Food Bioscience 51 (2023) 102293

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Incorporation of phenolic extracts from different by-products in yoghurts to create fortified and sustainable foods

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ARTICLE INFO

Keywords:
Antioxidants
By-products
Sustainability
Circular economy
Fortified foods
Yoghurts
Preservatives

ABSTRACT

This work aimed to study and compare the effect of the incorporation of agro-industrial by-products (chestnut shell, grapeseed, and pomegranate peel) into yoghurts to fortify them while assessing their potential to replace synthetic preservatives. From each by-product, phenolic extracts were obtained and characterized. All extracts demonstrated antioxidant and antibacterial properties, and the capacity to inhibit α-amylase. Chestnut shell extract stood out regarding antioxidant capacity, displaying values of 1128 and 972 $mg_{Trolox} \cdot g_{extract}^{-1}$, for the assays with DPPH and ABTS, respectively. Ten yoghurts were produced (negative control, positive control with 0.1% of sorbic acid, two for each extract with 0.1% and 0.2% of extract, a mixture of extracts and a mixture of extracts with sorbic acid), and stored at 4 °C. The incorporation of the extracts into the yoghurts maintained their physical-chemical properties and microbiological safety. The samples inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*. Oxidative analysis proved that higher concentrations of extract had similar results to synthetic antioxidants. The results showed the viability to fortify yoghurts with the incorporation of by-product extracts, developing a value-added food. Furthermore, revealed the possibility of these extracts replacing synthetic preservatives and antioxidants.

1. Introduction

Over the last decades, the human population has grown significantly, which has led to an increase in food processing. Even though food processing is a helpful technique that aids to maximize the use of raw materials, it is also one of the main responsible for the generation of agro-industrial by-products. Most of these by-products are pomace, peels, seeds, and stems, among others, that display interesting characteristics due to their composition rich in bioactive compounds (Ferreira & Santos, 2022a). Despite their characteristics, agro-industrial by-products are still treated as residues, whose incorrect disposal and poor management originate environmental, social and economic problems (Gómez-García et al., 2021).

One of the goals of the United Nations for 2030 is to reduce food waste production, to achieve a more sustainable world. This increased the concern and awareness over this problem, which led the scientific community to study and develop ways to valorise these by-products, reducing their negative effects. The recovery of substances and compounds from these matrices using different extraction techniques is one of the main solutions used (Rodriguez-Lopez et al., 2020). Indeed, by-products are rich in bioactive compounds, such as antioxidants, fibres, vitamins, and other compounds, that can be incorporated into the food, pharmaceutical and cosmetic industry (Capanoglu et al., 2022).

Some of the main fruits cultivated in Europe are chestnuts, grapes, and pomegranate, whose production has increased over the years. Regarding chestnut, the main variety cultivated is *Castanea sativa* Mill. This fruit is mainly used as human and animal food due to its fascinating nutritional value and characteristics. The industrial processing of chestnuts is responsible for the production of by-products such as flowers, leaves, chestnut wood and shells, where the outer chestnut shells (CS) represent 8.9%–13.5% of the processing yields (Echegaray et al., 2018). In the same line of thought, the annual production of grapes exceeds 60 thousand tons, mostly used for wine production (Ferreira & Santos, 2022a). The winemaking process generates wide amounts of by-products, such as bagasse, peels, and seeds. Around 4.2 to 5.5 thousand tons of grapeseed (GS) are produced every year, globally, contributing to environmental problems (Teixeira et al., 2014).

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https://doi.org/10.1016/j.fbio.2022.102293
Received 30 September 2022; Received in revised form 7 December 2022; Accepted 8 December 2022
Available online 10 December 2022

Abbreviations

CS	Chestnut Shell
GAE	Gallic Acid Equivalents
GS	Grapeseed
PP	Pomegranate Peel
SC	Scavenging Capacity
TE	Trolox Equivalents
TEAC	Trolox Equivalent Antioxidant Capacity
TPC	Total Phenolic Content

Considering pomegranate, the annual production of this fruit has increased over the years due to its interesting nutritional and medicinal value, since it is associated with benefits to human health due to the presence of antioxidants (Smaoui et al., 2019). Pomegranate is commonly used for food products, e.g., juices, infusions, and jams, among others (Andrade et al., 2019). The processing of this fruit to obtain food products leads to the development of by-products, such as peels that represent around 40%–50% of the total weight of the fruit (Smaoui et al., 2019).

The mentioned by-products are rich sources of bioactive compounds, mainly phenolic compounds that are recognized for their high antioxidant capacity. Fig. 1 shows the main phenolics present in chestnut shells (CS), grapeseed (GS), and pomegranate peel (PP).

Phenolic compounds exhibit a wide array of biological properties, which makes them extremely alluring to different types of industries. Indeed, studies have shown that these compounds display biological properties, such as antimicrobial, antiallergic, anticancer, antiaging, and inhibitory activity towards enzymes, e.g., α-amylase and β-glucosidase. The inhibition of these enzymes is important to delay the increase in blood glucose levels, and this is a strategy that can be used to prevent diseases such as diabetes type 2 (Poovitha & Parani, 2016). However, the antioxidant capacity exhibited by these compounds is what makes them so appealing. Phenolics can act as a natural antioxidant by neutralizing oxidative stress, interrupting reactions of chain auto-oxidation, and inhibiting the production of free radicals, among other ways (Ferreira & Santos, 2022a; Soto et al., 2015; Teixeira et al., 2014).

Nowadays, consumers are more aware and concerned about the composition of the food they purchase, preferring foods with natural ingredients or from natural sources while avoiding synthetic additives. At the same time, the demand for nutraceutical products has increased leading to the creation of new sources of healthy food resources, such as natural food products and products with natural ingredients, such as fortified foods (Rodriguez-Lopez et al., 2020). Therefore, to fulfil new market trends, studies have shown that some phenolic compounds can be incorporated into different food matrixes to fortify them, by increasing the nutritional content (Rodriguez-Lopez et al., 2020; Villamil et al., 2021). Table 1 displays some studies regarding the fortification of foods with phenolic extracts obtained from natural sources. All the displayed studies are very recent, proving that this theme is a new trend; however, few studies report the fortification proving the need for more studies regarding this topic, which prove the importance of phenolic extracts from by-products.

Milk and dairy products are basic foods for human nutrition (Citta et al., 2017). Nevertheless, these types of foods are susceptible to oxidation which normally leads to the development of odd and unpleasant flavours and smell and decreases the nutritional properties; as consequence, the shelf life of these products is dependent on their oxidative stability. Yoghurt is a major dairy product, that consists of the fermentation of the milk by lactic acid bacteria that give the product its texture and different properties (Yoon et al., 2019). This food is consumed as healthy food due to its high nutritional value and benefits to human health since it is more nutritious than milk and is a rich source of proteins (Nguyen & Hwang, 2016). Therefore, following consumer trends in the search for food with nutritional characteristics and health effects, yoghurts are an interesting matrix to be studied in terms of the enhancement of their biological properties. Furthermore, the fortification of this food will also allow obtaining a yoghurt with longer shelf life due to the increase in the antioxidant content.

Hence the purpose of this work, consisted of the fortification of yoghurts with natural extracts, obtained from different by-products (chestnut shells, grapeseed, and pomegranate peels). The study focused on the comparison of the performance of each extract and the mixture of extracts in the yoghurts, evaluating their physicochemical, antioxidant, and antimicrobial characteristics. Furthermore, the developed work intended to assess the potential of phenolic extracts to be used as a sustainable food ingredient, to create more nutritional foods.

