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Comparative Study of the Consumer Acceptance and Health-Promoting Properties of Yogurts Containing Coffee and Wine-Making Byproduct Extracts

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Abstract: This study compared yogurts containing coffee (cascara and silverskin) and wine-making (pomace, skin, and seed) byproduct extracts as novel ingredients. For this purpose, the analysis of the sensory acceptance, basic information on phytochemical profile, and health-promoting properties of novel yogurt formulations were carried out. The antioxidant (ORAC, ABTS, DPPH, and intracellular ROS), antidiabetic (α-glucosidase inhibition), and anti-inflammatory (NO assay) properties of the yogurts depended on the type of byproduct extract and concentration used. Among the studied formulations, coffee cascara yogurt showed a high sensory acceptance (6.96), high overall antioxidant capacity (significantly higher ($p < 0.05$) values of TPC and antioxidant capacity measured by ORAC, ABTS, and DPPH than control yogurt), the best antidiabetic properties (inhibition of α-glucosidase activity of 83%), and a significant ($p < 0.05$) anti-inflammatory effect used as an ingredient at a final concentration of 10 mg/mL of food. The antioxidant and antidiabetic properties of cascara yogurt were also observed after in vitro digestion, which may be ascribed to unidentified bioactive compounds such as metabolites of phytochemicals and proteins generated during the physiological process. Overall, we developed a healthy, tasty, and sustainable coffee cascara yogurt containing antioxidant and antidiabetic compounds, which may be bioaccessible for their in vivo effects. The cascara yogurt can be consumed by the general public since the caffeine concentration in the food is within the recommended range for all population groups and it does not seem bioaccessible after the digestion of the food.

Keywords: coffee and wine byproducts; dietary fiber; bioaccessibility; novel ingredient; phytochemicals; yogurt

Academic Editor: Thomas Bintsis

Received: 28 February 2025
Revised: 10 April 2025
Accepted: 15 May 2025
Published: 20 May 2025

Citation: Iriondo-DeHond, M.; Iriondo-DeHond, A.; Herrera, T.; Miguel, E.; del Castillo, M.D. Comparative Study of the Consumer Acceptance and Health-Promoting Properties of Yogurts Containing Coffee and Wine-Making Byproduct Extracts. *Fermentation* **2025**, *11*, 291. https://doi.org/10.3390/fermentation11050291

1. Introduction

The improvement of the nutritional quality of dairy foods has become a key strategy for reducing the risk of developing diet-related non-communicable diseases. Among dairy products, fermented beverages such as yogurt have received particular attention

due to their proven nutritional value and beneficial effects on human health. Yogurt is a widely consumed food that serves as an excellent carrier for functional ingredients due to its favorable matrix and consumer acceptability [1]. Moreover, yogurt contains live cultures and essential nutrients, including calcium, vitamin B2, and high-quality protein. According to the European Food Safety Authority (EFSA), health claims authorized for yogurts containing specific live cultures (e.g., *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus*) include the improvement of lactose digestion. Additionally, yogurt is recognized as contributing to bone health and the maintenance of normal tooth and muscle function, due to its calcium content. These attributes make yogurt an ideal candidate for the development of functional foods aimed at promoting health and preventing chronic diseases. To further enhance the health-promoting properties of yogurt, the incorporation of bioactive-rich byproducts from grape and coffee processing has emerged as a promising strategy, aligning with current trends in functional food development and circular economy principles.

Coffee and wine are two of the most consumed beverages all over the world. In 2023, more than 11 and 72 million metric tons of green coffee beans and grapes were produced, respectively [2]. The conversion of the coffee and grape berries into the popular beverages is responsible for the generation of a large amount of byproducts which represent an environmental issue. Approximately 90% of the edible parts of the coffee berry are discarded during its conversion into the coffee beverage [3], whereas wine-making byproducts account for about 20–30% of the weight of the grape [4]. Therefore, the valorization of byproducts from the coffee and wine industries is essential to promote the development of a more sustainable food system.

Coffee fruit cascara, which is the skin and pulp of the coffee cherry, has been authorized as a novel food for commercialization in the European Union [5]. In April 2021, the dried coffee cascara, the outer skin and pulp obtained from the wet processing of coffee berries, from *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner was designated a safe traditional food by the EFSA [6]. The composition of cascara extracts, also called instant cascara, and its potential to fight key biological events associated with the pathogenesis of chronic diseases (oxidative stress and inflammation) has been recently documented [7]. This cascara extract was proposed as an ingredient in the development of sustainable yogurts with health-promoting properties [8,9]. Coffee silverskin (CS), the thin layer surrounding the coffee bean that detaches during roasting, has attracted growing interest for its valorization within circular economy approaches. It is currently considered a non-authorized novel food under European Union legislation, as no evidence of significant consumption prior to May 1997 is available. Consequently, its use in food products requires a prior safety evaluation and formal authorization under Regulation (EU) 2015/2283. Despite this regulatory status, aqueous extracts derived from coffee silverskin have been characterized and studied for their chemical composition, safety profile, and biological activity, providing a foundation for future applications [10,11]. Notably, silverskin can be used in yogurt production to increase the nutraceutical value of the products, and its bioactive compounds are bioaccessible during the digestion process [12]. These results support the potential of using aqueous coffee silverskin extract in yogurt formulations. In Europe, several developed products using coffee husks as an ingredient are already marketed [13]. Additionally, the use of coffee byproducts as food ingredients for different types of foods is supported by numerous studies [14–17]. However, further scientific evidence is required to reinforce their safety at different levels of exposure and to establish the causal relationships needed to substantiate health claims related to their use as a functional ingredient.

Grape pomace, which contains seeds, pulp, and skins, is the main residue derived from winemaking activities. According to EU regulations, grape seeds, pulp, skins, and other grape-derived byproducts are considered not novel in food as they have been consumed to a significant degree before 15 May 1997. Therefore, their use in food products does not require pre-market authorization according to Regulation (EU) 2015/2283. However, some specific uses of these byproducts may be subject to further regulatory checks, depending on the Member State's legislation. This regulatory status allows for the broader incorporation of grape pomace in food formulations, including as an ingredient in yogurt, where it can enhance the product's functional properties. The use of grape pomace and extracts from the seeds, skins, and pulp as a functional ingredient in yogurt has been explored in several studies, showing promise in enhancing antioxidant activity, providing dietary fiber, and contributing to the management of chronic diseases such as diabetes [18–21].

The aim of the present study was to compare coffee and wine-making byproducts and to identify the best candidate for the development of sustainable and health-promoting yogurts focusing on their biological and sensory properties. In this context, we analyzed the following: (a) the phytochemical profile and antioxidant, antidiabetic, and anti-inflammatory properties of yogurts containing coffee and wine-making byproducts as novel food ingredients; (b) the sensory acceptance of the formulated yogurts; and (c) the effect of the oral gastrointestinal digestion process on the bioaccessibility of health-promoting compounds in a yogurt formulation selected as the best functional food candidate among those studied in the present research based on their consumer acceptance, bioactive composition, and health-promoting properties.

2. Materials and Methods

2.1. Byproduct Extracts

2.1.1. Laboratory Prepared Extracts

Coffee cascara and silverskin from Arabica species and Colombian origin were kindly provided by Supracafé S.A. (Móstoles, Spain). The coffee cascara was generated during the obtention of the green coffee beans processed with the wet method. The coffee cascara used in this study was composed of the outer skin of the coffee berry and pulp. The coffee cascara was subjected to a sanitation process involving the use of a carbon dioxide atmosphere (MABA-PEX process). The coffee cascara was certified as safe for consumption by Coffee Consulting S.L., as no chemical nor biological contaminants were detected in the samples. The coffee beans were roasted using a PROBAT roaster (Emmerich am Rhein, Germany) for 13 min at 220 °C, and coffee silverskin was generated during this process. The origin, type of processing, processing steps, and the nutritional and bioactive compound composition of the coffee byproducts were described previously [7,8,10,11].

