non-covalent dietary fiber - polyphenol interactions and their influence on polyphenol bioaccessibility. Trends Food Sci. Technol. 83, 235–247. https://doi.org/10.1016/j.tifs.2018.11.024.

Kennas, A., Amellal-Chibane, H., Kessal, F., Halladj, F., 2020. Effect of pomegranate peel and honey fortification on physicochemical, physical, microbiological and antioxidant properties of yoghurt powder. J. Saudi Soc. Agric. Sci. 19, 99–108. https://doi.org/10.1016/j.jssas.2018.07.001.

Kranz, P., Braun, N., Schulze, N., Kunz, B., 2010. Sensory quality of functional beverages: bitterness perception and bitter masking of olive leaf extract fortified fruit smoothies. J. Food Sci. 75, S308–S311. https://doi.org/10.1111/j.1750-3841.2010.01698.x.

Liu, S., Jia, M., Chen, J., Wan, H., Dong, R., Nie, S., Xie, M., Yu, Q., 2019. Removal of bound polyphenols and its effect on antioxidant and prebiotics properties of carrot dietary fiber. Food Hydrocolloids 93, 284–292. https://doi.org/10.1016/j.foodhyd.2019.02.047.

Lopez-Huertas, E., 2010. Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. Pharmacol. Res. 61, 200–207. https://doi.org/10.1016/j.phrs.2009.10.007.

Lucas-Gonzalez, R., Navarro-Coves, S., Pérez-Álvarez, J.A., Fernández-López, J., Muñoz, L.A., Viuda-Martos, M., 2016. Assessment of polyphenolic profile stability and changes in the antioxidant potential of maqui berry (Aristotelia chilensis (Molina) Stuntz) during in vitro gastrointestinal digestion. Ind. Crop. Prod. 94, 774–782. https://doi.org/10.1016/j.indcrop.2016.09.057.

Marand, Ardabilchi, Mahsa, Amjadi, S., Ardabilchi Marand, Mahdis, Roufegarinejad, L., Jafari, S.M., 2020. Fortification of yogurt with flaxseed powder and evaluation of its fatty acid profile, physicochemical, antioxidant, and sensory properties. Powder Technol. 359, 76–84. https://doi.org/10.1016/j.powtec.2019.09.082.

Mizuno, M., Nishitani, Y., 2013. Antioxidant, luteolin exhibits anti-inflammatory effect in in vitro gut inflammation model. Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease. Elsevier, pp. 227–234. https://doi.org/10.1016/B978-0-12-397154-8.00028-2.

Mordor Intelligence, 2019. GLOBAL YOGURT MARKET (2020-2025).

Moreno-Maroto, J.M., Uceda-Rodríguez, M., Cobo-Ceacero, C.J., de Hoces, M.C., MartínLara, M.Á., Cotes-Palomino, T., López García, A.B., Martínez -García, C., 2019. Recycling of 'alperujo' (olive pomace) as a key component in the sintering of lightweight aggregates. J. Clean. Prod. 239, 118041. https://doi.org/10.1016/j.jclepro.2019.118041.

Najgebauer-Lejko, D., Sady, M., Grega, T., Walczycka, M., 2011. The impact of tea supplementation on microflora, pH and antioxidant capacity of yoghurt. Int. Dairy J. 21, 568–574. https://doi.org/10.1016/j.idairyj.2011.03.003.

Nunes, M.A., Pimentel, F.B., Costa, A.S.G., Alves, R.C., Oliveira, M.B.P.P., 2016. Olive by-products for functional and food applications: challenging opportunities to face environmental constraints. Innovat. Food Sci. Emerg. Technol. 35, 139–148. https://doi.org/10.1016/j.ifset.2016.04.016.

Nunes, M.A., Costa, A.S.G., Bessada, S., Santos, J., Puga, H., Alves, R.C., Freitas, V., Oliveira, M.B.P.P., 2018. Olive pomace as a valuable source of bioactive compounds: a study regarding its lipid- and water-soluble components. Sci. Total Environ. 644, 229–236. https://doi.org/10.1016/j.scitotenv.2018.06.350.

Oliveira, A., Pintado, M., 2015. Stability of polyphenols and carotenoids in strawberry and peach yoghurt throughout in vitro gastrointestinal digestion. Food Funct 6, 1611–1619. https://doi.org/10.1039/C5FO00198F.

Paz-Yépez, C., Peinado, I., Heredia, A., Andrés, A., 2019. Lipids digestibility and polyphenols release under in vitro digestion of dark, milk and white chocolate. J. Funct. Foods 52, 196–203. https://doi.org/10.1016/j.jff.2018.10.028.

Peker, H., Arslan, S., 2017. Effect of olive leaf extract on the quality of low fat apricot yogurt. J. Food Process. Preserv. 41, e13107 https://doi.org/10.1111/jfpp.13107.

Pimentel, L.L., Fontes, A.L., Gomes, A.M., Rodríguez-Alcalá, L.M., 2015. Considerations about the in situ derivatization and fractionation of EFA and NEFA in biological and food samples. Methods 2, 475–484. https://doi.org/10.1016/j.mex.2015.11.006.