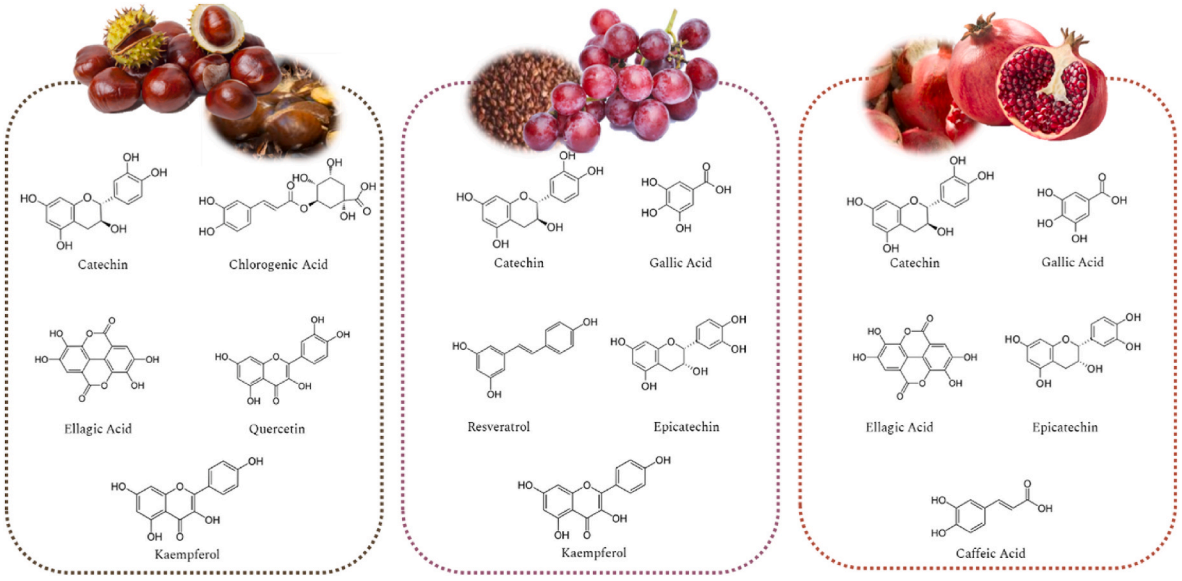

Fig. 1. Main phenolic compounds present in chestnut shells, grapeseed, and pomegranate peels.

Table 1
Literature studies regarding the fortification of different food matrices with extracts from natural sources and by-products.

Source	Matrix	Objectives	Results	Reference
Grape and olive pomace	Tagliatelle Pasta	Study the profile of fortified tagliatelle pasta with grape pomace or olive pomace	- Enriched tagliatelle from an organoleptic and nutritional viewpoint; - Increase in fibre and phenolic content.	Balli et al. (2021)
Olive mill wastewater (OMWW)	Blood Orange Juice	Select the best concentrate of olive mill wastewater to fortify blood orange juice; evaluation of the physicochemical, antioxidant, microbiological, and antimicrobial properties	- The addition of the OMWW concentrates leads to an increase in phenolic content; The juice provides a suitable amount of molecules with a healthy effect on consumers.	Foti et al. (2022)
Olive mill wastewater (OMWW) and Olive Pomace (OP)	Bread and Pasta	Study the effects of OMWW and OP addition to bread and pasta, separately and combined	- Fortification of bread and pasta with OMWW improved slightly the chemical quality without compromising sensory properties; - Enrichment with OP improved the chemical quality, nonetheless the acceptability was worse; - Bread was better than pasta for reusing olive oil by-products and OP was more suitable for food fortification - The combination of OMWW and OP showed better results for bread.	Cedola et al. (2020)
Kiwifruit	Beef	Incorporation of polyphenols extracted from deserted thinned young kiwifruits on beef and evaluation of the antioxidant and preservative effects on its quality during 7 days of refrigerated storage.	- Kiwifruit extract reduce fatty acid oxidation, alleviate discolouration, stabilize textural properties of beef and was able to inhibit TBARS; - After 7 days there was no significant alteration of sensory properties; - Kiwifruit extract showed potential to act as a natural preservative replacing synthetic ones in beef.	Jiao et al. (2020)
Chestnut Flour (CF)	Soft Wheat Fresh Pasta	Evaluation of the physicochemical properties of traditional fresh pasta enriched with different levels of chestnut flour.	- CF revealed inferior macroscopic quality properties compared to wheat flour; - CF in the pasta formulation delivered a pleasant brown colour and nutritional value; - The enrichment with CF increased the antioxidant capacity even after cooking.	Littardi et al. (2020)
Pomegranate Seed Powder (PSD)	Gluten-free Bread	Study the effect of the addition of pomegranate seed powder on the physical, sensorial and antioxidant properties of gluten-free bread	- PSD increased the specific volume and springiness of gluten-free bread and decreased hardness and chewiness - The addition of the PSD led to colour changes - Increased the total phenolic content - Antioxidant activity increased significantly with the addition of PSD, where higher percentages of powder exhibited more antioxidant activity.	Bourekoua et al. (2018)
Avocado Peel Extract (AVP)	Mayonnaise	Study the effect of the incorporation extracts from AVP in mayonnaise, evaluating the physicochemical, sensorial, antioxidant, and antimicrobial characteristics of the mayonnaises, assessing its potential to be used as a sustainable food ingredient.	- All mayonnaises exhibited no microbial growth and inhibited the growth of *Escherichia coli* and *Staphylococcus aureus;* - Oxidative stability revealed that the samples with extract displayed analogous results to those with synthetic antioxidants; - The results revealed that the incorporation o AVP extracts in mayonnaises do not compromise their stability.	Ferreira and Santos (2022b)
Moringa leaves	Yoghurt	Evaluate the effects of *M. oleifera* leaves extract on the fermentation, bioactive properties and quality characteristics of yoghurt	- The addition of the extract accelerated yoghurt fermentation by promoting the growth of lactic acid bacteria; - Changes in the colour of the yoghurt; - Increased viscosity and free radical scavenging during 21 days of cold storage; - The overall acceptability was not significantly influenced by the addition of 0.5% moringa extract; - Antioxidant capacity in human colorectal epithelial cells.	Zhang et al. (2019)

TBARS: Thiobarbituric acid reactive substance.

2. Materials and methods

2.1. Samples and reagents

Grapeseed samples were obtained from a Portuguese wine company, CancelaFé, located in Alfândega da Fé, Bragança, Portugal. Chestnut shells and pomegranate peels were obtained from a local Portuguese company and a local supermarket, respectively.

The extraction solvent ethanol (Ref. 1.02371.1000, C_2H_6O, CAS 64-17-5) was obtained from VWR (Fontenay-sous-Bois, France). For the total phenolic content, antioxidant and antimicrobial capacity and α-amylase assays, Folin Reagent (Ref. 47,641), Gallic Acid (Ref. 398,225, $C_7H_6O_5{\cdot}H_2O$, CAS 5995-86-8), DPPH (Ref. D9132, $C_{18}H_{12}N_5O_6$, CAS 1898-66-4), ABTS (Ref. A1888 $C_{18}H_{24}N_6O_6S_4$, CAS 30931-67-0), Trolox (Ref. 238,813, $C_{14}H_{18}O_4$, CAS 53188-07-1), Sorbic Acid (Ref. S1626, $C_6H_8O_2$, CAS 110-44-1), α-amylase (Ref. A3176, CAS 9000-90-2), starch from corn (Ref. S4180, CAS 9005-25-8), 3,5-Dinitrosalicylic acid (Ref. D0550, $C_7H_4N_2O_7$, CAS 609-99-4) and Potassium

sodium tartrate tetrahydrate (Ref. 217,255, $C_4H_4KNaO_6{\cdot}4H_2O$, CAS 6381-59-5) were used and purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol (Ref. 414,816, CH_4O, CAS 67-56-1) was purchased from Carlo Erba (Barcelona, Spain). A Merck Millipore Mill-Q water purification equipment, with 18.2 Ω of electric resistance (Billerica, MA, USA), is used for deionized water.