Aqueous extracts were produced, as described in the patent WO 2013/004873 Al [22]. Briefly, 50 g of coffee cascara or silverskin were added per water liter. These mixtures were stirred for 10 min at 100 °C, filtered, and the filtrates were freeze-dried. The powdered ingredients were stored at room temperature until further use. Extraction yields were 28% and 14% for coffee cascara and silverskin, respectively [3].

2.1.2. Commercial Extracts

Commercial whole grape pomace, grape seed, and grape skin hydroalcoholic extracts derived from byproducts of the wine-making process were purchased from Natac (Madrid, Spain). These extracts were supplied as fine powders that are partially soluble in water.

The bioactive compounds and antioxidant and antidiabetic properties of the coffee and wine-making byproduct extracts are summarized in Table S1.

2.2. Food Samples

2.2.1. Yogurt Samples

Six set-type yogurt formulations were prepared: coffee cascara, coffee silverskin, grape pomace, grape skin, and grape seed yogurts, and a control yogurt without byproduct extract. Yogurt samples were made using whole UHT cow milk (3.6% fat, 3% protein, and 4.8% sugar), starter culture YO-MIX 300 (Danisco, Copenhagen, Denmark), and 6% (*w*/*v*) inulin (Orafti®GR, Beneo, Tienen, Belgium). Inulin, a soluble, non-digestible, fermentable fiber, was added for technological and nutritional purposes to improve the texture properties of the yogurts and to achieve the dietary reference value (DRV) of 25 g of dietary fiber established by the European Food Safety Authority (EFSA). Byproduct extracts were dissolved in water, and the resulting soluble fractions were used as ingredients in the yogurt formulation, added to the milk at a final concentration of 4 mg/mL (coffee silverskin), 5 mg/mL (wine byproducts), and 10 mg/mL (coffee cascara) (Table 1). The aqueous extracts of the byproducts were prepared at a concentration 10 times higher than the final concentration in the yogurt. Yogurt samples were produced by heating the milk up to 45 °C, to inoculate the lyophilized starter culture (45 mg starter culture/L of milk). Then, inulin (6 g/100 mL) was added. Winery byproduct extracts were dissolved in water and added at a concentration of 5 mg/mL in the milk. In control yogurts, the corresponding amount of water was added without extract. The milk was stirred, separated into pots, and incubated at 45 °C for 5 h, which was the time needed for the yogurts to reach a pH value of approximately 4.6. The content of polyphenols in the extracts depended on their nature. These concentrations were determined by a preliminary pilot sensory acceptance test with 10 volunteers in which yogurts with different byproduct extract concentrations were analyzed. Coffee and wine-making byproduct extracts presented bioactive compounds (Section 2.3) with antioxidant capacity determined by the assays described in Section 2.4.1 (Table S1). Yogurt manufacturing was performed in triplicate in three independent sessions to assure reproducibility of the results.

Table 1. List of ingredients of yogurts formulated with coffee and wine-making byproduct extracts.

Type of Yogurt	Ingredients * (%)	Total Phenolic Content Incorporated (mg GAE/100 mL)
Yogurt control	Milk (85) + SC + inulin (6) + water (10)	
Yogurt coffee cascara	Milk (85) + SC + inulin (6) + coffee cascara extract (100 mg/mL) (10)	760.2
Yogurt coffee silverskin	Milk (85) + SC + inulin (6) + coffee silverskin extract (40 mg/mL) (10)	151.76
Yogurt grape pomace	Milk (85) + SC + inulin (6) + grape pomace extract (50 mg/mL) (10)	1237.55
Yogurt grape skin	Milk (85) + SC + inulin (6) + grape skin extract (50 mg/mL) (10)	1589.45
Yogurt grape seed	Milk (85) + SC + inulin (6) + grape seed extract (50 mg/mL) (10)	1964.55

SC: Starter culture. * Describes the ingredients in the order in which they were added in the yogurt production process.

2.2.2. Yogurt Digests

The coffee cascara yogurt was selected due to its sensory, chemical, and health-promoting properties to evaluate the bioaccessibility of phytochemicals naturally present in the cascara extract and other food components, which possessed antioxidant, antidiabetic, and anti-inflammatory potential after in vitro oral gastrointestinal digestion processes. A yogurt without any extract was used as the control. Cascara and control yogurts were digested in triplicate, mimicking human digestion conditions, as described previously [7,23].

2.2.3. Sample Preparation

Yogurt samples were prepared for further analysis, as described by Marchiani et al. (2016) [24], on day 1 after yogurt manufacture. Briefly, each sample (10 g) was diluted in distilled water (10 mL) and filtered in two steps with Whaltman no. 1 paper followed by a 0.45 µm filter (Symta, Madrid, Spain). The filtered extracts were stored at −20 °C.

Free phytochemicals recovered by sample preparation were analyzed by CZE. In addition, the health-promoting properties (antioxidant and anti-inflammatory) of the samples were assessed. Sample preparation for the analysis of α-glucosidase inhibition followed the same procedure, increasing the yogurt concentration (2.5 g in 10 mL of distilled water).

The resulting yogurt digests from the in vitro oral gastrointestinal digestion process were freeze-dried. Yogurt digests were diluted in distilled water to 10 mg/mL, filtered (0.45 µm filter), and their compositions of free phytochemicals and antioxidant, antidiabetic, and anti-inflammatory compounds were analyzed.

In relation to the byproduct extracts, coffee cascara and silverskin extracts were diluted in distilled water to a final concentration of 1 mg/mL and filtered with a 0.45 µm filter. Grape pomace, seed, and skin extracts were diluted in a dimethyl sulfoxide/water mixture (1:10) at a final concentration of 1 mg/mL and filtered with a 0.45 µm filter. Coffee and wine-making samples were stored at −20 °C until further analyses.

2.3. Chemical Characterization of Byproduct Extracts and Yogurts

2.3.1. Total Phenolic Content (TPC)

TPC analysis in extracts and yogurts was carried out by the Folin–Ciocalteu method [25] adapted to the microplate method. Briefly, 10 µL of each sample extract or gallic acid standard solution were transferred into the wells of a 96-well microplate. Then, 150 µL of Folin–Ciocalteu phenol reagent (previously diluted 1:15, *v*/*v*, in water) were added, and the mixture was incubated at room temperature for 3 min. Subsequently, 50 µL of sodium carbonate (Na_2CO_3) were added to each well, and the plate was incubated in the dark at 37 °C for 120 min to allow color development. A gallic acid calibration curve (0.01–1 mg/mL) was used for quantification and the results were expressed as mg GAE/g sample. Measurements were performed in triplicate.

2.3.2. Total Anthocyanin Content

The total anthocyanin content of the byproduct extracts and yogurts was estimated using the spectrophotometric pH differential method [26]. To analyze the sample extracts and cyanidin-3-O-glucoside chloride standard solutions, 10 µL of each were transferred into the wells of a 96-well microplate. Then, 190 µL of pH 1.0 potassium chloride buffer and 190 µL of pH 4.5 sodium acetate buffer were added separately to prepare the test portions. After a 15 min incubation, absorbance was measured at both 520 nm and 700 nm. Results were expressed as mg cyanidin-3-O-glucoside chloride (C3G) equivalents/g sample and all measurements were performed in triplicate.