Ribeiro, T.B., Oliveira, A., Campos, D., Nunes, J., Vicente, A.A., Pintado, M., 2020a. Simulated digestion of an olive pomace water-soluble ingredient: relationship between the bioaccessibility of compounds and their potential health benefits. Food Funct 11, 2238–2254. https://doi.org/10.1039/C9FO03000J.

Ribeiro, T.B., Oliveira, A., Coelho, M., Veiga, M., Costa, E.M., Silva, S., Nunes, J., Vicente, A.A., Pintado, M., 2020b. Are olive pomace powders a safe source of bioactives and nutrients? J. Sci. Food Agric. n/a, jsfa, 10812. https://doi.org/10.1002/jsfa.10812.

Rodrigues, F., Pimentel, F.B., Oliveira, M.B.P.P., 2015. Olive by-products: challenge application in cosmetic industry. Ind. Crop. Prod. 70, 116–124. https://doi.org/10.1016/j.indcrop.2015.03.027.

Román, G.C., Jackson, R.E., Gadhia, R., Román, A.N., Reis, J., 2019. Mediterranean diet: the role of long-chain ω-3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and Alzheimer disease. Rev. Neurol. (Paris). 175, 724–741. https://doi.org/10.1016/j.neurol.2019.08.005.

Romani, A., Ieri, F., Urciuoli, S., Noce, A., Marrone, G., Nediani, C., Bernini, R., 2019. Health effects of phenolic compounds found in extra-virgin olive oil, by-products, and leaf of olea europaea L. Nutrients 11. https://doi.org/10.3390/nu11081776.

Romero-García, J.M., Niño, L., Martínez-Patiño, C., Álvarez, C., Castro, E., Negro, M.J., 2014. Biorefinery based on olive biomass. State of the art and future trends. Bioresour. Technol. 159, 421–432. https://doi.org/10.1016/j.biortech.2014.03.062.

Ruiz, E., Romero-García, J.M., Romero, I., Manzanares, P., Negro, M.J., Castro, E., 2017. Olive-derived biomass as a source of energy and chemicals. Biofuels, Bioprod. Biorefining 11, 1077–1094. https://doi.org/10.1002/bbb.1812.

Sadeer, N.B., Montesano, D., Albrizio, S., Zengin, G., Mahomoodally, M.F., 2020. The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. Antioxidants 9, 709. https://doi.org/10.3390/antiox9080709.

Saura-Calixto, F., 1998. Antioxidant dietary fiber product: a new concept and a potential food ingredient. J. Agric. Food Chem. 46, 4303–4306. https://doi.org/10.1021/jf9803841.

Schaich, K.M., Tian, X., Xie, J., 2015. Hurdles and pitfalls in measuring antioxidant efficacy: a critical evaluation of ABTS, DPPH, and ORAC assays. J. Funct. Foods 14, 111–125. https://doi.org/10.1016/j.jff.2015.01.043.

Servili, M., Rizzello, C.G., Taticchi, A., Esposto, S., Urbani, S., Mazzacane, F., Di Maio, I., Selvaggini, R., Gobbetti, M., Di Cagno, R., 2011. Functional milk beverage fortified with phenolic compounds extracted from olive vegetation water, and fermented with functional lactic acid bacteria. Int. J. Food Microbiol. 147, 45–52. https://doi.org/10.1016/j.ijfoodmicro.2011.03.006.

Simopoulos, A., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 56, 365–379. https://doi.org/10.1016/S0753-3322(02)00253-6.

Tseng, A., Zhao, Y., 2013. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. Food Chem. 138, 356–365. https://doi.org/10.1016/j.foodchem.2012.09.148.

Van Nieuwenhove, C.P., Moyano, A., Castro-Gómez, P., Fontecha, J., Sáez, G., Zárate, G., Pizarro, P.L., 2019. Comparative study of pomegranate and jacaranda seeds as functional components for the conjugated linolenic acid enrichment of yogurt. LWT 111, 401–407. https://doi.org/10.1016/j.lwt.2019.05.045.

Villalva, M., Jaime, L., Arranz, E., Zhao, Z., Corredig, M., Reglero, G., Santoyo, S., 2020. Nanoemulsions and acidified milk gels as a strategy for improving stability and antioxidant activity of yarrow phenolic compounds after gastrointestinal digestion. Food Res. Int. 130, 108922. https://doi.org/10.1016/j.foodres.2019.108922.

Visioli, F., Galli, C., Grande, S., Colonnelli, K., Patelli, C., Galli, G., Caruso, D., 2003. Hydroxytyrosol excretion differs between rats and humans and depends on the vehicle of administration. J. Nutr. 133, 2612–2615. https://doi.org/10.1093/jn/133.8.2612.

Zoidou, E., Melliou, E., Moatsou, G., Magiatis, P., 2017. Chapter 11 - preparation of functional yogurt enriched with olive-derived products. In: Yogurt in Health and Disease Prevention. Elsevier, pp. 203–220. https://doi.org/10.1016/B978-0-12-805134-4.00011-0.