2.2. Methods

2.2.1. Extraction of phenolic compounds from different by-products

Phenolic compounds were extracted using the solid-liquid extraction, with a Soxhlet apparatus, method. The different by-products (chestnut shells, grapeseed and pomegranate peels) were previously subjected to a pre-treatment here the samples were washed, to remove impurities and freeze-dried to remove the water present in the samples. Later the samples were milled and sieved to obtain homogenized samples. Afterwards, the extractions were performed using ethanol as extraction solvent, with a mass:volume ratio of 1:20 (m/V), for 3 h. The extraction solvent was removed with the help of a rotary evaporator (Büchi R-200, Flawil, Switzerland) with a bath temperature of 40 °C. Total evaporation of the solvent was achieved with a constant stream of nitrogen.

2.2.2. Total phenolic content

The determination of the Total Phenolic Content (TPC) was assessed accordingly to the literature (Silva et al., 2007). For that, 20 μL of the sample solution, and 100 μL of Folin-Ciocalteu reagent, followed by 1.58 mL of distilled water were added in a 2 mL cuvette. Afterwards, saturated sodium carbonate solution (333.3 mg L^{-1}) was added and the cuvette was left to incubate for 2 h in the dark. The absorbance was measured at 750 nm with a spectrophotometer (V-530, Jasco, OK, USA). The results were expressed in gallic acid equivalents (GAE), using Equation (1).

$$TPC = \frac{\left(Abs_{sample} - Abs_{blank}\right) \times 1000}{m \times c_{sample}} \quad (1)$$

where Abs_{sample} and Abs_{blank}, refer to the absorbance of the sample and blank (made using 20 μL of water instead of the sample). C_{sample} refers to the sample concentration in the cuvette, and m = 0.0748 and refers to the slope of the calibration curve prepared using different concentrations of gallic acid.

2.2.3. Antioxidant capacity

The antioxidant capacity of the phenolic extracts was determined using two methods the assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the assay with 2,2-azinobis (3-ethyl-benzothiazolin-6-sulfonic acid) (ABTS), commonly known as Trolox Equivalent Antioxidant Capacity (TEAC).

The DPPH assay was performed accordingly to literature protocols (Bobo-García et al., 2015). For that, a DPPH solution of 150 μM solution was prepared in methanol:water (80:20). Afterwards, 20 μL of the sample was added to a 96-well microplate, followed by 180 μL of DPPH solution. The plate was left to incubate for 40 min, in the dark, and the absorbance was read using a spectrophotometer (V-530, Jasco, OK, USA) at 515 nm. The results were expressed in terms of the percentage of DPPH inhibition (%) using Equation (2), and Trolox Equivalents (TE), using a calibration curve prepared with different concentrations of Trolox.

$$\%\ DPPH\ Inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (2)$$

where $Abs_{control}$ and Abs_{sample}, refer to the control and sample absorbance, respectively, where the control was made using 20 μL of the methanol:water solution instead of sample.

The ABTS assay, also known as TEAC (Trolox equivalent antioxidant capacity) was performed according to the literature, with slight changes. (Xiao et al., 2020). A stock solution of ABTS and persulphate were mixed (1:1) and allowed to react for 16 h in the dark, at room temperature (20 °C). Afterwards, the 2.8 mL of the solution were diluted in 65 mL of acetate buffer (0.05 M, pH 4.6). The solution was left to incubate for 30 min in the dark and the absorbance was read at 734 nm using a spectrophotometer (V-530, Jasco, OK, USA). Acetate buffer was added to the solution until the absorbance of 0.72 ± 0.03 was achieved.

In a 96-well microplate, 20 μL of the sample was added followed by 180 μL of the reactive ABTS solution. The microplate was incubated for 15 min in the dark and the absorbance was read at 734 nm. The scavenging capacity (SC) was evaluated by resorting to Equation (3). The results were expressed in TE, using Trolox solutions of different concentrations, to create a calibration curve.

$$SC\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (3)$$

where $Abs_{control}$ and Abs_{sample}, refer to the control and sample absorbance, respectively, where the control was made using 20 μL of the acetate buffer instead of sample.

2.2.4. Alpha-amylase assay

The α-amylase assay was carried out accordingly to literature protocols (Kazeem et al., 2013). For that 250 μL of the sample (with concentrations within the range of 1.25–10 mg/mL) were mixed with 250 μL of a 0.5 mg/mL (6 U/mL) of α-amylase solution with phosphate buffer (0.02 M, pH 6.9), and left to incubate for 10 min at 25 °C. Subsequently, 250 μL of 1% starch from corn solution with phosphate buffer and left to incubate for 10 min at 25 °C. Later, 500 μL of DNS (3,5-Dinitrosalicylic acid) reagent and left to incubate in boiling water for 5 min. The solution was left to cool down to room temperature (20 °C) and 5 mL of water was added. To prepare the DNS reagent, 5 g of 3,5-dinitrosalicylic acid were dissolved in 250 mL of distilled water at 80 °C. Afterwards, the mixture was cooled down and 100 mL of 2 M NaOH were added. Subsequently, 150 g of La Rochelle salt (sodium and potassium tartrate) were added. The mixture was homogenized and brought up to 500 mL with distilled water (Miller, 1959). The solution was stored in a dark bottle. The absorbance was read at 540 nm and the percentage of inhibition was calculated according to Equation (4).

$$I\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (4)$$

where $Abs_{control}$ and Abs_{sample}, refer to the absorbance of the control and sample after the reaction with DNS respectively, where the control was made using 250 μL of water instead of sample.

2.2.5. Antimicrobial capacity

To assess the antimicrobial capacity of the phenolic extracts, disk diffusion tests were performed against *Staphylococcus aureus* (335 PF), and *Escherichia coli* (DSM 1103), two of the main bacteria responsible for human infections. Suspensions of microorganisms were prepared and adjusted to an optical density of ≈0.10 at 610 nm (Leal et al., 2020). After this step, PCA (Plate Count Agar) plates were inoculated by adding the suspension over the agar surface. Afterwards, sterile disks were added to the plate and 7 μL of the sample was added to it. The same was done with the negative and positive control, distilled water, and sorbic acid (SA), respectively. The plates were left to incubate for 48 h, at 37 °C, and, afterwards, the inhibition halos were measured.

2.2.6. Yoghurt production

For the production of the yoghurt, UHT milk and commercial yoghurt, with probiotics and milk ferments, were used. Firstly, the milk was heated to 90 °C (a temperature close to the milk's boiling point) and then allowed to cool down to the temperature of 42/45 °C. Secondly, 50 mL of milk was inoculated with 10 mL of commercial yoghurt. The

fortification of the different yoghurts was performed afterwards accordingly to Table 2. The yoghurts were left to incubate for 16 h at 37 °C. After incubation, the yoghurts were placed in a fridge at 4 °C, until further analysis. Sorbic acid was selected as a positive control since it is one of the main preservatives used in yoghurts, whose concentration is limited to 1000 mg kg^{-1} for fermented milk products (Food and Agriculture Organization of the United Nations (FAO), 2019).

Afterwards, the samples were subjected to stability studies for three weeks with three analysing times, t_1 (first week – same week of the production of the yoghurts), t_2 (second week) and t_3 (third week). The yoghurts were stored in a fridge at 4 °C, for 6 weeks.

2.2.7. pH determination

The pH of the yoghurt samples was determined by dissolving the sample in distilled water, in a 1:9 (m/V) ratio. After the dissolution, the samples were homogenized for 1 min, using an Ultra-Turrax (IKA T18 Digital ULTRA-TURRAX®, Staufen, Germany). The pH of the samples was measured using a digital pH meter.