2.3.3. Capillary Zone Electrophoresis (CZE)

Yogurt sample preparations (1 g/mL) and byproduct extracts at the concentrations used in yogurts (10 mg/mL coffee cascara, 4 mg/mL coffee silverskin, and 5 mg/mL grape pomace, seed, and skins) and digests (50 mg/mL) were subjected to CZE, as described by del Castillo et al. [27]. Determinations were carried out in an Agilent G1600 A (Santa Clara, CA, USA) capillary electrophoresis instrument equipped with ChemStation software (Product number G1601BA) and a diode array detector (DAD). The capillary was 48.5 cm long (40 cm to the detector) with an internal diameter of 50 µm and a ×3 bubble cell. Other conditions of analysis were as follows: 20 mM borate buffer at pH 9.3; voltage, 20 kV; temperature of analysis, 25 °C temperature; injection, 50 mbar for 5 s; and electroosmotic flow (EOF) marker, acetone. Electropherograms (e-grams) were monitored at 280 nm, and spectra were collected from 190 to 600 nm. Bioactive compounds were detected and quantified by comparison of migration times and UV/VIS spectra using the following as references: gallic acid, caffeic acid, ferulic acid (FA), chlorogenic acid (CGA), rutin hydrate,

catechin (CAT), C3G, procyanidin B2, genistein, glycitein, trigonelline hydrochloride, melatonin, and caffeine (CAF) (Table S2). All analyses were performed in triplicate.

2.4. Health-Promoting Properties of Byproduct Extracts and Yogurts

2.4.1. Antioxidant Properties

The overall antioxidant capacities of the extracts, yogurts, and yogurt digests (control and cascara) were analyzed using the following methods:

- 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

The DPPH free radical scavenging capacities of extracts and yogurts were evaluated as according to Brand-Williams, Cuvelier and Berset (1995) [28], adapted to the microplate method. For the analyses of sample extracts and cyanidin-3-O-glucoside chloride standard solutions, 10 µL of each were dispensed into the wells of a 96-well microplate. Subsequently, 190 µL of pH 1.0 potassium chloride buffer and 190 µL of pH 4.5 sodium acetate buffer were added separately to prepare the test solutions. After incubating for 15 min at room temperature, absorbance was recorded at 520 nm and 700 nm. Trolox was used as a reference standard (0.25–2.5 mM in ethanol). All measurements were performed in triplicate and results were expressed as µmol TE/g sample.

- 2,2′-azinobis-(3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) assay

The trapping capacity of cationic free radicals was evaluated using the method of radical ABTS+ bleaching described by Re et al. [29], and modified by Oki et al. [30] for its use in a microplate. The extract, yogurt, and digest samples (30 µL) were added to 270 µL of ABTS radical. The absorbance of mixture was read at 734 nm using a UV–Visible Spectrophotometer (BioTek Instruments, Winooski, VT, USA). Aqueous solutions of Trolox (0.15–2.0 mM) were used for calibration. All measurements were performed in triplicate and results were expressed as µmol TE/g sample.

- Oxygen Radical Absorbance Capacity (ORAC) assay

The ORAC assay was applied according to the method of Ou et al. [31], as modified by Dávalos et al. [32]. The reaction was carried out in 75 mmol/L phosphate buffer (pH 7.4). The extract, yogurt, and digest samples (20 µL) and fluorescein (120 µL; 70 nM final concentration) solutions were added. The mixture preincubated for 10 min. Finally, AAPH solution (60 µL, 12 mM final concentration) was added. The fluorescence was recorded every 104 min at 37 °C. The excitation and emission filters were 485 and 520, respectively. All measurements were performed in triplicate and results were expressed as µmol TE/g sample.

- Physiological intracellular reactive oxygen species (ROS)

Normal rat small intestine epithelial cells (IEC-6) were kindly provided by the Bioanalytical Techniques Unit (BAT) of the Instituto de Investigación en Ciencias de la Alimentación (CIAL) (Madrid, Spain). Cells were cultured as a monolayer in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% *v*/*v* heat-inactivated fetal calf serum (FBS), 50 U/mL penicillin, and 50 µg/mL streptomycin and 1% *v*/*v* L-glutamine, at 37 °C and in 5% CO_2 in a humidified incubator (BINDER CB series 2010, Tuttlingen, Germany).

Prior to the study of physiological intracellular ROS, the effect of different concentrations of extracts, yogurts, and yogurt digests (control and cascara) on cell viability was measured by the MTT assay [33] in order to select non-cytotoxic doses (Tables S4 and S5). IEC-6 cells were treated with extracts in concentrations present in yogurts (coffee cascara extract (10, 100, and 1000 µg/mL), coffee silverskin extract (4, 40, and 400 µg/mL) and wine byproduct extracts (5, 50, and 500 mg/mL)), yogurts (1, 10, and 100 mg/mL), and yogurt digests (0.01, 0.1, and 1 mg/mL). DMSO (50%) was used as the death control.

Subsequently, IEC-6 cells were incubated with MTT solution (0.5 mg/mL) at 37 °C for 3 h. After incubation, the supernatant was discarded, and 100 µL of DMSO were added to dissolve the formazan crystals. The optical density was then measured at 570 nm using a microplate reader (Epoch 2, BioTek Instruments, Winooski, VT, USA).

The determination of physiological intracellular ROS was performed following the same procedure used by Iriondo-DeHond et al. [11]. Tert-butyl hydroperoxide (tBOOH) 1 mM was used as a positive oxidation control and vitamin C as an antioxidant control. Then, a MTT assay was performed to normalize the data by the number of cells per well. Non-treated control cells produced 100% of physiological ROS. Experiments were carried out in triplicate.

2.4.2. Antidiabetic Properties

The α-glucosidase inhibition assay was carried out to study the antidiabetic properties of samples. The α-glucosidase inhibitory capacities of byproduct extracts, yogurts, and digested yogurts (control and cascara), were analyzed as described previously [18,34,35]. In a 96-well microplate, 100 µL of sample dissolved in PBS (100 mM, pH 6.9) were mixed with 100 µL α-glucosidase (diluted 1/10) and 100 µL 4-MUG (2 mM). Fluorescence was then monitored at an excitation wavelength of 360 nm and an emission wavelength of 460 nm for 30 min at 37 °C. Results were expressed as percentage of α-glucosidase inhibition. All measurements were performed in triplicate.

2.4.3. Anti-Inflammatory Properties

Mouse macrophages (RAW 264.7) were cultured in the same conditions as intestinal cells. RAW 264.7 cells were treated with the same concentrations used for intestinal cells and only non-cytotoxic concentrations were used for the analysis of anti-inflammatory properties (Tables S3 and S4). Anti-inflammatory properties were studied by measuring nitric oxide (NO) production in RAW 264.7 cells by inducing inflammation with *E. coli* lipopolysaccharide (LPS) [36]. Cells were plated at a density of 80,000 cells per well in a 96-well plate. After 24 h, cells were pre-treated with extracts, yogurts, or yogurt digests in the same concentrations used for viability assays for 24 h. Then, cells were treated with LPS (1 µg/mL) and samples for 24 h. After incubation, 100 µL of supernatant or standard curve (NO, 0–10 µg/mL) were mixed with 100 µL of Griess reagent (1% (*w*/*v*) sulfanilamide and 0.1% (*w*/*v*) N-1-(naphthyl) ethylenediamine-diHCl in 2.5% (*v*/*v*) H3PO4). Absorbance at 550 nm was measured (BioTek Epoch 2 Microplate Spectrophotometer, Winooski, VT, USA) after a 15 min incubation in the dark and at room temperature. Experiments were carried out in triplicate.

2.5. Consumer Acceptance Analysis

Consumers (n = 70) were recruited at the Instituto de Investigación en Ciencias de la Alimentación (CIAL) (Madrid, Spain). The test consisted of a hedonic evaluation of the coffee and wine-making byproduct yogurts and the control yogurt. Yogurt samples (30 mL) were served at 7 °C using individual plastic containers identified with a random 3-digit code. Samples were given in blind conditions and were served in completely randomized order. Consumers were asked to rate the acceptance on appearance, smell, flavor, texture, and overall acceptance of the samples by using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely).

2.6. Statistical Analysis

A one-way analysis of variance (ANOVA) followed by Tukey's test for mean comparisons were used to highlight significant differences among samples. All statistical analyses were performed using IBM SPSS Statistics 24.

3. Results

3.1. Chemical Characterization of Yogurts

Bioactive compounds present in yogurts were preliminary identified by CZE based on their spectrum and migration time (Figure 1A) and quantified (Table 2). CAF was detected in yogurts made with coffee cascara and silverskin extracts. Free FA and CGA were detected in the original coffee byproduct extracts (Figure 1B, Table S1), but not in the yogurts. CAF was significantly higher ($p < 0.001$) in coffee silverskin extract compared to the coffee cascara extract (Table S1), and was the major phytochemical found in both samples. The content of CGA was also significantly higher ($p < 0.001$) in coffee silverskin extract. FA was only detected in coffee cascara extract.

Anthocyanins were identified in yogurts containing wine-making byproduct extracts. C3G was identified at 4.6 min in the grape pomace, skin, and seed yogurts. CAT was identified at 4.7 min in the grape pomace yogurt (Figure 1A) and grape pomace and seed extracts (Figure 1B). The grape skin extract showed a significantly ($p < 0.005$) higher content of C3G than the grape pomace and seed extracts.

Yogurts containing coffee cascara or grape pomace, skin, or seed extracts presented a significant increase ($p < 0.05$) in their TPC compared to the control yogurt (Table 2). The lowest TPC was observed in coffee silverskin yogurt (0.05 ± 0.00 mg GAE/g yogurt), which was in the same range as the control yogurt ($p > 0.05$).

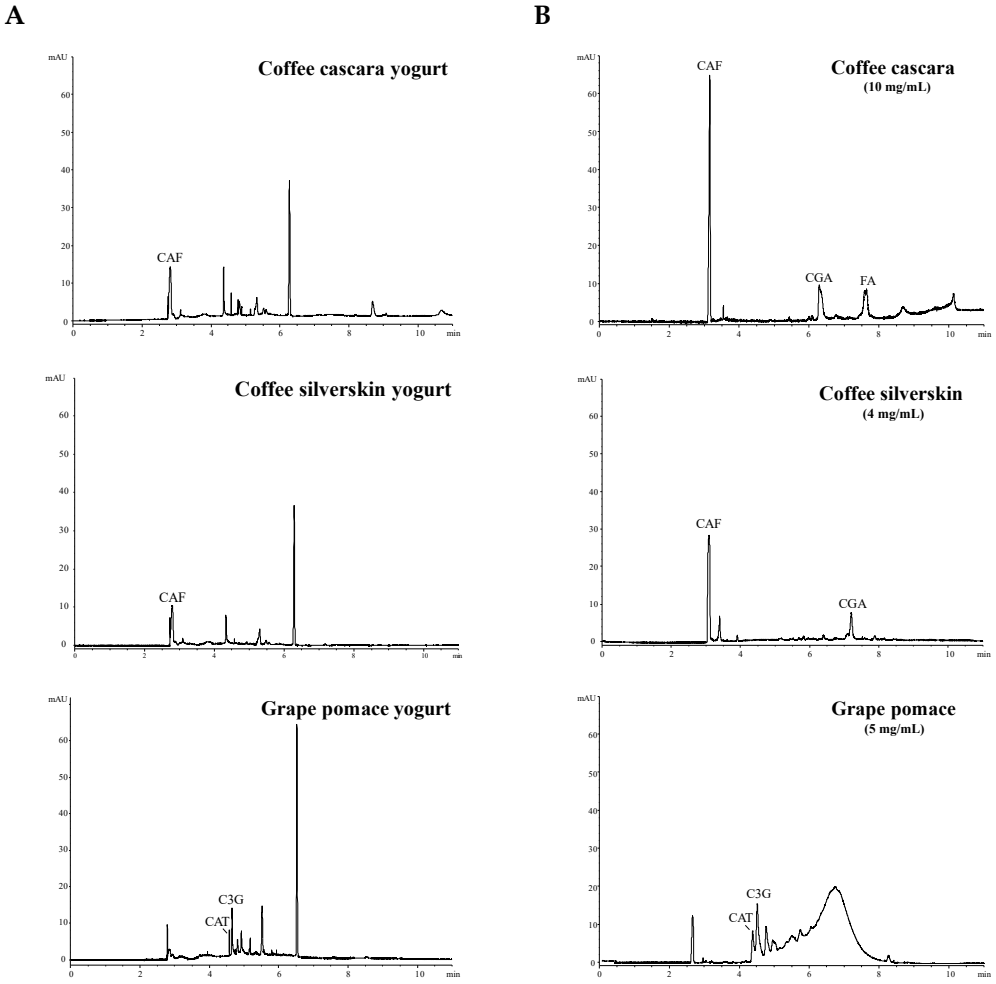

Figure 1. *Cont.*

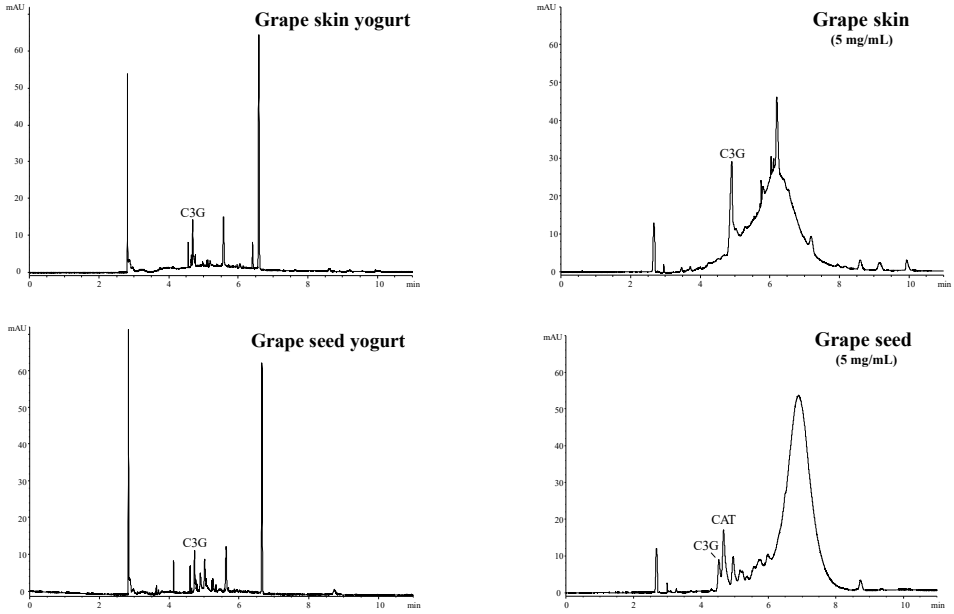

Figure 1. E-grams recorded at 280 nm showing the identified compounds from (**B**) coffee and wine-making byproduct extracts (at the concentrations used in the corresponding yogurts) and (**A**) yogurts containing the extracts as food ingredients (1 g/mL). Peak identification: CAF, caffeine; CGA, chlorogenic acid; FA, ferulic acid; CAT, catechin; C3G, cyanidin-3-O-glucoside chloride.