2.2.8. Viscosity

The samples were subjected to a viscosity analysis. For that, the sample was placed in a rheometer (MCR 92, Anton Paar, Graz, Austria) and the apparent viscosity (mPa•s) was measured in function of different shear rates (s^{-1}), at room temperature (20 °C). The effect of temperature was also assessed, where the apparent viscosity was measured for different temperatures (in the range of 2–25 °C), at a constant shear rate.

2.2.9. Syneresis and water holding capacity

The syneresis, which means the amount of released whey, was determined by weighing 10 g of sample. Afterwards, the samples were centrifuged at 700 rpm (82×*g*) for 20 min. The supernatant was collected and weighed (Cho et al., 2020). The syneresis was evaluated using Equation (5).

$$Syneresis = \frac{m_{supernatant}}{m_{yoghurt}} \times 100 \quad (5)$$

For the water holding capacity (WHC) assay 0.25 g of sample were weighed and 7.5 mL of distilled water was added. Afterwards, the mixture was vortexed for 1 min and left to hydrate for 18 h. Subsequently, the sample was centrifuged at 3000 rpm (1510×*g*), for 20 min, the supernatant was removed the solid fraction was weighed (Bakirci et al., 2017). The solid fraction was dried at 105 °C for 6 h and weighed. The WHC was determined using Equation (6).

$$WHC = \frac{m_{fresh\ sample} - m_{dried\ sample}}{m_{dried\ sample}} \times 100 \quad (6)$$

Table 2
Percentage of additives incorporated in the different yoghurts.

Yoghurt	Sorbic Acid	CS Extract	GS Extract	PP Extract
	Percentage (%)			
NC	–			
PC	0.1	–		
CS 1	–	0.1	–	
CS 2	–	0.2	–	
GS 1	–		0.1	–
GS 2	–		0.2	–
PP 1	–			0.1
PP 2	–			0.2
MIX E	–	0.1	0.1	0.1
MIX E + PC	0.1	0.1	0.1	0.1

NC - Negative control (yoghurt without additives); PC - Positive control (yoghurt with Sorbic Acid); CS 1 –yoghurt with 0.1% of CS; CS 2 –yoghurt with 0.2% of CS; GS 1 –yoghurt with 0.1% of GS; GS 2 –yoghurt with 0.2% of GS; PP 1 –yoghurt with 0.1% of PP; PP 2 –yoghurt with 0.2% of PP; MIX E – yoghurt with all extracts; MIX E + PC – yoghurt with all extracts and sorbic acid.

2.2.10. Microbiological analysis

The antimicrobial capacity of the yoghurt samples was assessed using the method described in Section 2.2.5, but instead of using sterile disks, a well was made in the agar using a Pasteur pipette to insert the samples (Ferreira et al., 2022).

For the microbiological safety of the samples, a solution of 10% of yoghurt was prepared with water distilled for each sample. This solution was diluted 2 times. Subsequently, 100 μL of the solution was plated on Laurysulphate Agar (LSA) and Rose Bengal Agar (RBC), adequate medium for coliform microorganisms, and yeast and molds, respectively. The plates were left to incubate for 48 h at 37 °C for the LSA plates and 25 °C for the RBC. Posteriorly, the culturable cells were enumerated.

2.2.11. Statistical analysis

For the statistical analysis, the analysis of variance (ANOVA) was used. The null hypothesis was considered valid when all the sample values are equal or do not have any significant difference. The alternative hypothesis is valid when at least one of the sample values is different from the rest of them. The software used as GraphPad and the values were considered statistically significant for $p < 0.05$ (95% confidence interval).

3. Results and discussion

3.1. Bioactive characterization of the extracts

The present study pretended to evaluate the effect of the incorporation of phenolic extracts, from different by-products, in yoghurts. For that, chestnut shells (CS), grapeseed (GS) and pomegranate peels (PP) were used to obtain extracts rich in phenolic compounds, where ethanol was selected as the extraction solvent since it is a GRAS (Generally Recognized as Safe) solvent. After the extraction, the extracts were characterized and evaluated regarding the TPC, antioxidant capacity (by the assays with DPPH and ABTS), α-amylase inhibition, and antibacterial capacity. Results for the characterization of the extracts are displayed in Table 3.

The total phenolic content ranged between 223.5 and 515.9 $mg_{GAE•}g^{-1}_{dried\ extract}$ for the three extracts. The extract from CS displayed the highest phenolic content, and it is possible to observe, from Table 3, that the result was significantly higher when compared to the extracts from GS and PP. This result can be associated with the presence of

Table 3
Results for the characterization of the extracts from chestnut shells, grapeseed, and pomegranate peels.

		Extract		
		Chestnut Shell (CS)	Grapeseed (GS)	Pomegranate Peel (PP)
TPC ($mg_{GAE•}g^{-1}_{dried\ extract}$)		515.9 ± 3.2[a]	223.5 ± 2.0[b]	275.0 ± 1.3[c]
DPPH	IC_{50} ($mg_{extrat•}mL^{-1}$)	5.46 ± 0.01[a]	13.25 ± 0.35[b]	9.96 ± 0.96[c]
	TE ($mg_{Trolox•}g^{-1}_{extract}$)	1128.4 ± 2.5[a]	353.2 ± 2.6[b]	424.2 ± 7.7[c]
TEAC ($mg_{Trolox•}g^{-1}_{extract}$)		972.0 ± 12.7[a]	353.9 ± 10.5[b]	873.9 ± 3.5[c]
α-amylase Inhibition (IC_{50} $\mu g_{extrat•}mL^{-1}$)		113.5 ± 0.3[a]	312.6 ± 1.0[b]	281.1 ± 0.3[c]
Microorganism Inhibition (d_{halo} mm)	*S. aureus*	14.7 ± 0.5[a]	15.3 ± 0.5[a]	20.0 ± 0.0[b]
	E. coli	<5.0	<5.0	<5.0

The results are expressed as means ± standard deviations of n = 3 independent measurements. a, b, and c values represented with different letters in the same row are statistically different. ($p < 0.05$).
Total Phenolic Content; GAE: Gallic Acid Equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: Needed concentration of extract to inhibit 50% of the DPPH; TE: Trolox Equivalents; TEAC: Trolox equivalent antioxidant capacity.

pigments, such as anthocyanins, in the CS that contribute significantly to the total phenolic content of the extract (Babbar et al., 2011). Regarding the antioxidant capacity, it is possible to conclude that the CS extract was the one that exhibited better values. Considering the IC_{50} (necessary concentration of extract to inhibit 50% of DPPH), this was the extract that revealed a lower value, when compared with GS and PP extracts. The results show that to inhibit the same percentage of the DPPH radical and have the same biological effect, lower concentrations of the extract are needed, which results in higher antioxidant capacity. On the other hand, observing the values of Trolox equivalents in both assays (TE in the DPPH assay and TEAC in the ABTS), it is possible to conclude that the CS extract, indeed demonstrated higher values, showing significantly higher antioxidant capacity than GS and PP extracts.

Abundantly found in the pancreatic juice and saliva of humans, α-amylase is an enzyme that is responsible for the breakdown of insoluble starch molecules, such as oligosaccharides and disaccharides, into monosaccharides, soluble molecules and suitable for absorption (de Sales et al., 2012; Kazeem et al., 2013). One of the strategies to treat type 2 diabetes consists in the inhibition of the degradation of oligo and disaccharides through the inhibition of enzymes, such as α-amylase. The inhibition of this enzyme will prolong the digestion of carbohydrates and, consequently, the digestion time, leading to a reduction in the rate of glucose absorption reducing the glucose rise in the plasma (de Sales et al., 2012). Observing the results in Table 3, it is possible to conclude that the CS extract displayed better results in inhibiting α-amylase since the IC_{50} is inferior to the other extracts. *In vitro* studies revealed that polyphenols have inhibitory effects on amylolytic enzymes, such as α-amylase; this activity results from the binding interaction between the active site of the enzyme and the phenols (Giuberti et al., 2020). Therefore, the different results can be associated with the different compositions of the extracts, since the molecular structure influences the inhibitory effects of α-amylase.