Table 2. Antioxidant and antidiabetic properties of yogurts formulated with coffee and wine-making byproduct extracts.

		Coffee Byproducts		Wine-Making Byproducts		
	Control	**Cascara**	**Silverskin**	**Grape Pomace**	**Skin**	**Seed**
Chemical characterization						
TPC (mg GAE/g yogurt)	0.04 ± 0.01^{a}	0.13 ± 0.02^{c}	0.05 ± 0.00^{ab}	0.10 ± 0.01^{bc}	0.09 ± 0.03^{bc}	0.09 ± 0.02^{bc}
Anthocyanins (C3G eq./g yogurt)	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.01	n.d.
CAF (mg/g)	n.d.	0.11 ± 0.03^{a}	0.06 ± 0.01^{a}	n.d.	n.d.	n.d.
CGA (mg/g)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FA (mg/g)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C3G (mg/g)	n.d.	n.d.	n.d.	+	+	+
CAT (mg/g)	n.d.	n.d.	n.d.	+	n.d.	n.d.
Antioxidant capacity						
ORAC (μmol TE/g yogurt)	1.79 ± 0.23^{a}	5.03 ± 0.11^{b}	2.82 ± 0.19^{a}	6.15 ± 0.36^{bc}	5.24 ± 1.07^{bc}	7.26 ± 1.32^{c}
ABTS (μmol TE/g yogurt)	0.42 ± 0.04^{a}	3.26 ± 0.13^{c}	1.01 ± 0.29^{ab}	1.63 ± 0.05^{b}	1.59 ± 0.36^{b}	1.67 ± 0.38^{b}
DPPH (μmol TE/g yogurt)	0.00 ± 0.00^{a}	0.75 ± 0.17^{c}	0.22 ± 0.07^{ab}	0.73 ± 0.12^{c}	0.47 ± 0.07^{b}	0.90 ± 0.09^{c}
Antidiabetic properties *						
α-Glucosidase inhibition (%)	41.47 ± 5.72^{a}	82.87 ± 2.14^{c}	64.54 ± 4.05^{b}	54.11 ± 3.96^{ab}	63.81 ± 5.62^{b}	53.10 ± 5.16^{ab}

* Concentrations for antidiabetic analyses: α-glucosidase inhibition was conducted at 4 g/mL in yogurts containing coffee and wine-making byproducts. Different letters denote statistically significant differences between formulated yogurts (Tukey test, $p < 0.05$). + compound detected through migration time and UV/VIS spectra but not quantified. n.d.: not detected.

3.2. Health-Promoting Properties of Yogurts

3.2.1. Antioxidant Properties

Results from the antioxidant capacity in vitro determinations in yogurts (Table 2), showed that the addition of 4 mg/mL of coffee silverskin extract did not significantly increase ($p > 0.05$) the basal formulation (control yogurt) antioxidant capacity. Yogurts containing coffee cascara or grape pomace, skin, or seed extracts showed a significant increase ($p < 0.01$) in their antioxidant capacity compared to the control yogurt, independently from the method used for their analysis. Regarding the study of intracellular ROS formation (Table S3), the correct physiological redox status of cells was confirmed, since the oxidation control (t-BOOH 1 mM) significantly increased ($p < 0.05$) ROS and the antioxidant standard (vitamin C 10 μg/mL) significantly reduced ($p < 0.05$) ROS levels. Coffee and wine-making byproduct extracts significantly reduced ($p < 0.05$) physiological intracellular ROS. The

decrease in physiological intracellular ROS levels was similar ($p > 0.05$) to that observed for vitamin C (10 µg/mL, 32.40 ± 12.01% ROS). However, no significant effects ($p > 0.05$) were observed when cells were treated with yogurts (1, 10, and 100 mg/mL) compared to control non-treated cells (Table S3).

3.2.2. Antidiabetic Properties

Yogurts containing coffee byproduct extracts and grape skin extract significantly inhibited the activity of the enzyme ($p < 0.01$) compared to the control (Table 2). However, all coffee and wine-making byproduct extracts inhibited the activity of α-glucosidase (Table S1). Among the studied formulations, coffee cascara yogurt showed the highest significant ($p < 0.01$) inhibition of the activity of α-glucosidase (83%).

3.2.3. Anti-Inflammatory Properties

Yogurts containing coffee cascara and coffee silverskin significantly reduced ($p < 0.05$) levels of induced NO (Figure 2A). This effect was also observed when cells were treated with pure extracts (Figure 2B). No significant changes ($p > 0.05$) in NO levels were observed in macrophages treated with yogurts containing wine-making byproduct extracts. However, grape pomace and grape seed extracts at 50 µg/mL significantly reduced ($p < 0.05$) the production of NO.

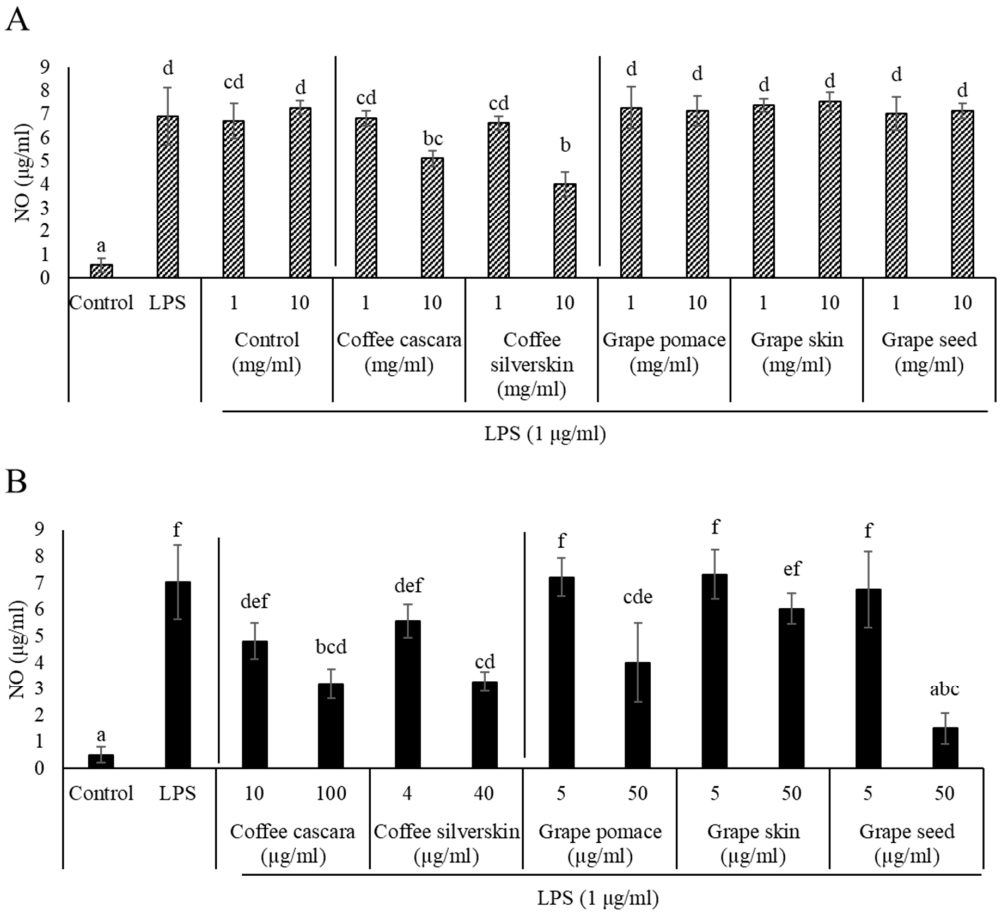

Figure 2. Anti-inflammatory effects of coffee and wine-making byproduct extracts (**A**) and yogurts containing the extracts as food ingredients (**B**) on nitric oxide (NO) generation in RAW264.7 induced by LPS (1 µg/mL). Cells were pre-treated with extracts or yogurts for 24 h. Then, macrophages were treated with samples and LPS for 24 h. Supernatants from treated cells and NO standards were mixed with Griess reagent (1:1) and absorbance was measured at 550 nm. Data represent means ± SD of three independent experiments. Different letters denote statistically significant differences between all treatments (Tukey test, $p < 0.05$).

3.3. Consumer Analysis of Yogurts

Results from the hedonic test with untrained consumers (n = 70) are shown in Table 3. The basal formulation obtained average acceptance scores > 6.8 for all its attributes. The smell of the grape seed yogurt was significantly lower ($p < 0.01$) than the control. Coffee silverskin yogurt showed a significantly lower ($p < 0.05$) taste score than the rest of the studied yogurts. No differences were observed in the texture properties between yogurts ($p > 0.05$). Overall, the control yogurt and yogurts containing coffee cascara and wine-making byproduct extracts showed high overall liking values (6.22–7.08) (Figure 3). Only the coffee silverskin yogurt showed a significantly lower overall acceptance ($p < 0.01$) than the other formulations. The acceptance scores of coffee cascara and control yogurts were not significantly different for all their attributes ($p > 0.05$).

Table 3. Mean consumer acceptance scores on appearance, smell, taste, and texture of control yogurt and yogurts formulated with coffee and wine-making byproducts using a nine-point hedonic scale.

	Appearance	Smell	Taste	Texture
Control	7.69 ± 1.26 ^{c}	6.82 ± 1.71 ^{b}	7.38 ± 1.44 ^{c}	7.67 ± 1.26 ^{a}
Coffee cascara	6.95 ± 1.83 ^{abc}	6.87 ± 1.49 ^{b}	6.82 ± 1.55 ^{bc}	6.82 ± 1.47 ^{a}
Coffee silverskin	6.59 ± 1.60 ^{ab}	6.49 ± 1.62 ^{ab}	4.36 ± 2.08 ^{a}	6.72 ± 1.81 ^{a}
Grape seed	5.93 ± 1.49 ^{a}	5.44 ± 1.25 ^{a}	6.48 ± 1.63 ^{bc}	6.74 ± 1.43 ^{a}
Grape skin	7.30 ± 1.26 ^{bc}	5.78 ± 1.40 ^{ab}	5.89 ± 1.99 ^{b}	6.85 ± 1.35 ^{a}
Grape pomace	5.96 ± 1.34 ^{a}	5.96 ± 1.31 ^{ab}	6.52 ± 1.60 ^{bc}	7.04 ± 1.43 ^{a}

Data represent means ± SD. Different letters in columns denote statistically significant differences between yogurts (Tukey test, $p < 0.05$).

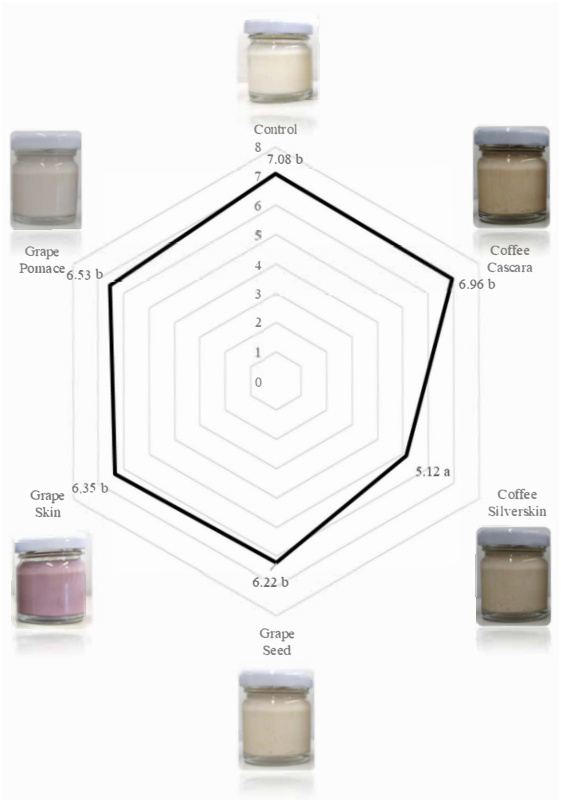

Figure 3. Consumer (n = 70) overall acceptance of yogurts formulated with coffee byproducts (cascara and silverskin) and wine-making byproducts (grape pomace, skin, and seed), measured on a nine-point hedonic scale. Different letters denote statistically significant differences between yogurts (Tukey test, $p < 0.01$).

3.4. In Vitro Oral Gastrointestinal Digestion of Coffee Cascara Yogurt

3.4.1. Bioaccessible Compounds in Cascara Yogurt Digests

The TPC of the digested cascara yogurt was 4.90 ± 0.13 mg GAE/g digest, which was similar ($p > 0.05$) to the control yogurt's 4.83 ± 0.64 mg GAE/g digest. No free individual phytochemicals in yogurt digests were found by CZE with UV–VIS detection (Figure 4).

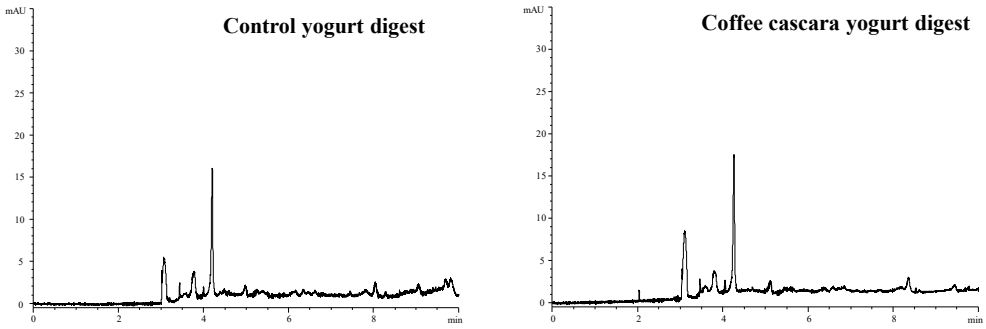

Figure 4. E-grams recorded at 280 nm from the digests of the control yogurt and yogurt containing coffee cascara extract (50 mg/mL).

3.4.2. Antioxidant, Antidiabetic, and Anti-Inflammatory Properties of Bioaccessible Compounds in Cascara Yogurt Digests

The antioxidant capacities of digests from the control and coffee cascara yogurts analyzed with the ABTS radical were not significantly different ($p > 0.05$) (186.43 ± 9.71 µmol TE/g digest and 187.04 ± 17.69 µmol TE/g digest, respectively). However, the antioxidant capacity measured with the ORAC method was significantly higher in cascara yogurt digests (100.34 ± 12.77 µmol TE/g digest) compared to that of the control (76.04 ± 10.94 µmol TE/g digest) ($p < 0.05$).