Considering the antibacterial capacity, from Table 3, it is possible to observe that, all three extracts could inhibit the growth and development of *S. aureus*; however, against *E. coli*, no inhibitory effect was detected. While CS and GS extracts displayed a similar action towards *S. aureus*, PP extract displayed significantly superior results regarding the inhibition of this bacteria. It was expected that the extracts were able to inhibit the growth of Gram-positive bacteria (such as *S. aureus*) and did not affect Gram-negative bacteria (such as *E. coli*). Indeed, Gram-negative bacteria have an extra protective outer membrane in their structure, which repulses the phenolic compounds present on the surface of the membrane (Asghar et al., 2020; Rodríguez-Carpena et al., 2011). Consequently, this occurrence increases the resistance of these bacteria, diminishing their susceptibility to preservatives.

Comparing the obtained results with those in literature, displayed in Table 4, it is possible to observe that the achieved values are within the literature ones, with slight differences. These can be explained by the variety of the fruits used, as well as the growing and cultivation conditions, and the maturation phase. Furthermore, the extraction techniques, samples pre-treatment and the protocols used to perform the assays are also variables that can interfere with the results.

Hereupon, observing the obtained results, it is possible to conclude that all the extracts exhibited interesting outcomes regarding their biological properties and capacities, with the CS extract displaying overall better scores, showing their potential to be incorporated into foods as fortifying agents.

3.2. Physical and chemical characterization of the yoghurts

After the production of the control and fortified yoghurts, these were subjected to a physical and chemical characterization, to compare the performance of each yoghurt. For that, parameters such as pH, syneresis, water holding capacity and the viscosity of the samples were determined.

To encounter the legal requirements of the National Yoghurt Association, the pH of yoghurts should have a pH of 4.6 or lower. Thereby, observing Fig. 2, it is possible to conclude that all ten yoghurts are under the established legal limits, making them suitable for human consumption. On the other hand, it can be perceived that all samples experienced a decrease in the pH value over time, where the highest decrease was detected in yoghurt NC (negative control). The main reason behind the pH reduction results in the breakdown of the lactose into lactic acid; the increase in these acids leads to a decrease in the pH value, during fermentation (Ranasinghe & Perera, 2016). Therefore, the results demonstrate that the incorporation of the different extracts on the yoghurts did not affect negatively the pH value of the samples. Furthermore, the increase in the extract concentration (samples CS2, GS2, PP2, MIX E and MIX E + PC) did not impact the pH of the yoghurts. Hence, results show that the incorporation of phenolic extracts from by-products into yoghurts is possible, without compromising the pH and maintaining the product suitable for consumption.

Table 4
Literature values regarding the TPC, antioxidant capacity, alpha-amylase inhibition and antibacterial capacity of the CS, GS, and PP extracts.

Extract	Extraction Conditions	TPC ($mg_{GAE} \bullet g^{-1}$)	Antioxidant Capacity ($mg_{Trolox} \bullet g^{-1}_{extract}$)	α-amylase Inhibition ($\mu g_{extrat} \bullet mL^{-1}$)	Antibacterial Capacity (d_{halo} mm)	Reference
CS	- EtOH:W (50:50) - SLE - T = 75 °C	53.5	TE = 1341.5 TEAC = 1148.8	–	- *S. aureus*: 5-9 - *E. coli*: 3-5	Fernández-Agulló et al. (2014)
	- MeOH, ACE - SLE - T = 60 °C	87.6–207.1	TE = 500.5–550.6 TEAC = 125.1–450.5	≈ 110 - 145	–	Liu et al. (2020)
GS	- EtOH - SLE; MAE	265.2–279.6	TE = 119-344 TEAC = 105-230	–	–	Brezoiu et al. (2019)
	- MeOH - Maceration	74–277	–	≈ 190 - 320	–	Lavelli et al. (2015)
	- ACE:W (80:20) - Maceration - T = Troom	92–153.8	–	–	- *S. aureus*: 12-7 - *E. coli*: N. D.	Xu et al. (2016)
PP	-EtOH - Maceration; SLE	251.1–549.1	TE = 547.5–1006.2 TEAC = 411.2–1374.1	–	–	Masci et al. (2016)
	- EtOH:W (70:30) - Maceration - T = Troom	173.5	–	122.93	–	Šavikin et al. (2018)
	- EtOH - Maceration	361.8	–	–	- *S. aureus*: 16.5 - *E. coli*: 16.4	Ismail et al. (2016)

CS: Chestnut Shell; GS: Grapeseed; PP: Pomegranate Peel; ACE: Acetone; EtOH: Ethanol; MAE: Microwave assisted extraction; MeOH: Methanol; N.D.: Non detected; SLE: Solid-liquid extraction; T: temperature; TE: Trolox Equivalents; TEAC: Trolox equivalent antioxidant capacity; TPC: Total Phenolic Content.

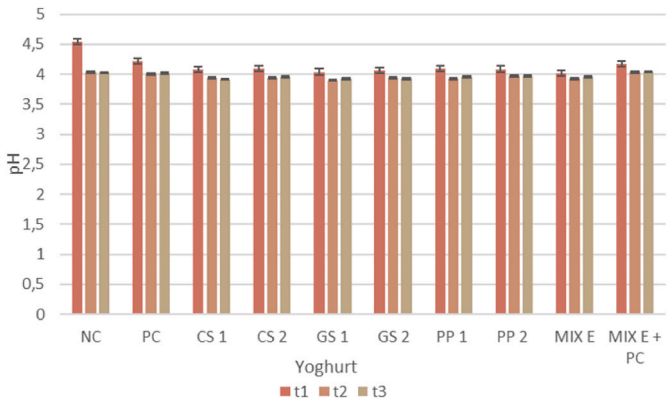

Fig. 2. Variation of the pH value throughout the study period. t_1 refers to the first week (week of the production of the yoghurts), t_2 refers to the second week and t_3 refers to the third week. NC - Negative control (yoghurt without additives); PC - Positive control (yoghurt with Sorbic Acid); CS 1 –yoghurt with 0.1% of CS; CS 2 –yoghurt with 0.2% of CS; GS 1 –yoghurt with 0.1% of GS; GS 2 –yoghurt with 0.2% of GS; PP 1 –yoghurt with 0.1% of PP; PP 2 –yoghurt with 0.2% of PP; MIX E − yoghurt with all extracts; MIX E + PC – yoghurt with all extracts and sorbic acid.

To make yoghurt appealing to consumers it is important to ensure the physical properties of the food. Syneresis is a major parameter to consider when evaluating the physical properties of yoghurts since this phenomenon consists in the separation of the whey, which can happen due to high concentrations of whey protein in comparison to the casein, to low concentrations of total solids and physical alterations during storage (da Silva et al., 2012). One strategy commonly used to diminish syneresis is to increase the water holding capacity (WHC). Therefore, the higher the value of WHC, the lower will the syneresis value be, and the physical properties and quality of the product will be reached. The obtained results are displayed in Fig. 3.

Observing Fig. 3, it is possible to conclude that the WHC increased throughout the study period, whilst the syneresis decreased. Indeed, the literature reveals that WHC increases during storage, whilst syneresis decreases. This phenomenon can be explained by the stable complexes with stronger internal bonds, that can reduce the rearrangement of proteins during storage, stabilizing the casein networks and,

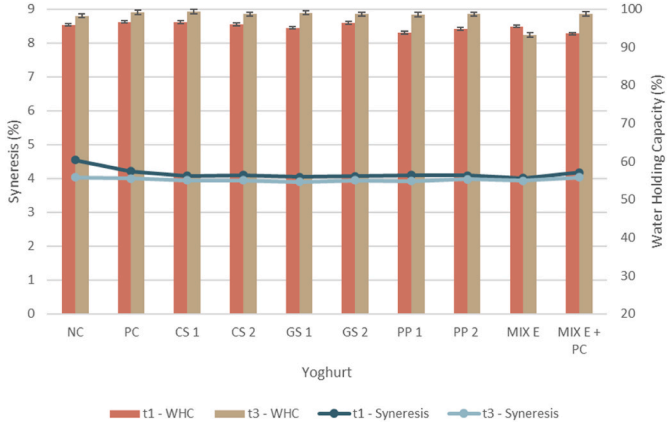

Fig. 3. Variation of the syneresis (%) and WHC (%), for the ten yoghurts, for t_1 and t_3. The lines refer to the syneresis values, while the bars refer to the WHC values. t_1 refers to the first week (week of the production of the yoghurts), t_2 refers to the second week and t_3 refers to the third week. NC - Negative control (yoghurt without additives); PC - Positive control (yoghurt with Sorbic Acid); CS 1 –yoghurt with 0.1% of CS; CS 2 –yoghurt with 0.2% of CS; GS 1 –yoghurt with 0.1% of GS; GS 2 –yoghurt with 0.2% of GS; PP 1 –yoghurt with 0.1% of PP; PP 2 –yoghurt with 0.2% of PP; MIX E − yoghurt with all extracts; MIX E + PC – yoghurt with all extracts and sorbic acid.

consequently, retaining water in yoghurt and reducing syneresis (Qiu et al., 2021). It is worth pointing out that yoghurts incorporated with phenolic extracts exhibited a superior increase in WHC when compared to NC and PC; however, in sample MIX E the WHC decrease over time, which can be associated with unstable bonds between phenolics and casein, retaining less the water. Regarding syneresis, it is noticeable that the values decreased over time. Therefore, it is possible to conclude that the incorporation of the extracts into yoghurts allowed to increase the WHC, and consequently, decrease the syneresis, improving the physical characteristics of the yoghurts, when compared to NC (negative control).

Another physical parameter that influences the properties of the yoghurt is its viscosity. Therefore, the apparent viscosity of the yoghurt samples was determined, as well as its behaviour towards different temperatures. The results are displayed in Fig. 4. The obtained values for the flow behaviour index, n, as well as the consistency index, K, for the yoghurt samples, are displayed in Table 5.

From the information displayed in Fig. 4(A), it is possible to observe that the incorporation of the extracts increases the viscosity of the yoghurts. Nevertheless, this variation did not modify the behaviour of the samples, since for higher shear rates the viscosity decreases and tends to the same value of the yoghurts NC and PC (around 5×10^2). On the other hand, observing Table 5, it is possible to conclude that all samples exhibit a flow behaviour index (n) inferior to 1, which demonstrates that the samples possess a non-Newtonian flow behaviour, exhibiting a pseudoplastic (shear-thinning) behaviour. It is also noticeable that, apart from yoghurt CS1 and GS1, all the yoghurts with extracts contributed to higher flow behaviour index values. This means that the incorporation of the by-product extracts reduces shear-thinning possibly due to inferior rupture of the intramolecular and intermolecular bonds in the system (Kim & Yoo, 2010).

Regarding the behaviour of viscosity in the function of the temperature, from Fig. 4(B), it is observable that, the increase in temperature leads to a decrease in the viscosity of the samples, making them more fluid. It is also noticeable that this is an irreversible phenomenon since when the temperature returns to lower values, the viscosity increases but it does not reach the initial values. This can be associated with changes in the structure of the yoghurts with temperature changes, that cannot be reversed.

3.3. Total phenolic content and antioxidant analysis

To assess the potential of the different extracts to act as antioxidants, the total phenolic content and antioxidant capacity of the yoghurts were evaluated. For the antioxidant capacity, the methods with the DPPH and ABTS radicals were selected, and the results were expressed in the percentage of inhibition for each radical. The obtained results for the three tests, throughout the study, are displayed in Fig. 5.

From Fig. 5(A), it is possible to observe that, the phenolic content increased in all the samples throughout the time, with yoghurts CS2 and MIX E + PC revealing higher values, at the end of the study. The results indicate that the fortification of yoghurts with phenolic extracts increases the TPC, which can be explained by the action between microorganisms and the phenolic compounds present in the extracts. It is possible that microorganisms, present in the yoghurt, use certain phenolics, mainly phenolic acids such as ferulic acid and *p*-coumaric acid, during fermentation originating other phenolics, contributing to the increase in the phenolic content. Additionally, milk protein hydrolysis and the fermentation by lactic bacteria, originate secondary metabolites, including tyrosine (an amino acid) that contains a phenolic side chain, contributing to the increase in TPC (Joung et al., 2016). The results show that the incorporation of phenolic extracts augments the total phenolic content of the yoghurts that, consequently, could originate an increase in the antioxidant capacity of the yoghurts, enlarging their shelf-life.

Considering the antioxidant capacity, it is possible to conclude from Fig. 5(B) and (C) that the radical scavenging percentage diminished