Considering the effect of yogurt digests on intracellular ROS levels, concentrations of 0.01 and 0.1 mg/mL were tested on intestinal cells (Table S5). Control yogurt digests did not alter the antioxidant status of IEC-6 cells compared to non-treated control cells. However, a dose of 0.01 mg/mL of the cascara yogurt digest significantly reduced ($p < 0.05$) physiological intracellular ROS levels to about 75%.

The antidiabetic properties of the cascara yogurt were observed after the in vitro digestion process. Coffee cascara yogurt digest significantly ($p < 0.001$) inhibited the activity of the α-glucosidase enzyme (20.06 ± 2.81%) compared to the control digest (7.79 ± 2.16%).

The two lower concentrations of yogurts (1 and 10 mg/mL) and all the concentrations tested for digests were used for NO determination. However, none of the tested concentrations were able to reduce NO levels induced by LPS.

4. Discussion

The yogurts developed in the present study showed health-promoting properties which may be attributed to the presence of phytochemicals derived from the coffee and wine-making byproduct ingredients (Figure 1). The observed content of bioactive compounds in yogurts containing coffee and wine-making byproduct extracts may be influenced by several factors, including compound degradation during milk fermentation [37], the food matrix effect, and sample preparation for analysis by CZE with UV/VIS detection. Bioactive compounds undergo significant changes after ingestion. These changes affect their bioavailability and potential therapeutic applications. The metabolic pathways that may be involved in these changes are complex and often produce metabolites with biological activities different from those of the original compounds. It is very important to understand these changes in order to draw conclusions about the health benefits of polyphenol-rich extracts and thus their implications for disease prevention [38].

There is limited information on the metabolic activity of yogurt starter culture bacteria on phenolic compounds. Some *Lactobacillus* species, such as *L. plantarum*, have been described to metabolize plant-derived phenolic compounds, such as hydroxycinnamic acids and tannins [39]. Fermentation with *Lactobacillus plantarum* significantly increased the content (accessibility) of phenolic compounds in several herbal infusions compared to non-fermented infusions, with enhanced antioxidant and anti-inflammatory activities [40]. Polyphenols present in yogurts fortified with byproducts from wine-making or coffee could be altered by fermentation by lactic acid bacteria, leading to their biotransformation. Microbial enzymes (such as tannases, esterases, phenolic acid decarboxylases, and glycosidases) can hydrolyze complex polyphenols into smaller phenolic compounds that may have altered bioactivity and bioavailability [41–43]. Therefore, further analyses are needed to study whether the starter culture could have modified the parental phytochemical structures of the extracts during milk fermentation.

On the other hand, there is evidence of a matrix effect concerning protein–polyphenol interactions between milk proteins and phytochemicals. An immediate decrease of polyphenol compounds was observed 24 h after adding a strawberry preparation to milk for yogurt elaboration, with a decrease within a range of 29% to 60% in individual polyphenols [44]. Polyphenols can establish molecular interactions with milk proteins, forming complexes. The association can alter the conformation of proteins, also modifying their physical and chemical properties and potentially influencing the stability and bioaccessibility of polyphenols [45,46]. Interaction with milk proteins is important for the antioxidant properties of polyphenols in milk matrices and also conditions their processing during digestion. Mao et al. (2024) showed that polyphenols from blackcurrant extracts were preferentially bound to caseins more than whey proteins, with noncovalent interactions causing secondary structural changes in the proteins. Additionally, the bioaccessibility and antioxidant activity of polyphenols were enhanced in the presence of milk proteins in milk-based blackcurrant samples when compared to polyphenol and protein-alone samples in the in vitro gastric phase [47].

The observed antioxidant capacity of yogurts containing coffee cascara and wine-making extracts may be associated with protein–polyphenol complexes, whose formation has been previously described in other dairy foods fortified with phenol compounds, such as strawberry yogurt formulations, Greek-style yogurt fortified with cheese whey- spent coffee ground powder and tea beverages with milk, or milk with chocolate [14,48–50].

The coffee cascara, silverskin, and grape skin yogurts showed significant ($p < 0.01$) inhibition of the enzyme α-glucosidase compared to the control. Intestinal α-glucosidases are key enzymes responsible for carbohydrate digestion and absorption. Their inhibition has been proven effective in both preventing and treating diabetes by targeting postprandial hyperglycemia [51]. The antidiabetic properties of yogurts have been less studied. The inhibition of α-glucosidase activity has been previously measured in yogurts containing winery byproducts [18], garlic, cinnamon, and other herbs [52–54], but there is no previous reference of the effect of yogurts containing coffee byproducts on the activity of diabetes' key enzymes. The antidiabetic effect of the coffee cascara extract is described in this study for the first time (Table S1), while that for coffee silverskin extract has been previously reported [55], and can be associated to the presence of phytochemicals such as CGA and FA [56,57]. Since these compounds were detected in the corresponding extract and not quantified in the coffee cascara and silverskin yogurts (Figure 1), its antidiabetic properties may be associated to their derivatives (metabolites) or other unknown compounds. Similarly, the antidiabetic properties of the grape skin yogurt may be attributed to C3G and other unidentified compounds [19,38,58,59].

In relation to their anti-inflammatory properties, coffee cascara and silverskin extracts at 10 mg/mL reduced LPS-induced inflammation (Figure 2). A recent study concerning the anti-inflammatory properties of coffee skin/pulp obtained from semi-dry processing [60] showed that coffee pulp was capable of inhibiting the release of the pro-inflammatory cytokine IL-8 in gastric epithelial cells when inflammation was induced by TNF-α [60]. CAF, a compound detected in yogurts containing coffee byproduct extracts (Figure 2), showed anti-inflammatory properties by reducing NO levels in LPS-induced RAW 264.7 cells, the same model used in our study [7,61,62]. Therefore, CAF may be one of the contributors to the overall anti-inflammatory effect observed in the novel foods.

Anti-inflammatory effects were observed for yogurts containing two wine-making byproducts. Grape pomace and seed extracts in concentrations of 50 µg/mL significantly reduced ($p < 0.05$) NO levels by 43% and 79%, respectively. The anti-inflammatory properties of grape pomace and seed extracts have been previously described in macrophages and in other cell lines [63–65]. The absence of anti-inflammatory properties in yogurts may be due to a possible matrix effect between wine-making byproducts and other components present in yogurts, and the degradation of phytochemicals during food fermentation and sample preparation, resulting in ineffective concentrations.

The ultimate challenge in functional foods is the development of food products that provide health-promoting properties beyond basic nutrition without compromising their organoleptic properties. Our results showed that all formulated yogurts achieved high overall acceptance scores except for the coffee silverskin yogurt, which would need to be reformulated to improve its taste. On the other hand, acceptance scores of the coffee cascara yogurt were among the highest for each sensory attribute evaluated (Table 3).

Coffee cascara is a highly demanded ingredient by the industry for its popularity in America and Europe. The health-promoting properties and high acceptance obtained for the coffee cascara yogurt support its potential as a functional food. Therefore, this food was selected for a bioaccessibility study.