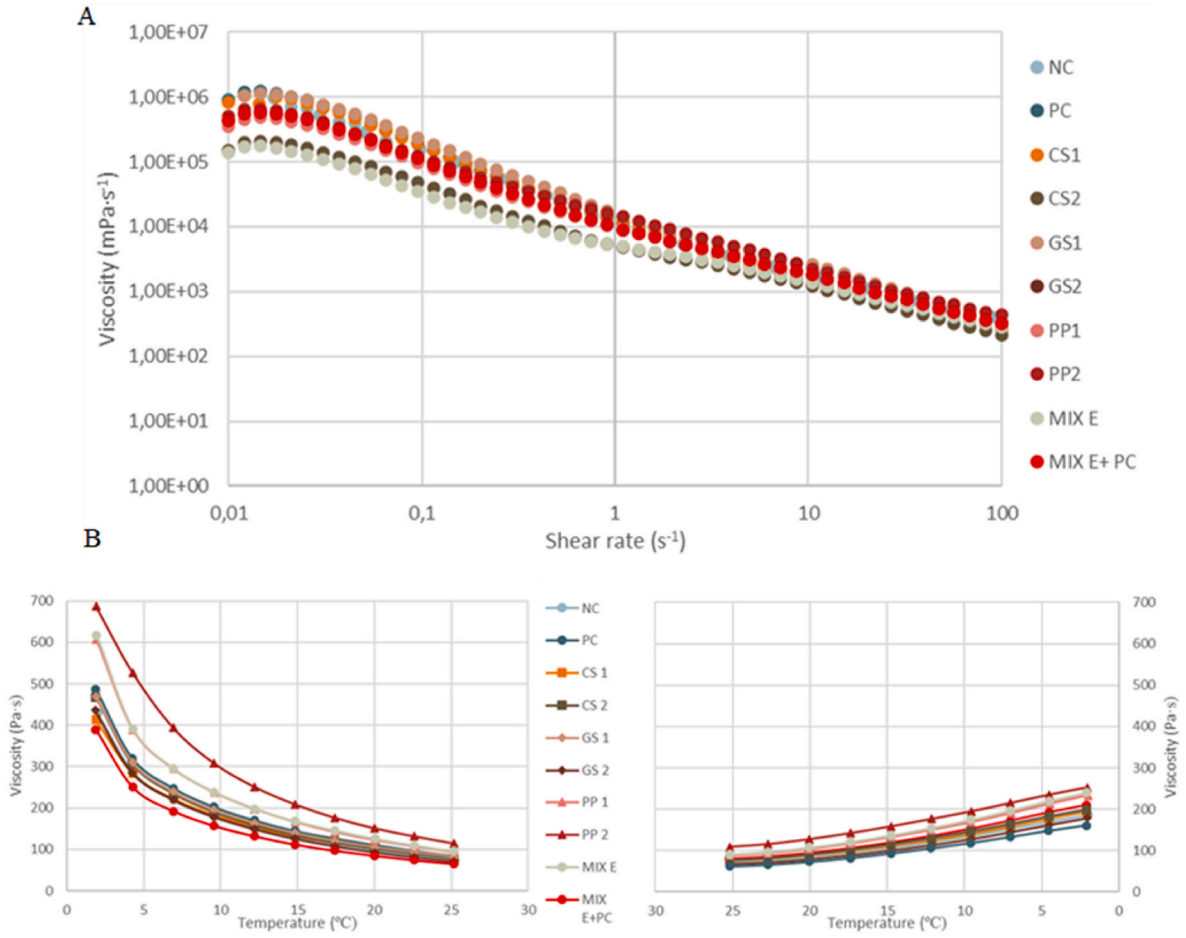

Fig. 4. Variation of viscosity in function of: (A) - shear rate (s-1); (B) – Temperature (°C). t_1 refers to the first week (week of the production of the yoghurts), t_2 refers to the second week and t_3 refers to the third week. NC - Negative control (yoghurt without additives); PC - Positive control (yoghurt with Sorbic Acid); CS 1 –yoghurt with 0.1% of CS; CS 2 –yoghurt with 0.2% of CS; GS 1 –yoghurt with 0.1% of GS; GS 2 –yoghurt with 0.2% of GS; PP 1 –yoghurt with 0.1% of PP; PP 2 –yoghurt with 0.2% of PP; MIX E − yoghurt with all extracts; MIX E + PC – yoghurt with all extracts and sorbic acid.

Table 5
Obtained parameters for the consistency index, K, and flow consistency index, n.

Yoghurt	K (mPa•s^n)	n
NC	16,019	0.06
PC	16,635	0.02
CS 1	16,488	0.03
CS 2	68,395	0.22
GS 1	20,773	0.06
GS 2	13,217	0.13
PP 1	12,034	0.15
PP 2	16,881	0.16
MIX E	6648.1	0.29
MIX E + PC	13,217	0.13

NC - Negative control (yoghurt without additives); PC - Positive control (yoghurt with Sorbic Acid); CS 1 –yoghurt with 0.1% of CS; CS 2 –yoghurt with 0.2% of CS; GS 1 –yoghurt with 0.1% of GS; GS 2 –yoghurt with 0.2% of GS; PP 1 –yoghurt with 0.1% of PP; PP 2 –yoghurt with 0.2% of PP; MIX E − yoghurt with all extracts; MIX E + PC – yoghurt with all extracts and sorbic acid.

during the storage period. Literature reports show that the decrease of the antioxidant capacity of the yoghurt is a common phenomenon, that relates to the damage of the phenolic compounds during fermentation and the presence of lactic bacteria during storage under refrigeration conditions (Tavakoli et al., 2018). Furthermore, this occurrence is also associated with the behaviour of the yoghurt, since the control sample also exhibited changes in the antioxidant capacity, which can occur due to proteolysis and formation of organic acids, with the metabolic performance of the lactic bacteria and with the antimicrobial activity (Tavakoli et al., 2018).

For the assay with DPPH, the values ranged from 8% to 40%, while on the assay with ABTS the values were between 19% and 28%. It is perceived that the antioxidant capacity of the fortified yoghurt samples was considerably higher than the one displayed for yoghurt NC. In the DPPH assay, yoghurts containing extracts exhibited a higher percentage of DPPH inhibition during storage, delaying the degradation of these samples. For the ABTS assay, the behaviour of the samples is analogous, however, only yoghurts containing higher concentrations of extract reach similar values to PC yoghurt. Even though milk protein hydrolysis contributes to the antioxidant capacity, the results indicate that the presence of the extracts increases the antioxidant potential, which is highly correlated to the presence of phenolic compounds (Cho et al., 2017). These findings seem to indicate that phenolic extracts, obtained from by-products, are a stable ingredient to be incorporated into yoghurts as an antioxidant, and have the potential to replace synthetic antioxidants.

Analysing the behaviour of the yoghurt samples in terms of TPC and antioxidant capacity, it is possible to conclude that the increase in the TPC did not reflect an enhancement of the antioxidant capacity. This can be associated with the fermentation of lactic bacteria, as previously mentioned, since it originates secondary metabolites that can contribute to amplifying the TPC, that do not display antioxidant properties and, consequently, do not contribute to the antioxidant capacity of yoghurts. Nevertheless, the results indicate that the incorporation of the phenolic

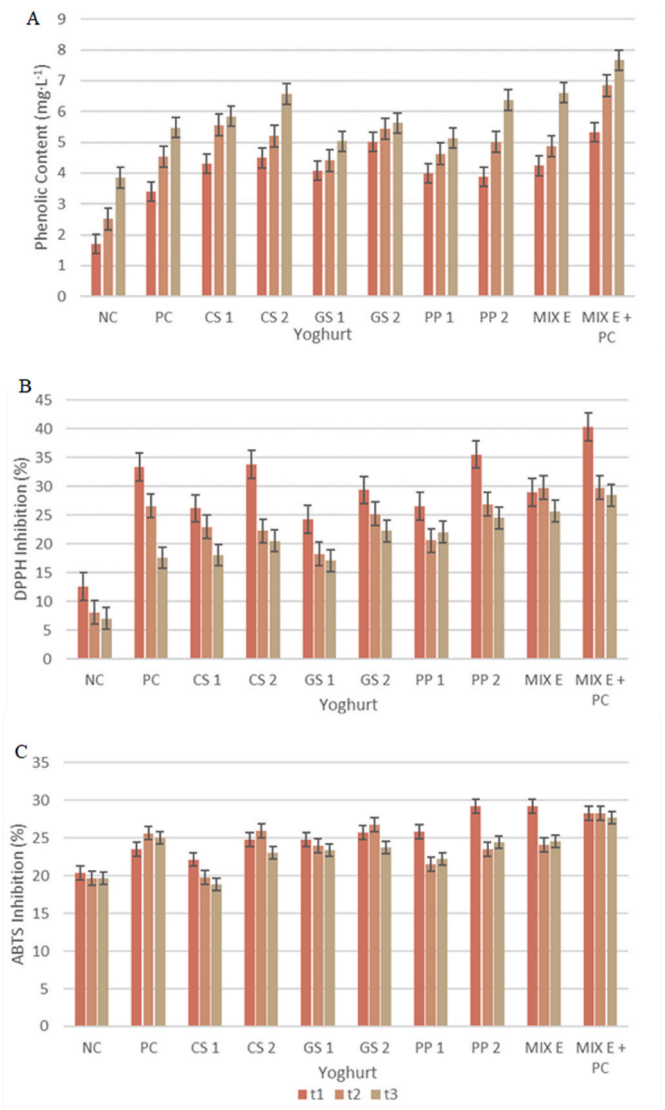

Fig. 5. Results obtained for: total phenolic content (A), DPPH (B) and ABTS(C), for the different yoghurt samples, for the three study times. t_1 refers to the first week (week of the production of the yoghurts), t_2 refers to the second week and t_3 refers to the third week. NC - Negative control (yoghurt without additives); PC - Positive control (yoghurt with Sorbic Acid); CS 1 –yoghurt with 0.1% of CS; CS 2 –yoghurt with 0.2% of CS; GS 1 –yoghurt with 0.1% of GS; GS 2 –yoghurt with 0.2% of GS; PP 1 –yoghurt with 0.1% of PP; PP 2 –yoghurt with 0.2% of PP; MIX E – yoghurt with all extracts; MIX E + PC – yoghurt with all extracts and sorbic acid.

extracts allowed for the enhancement of the antioxidant properties of the yoghurt, allowing the development of fortified food, whose consumption has potential health benefits due to high antioxidant potential.