The antioxidant capacity of the control yogurt increased after in vitro digestion, which may be due to further release of antioxidant peptides and amino acids encrypted in the milk proteins [66]. The antioxidant capacity of the coffee cascara yogurt increased by 93% after in vitro digestion. This increase in antioxidant capacity after in vitro digestion has also been observed in yogurts containing cinnamon, strawberry, and peach polyphenols [44,67], and has been attributed to the release of polyphenols from the protein/polysaccharide yogurt matrix. Gastrointestinal digestion could increase the antioxidant capacity of phenolic compounds by changing their molecular weight and chemical structure under simulated conditions [33,34]. The inhibition of the activity of α-glucosidase was significantly higher ($p < 0.001$) in coffee cascara yogurt digests than in the digested control yogurt. However, none of the free parental phytochemicals present in the coffee cascara extract were detected in the cascara yogurt or in its digest. These compounds may have been modified during the technological yogurt process, the in vitro oral gastrointestinal process, or the sample preparation. Further studies are needed to achieve a complete identification of the contributors to the health-promoting properties of the cascara yogurt and its digests.

In addition, the antidiabetic effect observed in the yogurts could be potentially enhanced by the presence of inulin in the formulation. Inulin is a soluble dietary fiber that has been recognized for its beneficial effects on postprandial glucose modulation and improved insulin sensitivity. According to Commission Regulation (EU) No 432/2012, inulin and fructooligosaccharides (FOSs) may contribute to the maintenance of normal bowel function by increasing stool frequency, and in some cases, may help reduce the postprandial blood glucose response [68]. These properties have been associated with delayed carbohydrate

digestion and improved glycemic profiles, which may synergize with the bioactive extracts used in this study.

From a sensory perspective, inulin also plays a key role. Its inclusion in fermented dairy products not only improves texture by providing creaminess akin to fat, but can also reduce astringency from certain polyphenols [69,70]. These effects may have contributed to the high sensory acceptance observed for the coffee cascara yogurt.

It is also important to consider that the phenolic compound content and nature of the byproduct extracts incorporated into the yogurt formulations differed substantially (Table 1). For instance, grape seed and skin extracts presented the highest total phenolic content (TPC), whereas coffee silverskin showed the lowest TPC among all formulations. These differences may be attributed to the intrinsic polyphenol composition of the raw materials, as well as the extract concentration used, which was adjusted according to sensory acceptance during preliminary trials. Consequently, the observed biological activities—especially antioxidant and antidiabetic properties—may reflect not only the bioactivity of specific compounds, but also the dose applied and the physicochemical compatibility of the phenolics with the yogurt matrix. Additionally, the total phenolic compounds during in vitro digestion could have increased due to the action of digestive enzymes making them more accessible and detectable. Additionally, this could cause a deprotonation of the hydroxyl groups present on the aromatic rings of the phenolic compounds [12].

Future studies should aim to standardize phenolic inputs across formulations or normalize biological activities per mg of phenolics to better compare extract efficacy. Moreover, identifying the dominant phenolic structures in each extract (e.g., chlorogenic acids in coffee cascara vs. proanthocyanidins in grape seed) would support a structure–function understanding of their roles in health promotion. Biologically active compounds recovered from wine-making byproducts include hydroxybenzoic acids (p-hydroxybenzoic acid, pro-tocatechuic acid, tannic acid, vanillic acid, gallic acid derivatives, and syringic acid), flavanols (catequin), flavonols, anthocyanins, and stilbenes (resveratrol) [58,59]. Grape pomace is a rich source of polyphenols, bioactive compounds with recognized antioxidant and anti-inflammatory properties [38].

Studies using in vitro digestion models have shown that caffeine from coffee byproducts remains largely unchanged during simulated gastrointestinal processes. This means that the initial amount of caffeine present in the coffee pulp remains relatively constant throughout the oral, gastric, and intestinal phases of digestion [71,72].

In relation to the cascara yogurt safety due to CAF intake, a portion of 125 g would have 13.75 mg of CAF, which is far below the European safety threshold of 400 mg per day for the healthy adult population and 200 mg per day for pregnant woman and children [73]. In comparison to some of the most popular caffeinated beverages, the CAF content of the formulated yogurts was lower than a 250 mL cup of coffee (66.88 mg of CAF), a 33 cl can of cola soda (31.05 mg of CAF), or a 250 mL cup of green tea (30.13 mg of CAF) [74]. In addition, CAF was not detected in the cascara yogurt digest, suggesting that this molecule is not bioaccessible for its absorption and in vivo metabolism. Therefore, the coffee cascara yogurt may be consumed by the general population.

5. Conclusions

The present study supported the feasibility of the application of coffee and wine-making byproducts as health-promoting ingredients in yogurts. The coffee cascara yogurt was selected for its health-promoting properties, high overall liking, and regulatory status, and can be considered the best potential functional food candidate among those studied in the present investigation. This food product can be consumed by all population groups. Future studies are necessary to gain insight on the identification of the main contributors to

the observed health-promoting properties of the newly formulated yogurts. However, the results suggest the phytochemicals of the byproducts and their metabolites/derivatives may play a role regarding their biological properties. In addition, the results seem to indicate that, although the chemical composition of the yogurts changed during food processing and digestion, their antidiabetic and antioxidant properties remained after the technological and physiological processes, while coffee cascara anti-inflammatory compounds also remained after the technological process.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fermentation11050291/s1, Table S1. Composition and in vitro bioactivity of coffee and wine-making byproduct extracts; Table S2. Characteristics of standards analyzed by CZE at 280 nm; Table S3. Effect of 24 h treatment with noted concentrations of yogurts and extracts on intracellular ROS generation in IEC-6 cells determined by the DCFH-DA probe. Oxidative damage was induced by t-BOOH (1 mM) and vitamin C (10 µg/mL) was used as an antioxidant control; Table S4. Effect of 24 h (for IEC-6 cells) and 48 h (RAW 264.7 cells) treatments with noted concentrations of yogurts and extracts determined by the MTT assay. Extract concentrations corresponded to the amount of extract in each yogurt. Control, non-treated cells. DMSO (50%) was used as a death control; Table S5. Effect of 24 h (for IEC-6 cells) and 48 h (RAW 264.7 cells) treatments with noted concentrations of yogurt digests on cell viability determined by the MTT assay. Control, non-treated cells. DMSO (50%) was used as a death control.

Author Contributions: M.I.-D. contributed to data curation, formal analysis, funding acquisition, investigation, and writing the original draft. A.I.-D. and T.H. contributed to data curation, formal analysis, and writing—review and editing. E.M. contributed to the investigation, supervision, funding acquisition, and writing—review and editing. M.D.d.C. contributed to conceptualization, formal analysis, funding acquisition, investigation, project administration, supervision, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the following projects: New insights to support health claims and consumer acceptance of sustainable foods" (202470E207, CSIC), and Aprovechamiento de subproductos de la industria agroalimentaria (sandía y melón) para reducir el coste de alimentación animal y/o mejorar el perfil lipídico y las propiedades funcionales de quesos y otros productos lácteos de oveja y de cabra de la Comunidad de Madrid (FP2024-LACTEOSBYPRODUCTS).

Institutional Review Board Statement: The participation of the consumers was voluntary. Sensory evaluations were performed in different sessions. For scientific research matters, the regional government and therefore our institution follow the Spanish Law 14/2011 on Science, Technology and Innovation.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article. A preprint of this article appears in the thesis document of Maite Iriondo de Hond, available on https://repositorio.uam.es/handle/10486/688893, according to the Spanish legislation regulating doctoral studies, accessed on 25 February 2025.

Acknowledgments: The authors thank SUPRACAFE for kindly providing the coffee byproducts and Beneo for kindly providing inulin Orafti®GR.

Conflicts of Interest: The authors declare no conflict of interest.

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