3.4. Microbiological analysis

Regarding the microbiological analysis, two assays were performed on the yoghurt samples. Initially, the samples were evaluated regarding their level of contamination. For that, the solution of the yoghurts was inoculated in two different culture mediums, LSA – a specific culture medium for coliform microorganisms, such as *E. coli* – and RSA – a medium specific to fungi and molds. The assay was performed at t_1 and t_3. After 48 h, the results revealed no microbiological growth in all yoghurt samples (0 CFU mL^{-1}). The established limit of microorganisms allowed, to guarantee hygienic-sanitary regulation and consumer safety, is 10^2 CFU g^{-1}, for yoghurts (European Commission, 2005). Therefore, it is possible to conclude that the results are within the legal limits, proving that the incorporation of the phenolic extracts did not interfere with consumers' safety. Additionally, the results allude that the physicochemical properties of the yoghurts, such as acid pH value, create an environment that does not support microbial growth, which helps to prevent the spoilage of the product.

The yoghurts samples were also analysed regarding their antibacterial capacity, to evaluate their possibility to inhibit the growth of bacteria. This biological capacity was assessed against *S. aureus* and *E. coli*, major infectious bacteria in humans (Bachir & Abouni, 2015). The obtained results are displayed in Table 6.

Observing the results present in Table 6, it is possible to perceive that, the antibacterial capacity of the extracts increased with time. This behaviour of the yoghurts can be associated with the diminishment of the pH value throughout the study time, as previously shown in Fig. 2. The pH range that allows these bacteria to grow is 4.0–9.8 and 4.5–9, for *S. aureus* and *E. coli*, respectively (Medveďová & Valík, 2012; Wilks & Slonczewski, 2007). Therefore, the results are the expected, for higher pH values (at t_1) the antibacterial capacity is inferior for both bacteria; when the pH slightly decreases (at t_3), the capacity to inhibit bacteria increases. On the other hand, it is possible to detect that the yoghurts displayed higher inhibition capacity towards *E. coli* than *S. aureus*, which can also be associated with the pH values since all yoghurts demonstrated pH inferior to 4.5.

Additionally, the incorporation of phenolic extracts allowed to increase in the antibacterial capacity of the yoghurts, when compared to NC and PC. Phenolic extracts typically present flavonoids (such as epicatechin, quercetin, and kaempferol, among others), and literature reveals that there is a relationship between the structure of these compounds and the antibacterial capacity, provoked by the reduction of the fluidity in the hydrophobic and hydrophilic regions of the membrane (Hamad et al., 2020). Yoghurt GS2 was the one that exhibited better results in inhibiting the growth of *S. aureus*, while yoghurt PP2 was the most effective one in the inhibition of *E. coli.* Both GS and PP extract are characterized for displaying high contents in flavonoids, which shows that the obtained results are in agreement with the literature. The results seem to indicate that, higher concentrations of extract result in a higher antibacterial effect; however, the same does not apply to yoghurt MIX E, which can be associated with antagonist effects between the extracts, as a result of their composition.

Hereupon, the incorporation of phenolic extracts, obtained from different by-products, into yoghurts, improves the capacity to inhibit bacteria, proving their potential to be incorporated in foods to extend

Table 6
Inhibition halos diameter, in mm, for t_1 and t_3, of the different yoghurts, against *S. aureus* and *E. coli*.

Yoghurt	*S. aureus*		*E. coli*	
	t1	t3	t1	t3
NC	<5.0	6.3 ± 0.5	<5.0	6.0 ± 0.8
PC	<5.0	6.3 ± 0.5	6.3 ± 1.9	8.0 ± 0.8
CS 1	<5.0[a]	7.3 ± 0.5[a]	6.0 ± 1.4	8.0 ± 0.0
CS 2	<5.0[b]	8.6 ± 0.5[b]	8.3 ± 0.5	9.0 ± 0.8
GS 1	<5.0[c]	8.6 ± 0.5[c]	8.3 ± 0.5	8.3 ± 1.2
GS 2	<5.0[d]	10.0 ± 1.4[d]	7.3 ± 0.5	8.6 ± 2.6
PP 1	<5.0	6.0 ± 1.4	8.3 ± 1.9	8.3 ± 0.5
PP 2	6.3 ± 1.9	8.6 ± 0.5	8.3 ± 0.5	9.3 ± 1.2
MIX E	<5.0[e]	9.0 ± 0.8[e]	6.3 ± 1.9	8.3 ± 0.5
MIX E + PC	6.6 ± 2.4	8.6 ± 1.2	<5.0	6.0 ± 1.4

NC - Negative control (yoghurt without additives); PC - Positive control (yoghurt with Sorbic Acid); CS 1 –yoghurt with 0.1% of CS; CS 2 –yoghurt with 0.2% of CS; GS 1 –yoghurt with 0.1% of GS; GS 2 –yoghurt with 0.2% of GS; PP 1 –yoghurt with 0.1% of PP; PP 2 –yoghurt with 0.2% of PP; MIX E – yoghurt with all extracts; MIX E + PC – yoghurt with all extracts and sorbic acid. The results are expressed as means ± standard deviations of n = 3 independent measurements. a, b, c, d, and e values represented with the same letters are statistically different. ($p < 0.05$).

their shelf-life while creating a fortified and value-added product.

The present study unveiled that chestnut shell, grapeseed and pomegranate peel extracts revealed to be an interesting source of bioactive compounds, mainly phenolics, with extremely high antioxidant and antibacterial capacity, revealing their potential to be incorporated in food products as an alternative to synthetic compounds. Nevertheless, to guarantee the safety and security of the consumers, complementary toxicological assays should be performed by preclinical and clinical trials, that are not within the scope of this manuscript.

4. Conclusion

The present study aimed to evaluate the possibility to incorporate extracts from agro-industrial by-products, mainly chestnut shell, grapeseed, and pomegranate peel, into yoghurts and their effect on stability, while assessing their potential to replace synthetic antioxidants. The characterization of the three extracts proved that chestnut shell extract exhibited higher phenolic content and antioxidant capacity, and a lower IC_{50} towards α-amylase inhibition; pomegranate peel extract revealed better results regarding antibacterial capacity. Incorporation of the extracts into yoghurts did not affect their physical-chemical stability, throughout the storage period. Considering the microbial safety, all yoghurt samples fulfilled the legal requirements and were able to inhibit the growth of *E. coli* and *S. aureus*. Yoghurts fortified with grapeseed and pomegranate peel extract exhibited the best results. Oxidative analysis revealed that higher extract concentrations had a similar performance to the synthetic antioxidant, sorbic acid. Therefore, this study highlighted the possibility to incorporate extracts from agro-industrial by-products into foods, such as yoghurts, as a replacement for synthetic preservatives and as a functional ingredient, creating value-added products. It would be interesting to compare the obtained results with the incorporation of microencapsulated extracts.

Author statement

L.S.: Conceptualization, Methodology, Resources, Data Curation, Writing—Review and Editing, Visualization, Supervision, Project Administration, Funding Acquisition. S. M. F.: Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing Original Draft Preparation, Visualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

The data that has been used is confidential.

Acknowledgements

This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC).

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