Research Article

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Coffee silverskin as nutraceutical ingredient in yogurt: its effect on functional properties and its bioaccessibility

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Abstract

BACKGROUND: Silverskin is a by-product obtained from coffee roasting. It is characterized by a high content of dietary fibre, phenolic compounds and caffeine. The aim of this study was to assess the silverskin obtained from two species of *Coffea* (Arabica and Robusta) at three percentages (2%, 4%, or 6%) into cow whole-milk yogurt to raise the nutraceutical value of the products and to verify the bioaccessibility of bioactive compounds during the shelf-life of 3 weeks.

RESULTS: The amount and origin of silverskin significantly influenced all the physicochemical parameters. Concerning the bioactive compounds, the highest levels were observed in yogurt supplemented with 6% of silverskin. Between the coffee species, Arabica yielded the highest 5-caffeoylquinic acid content and the strongest antioxidant activity, whereas Robusta gave the highest caffeine content.

The digestion increased antioxidant activity in the yogurt, possibly because of greater accessibility of compounds.

CONCLUSION: The results obtained highlighted that silverskin can be used in yogurt production to increase the nutraceutical value of the products and that the bioactive compounds are bioaccessible during the digestion process. The characteristics and bioaccessibility of the resulting yogurt were strongly correlated with the coffee species and with the percentage added.

Keywords: coffee silverskin; yogurt; *In vitro* digestion; bioactive compound; shelf-life

INTRODUCTION

Production of green coffee beans in 2016 was 9 million tons, of which 63% came from species Arabica (*Coffea arabica*) and 37% from Robusta (*Coffea canephora*).[1] During the transformation of green coffee beans into coffee brew, two by-products are obtained by the consuming countries: coffee silverskin (CS), derived from the roasting process, and spent coffee grounds from the brewing process. As suggested by circular economy principles, some researchers are seeking to utilize coffee waste products, particularly as a starting material of compounds with a positive action on the human body. CS representing ~4.2% (w/w) of coffee beans is rich in dietary fibre (50–70%), of which 85% is insoluble (IDF) and 15% is soluble dietary fibre (SDF) and phenolic compounds, both with antioxidant properties.[2–5] IDF has a bulking effect due to its water-holding capacity, whereas SDF reduces cholesterol and sugar absorption. Because of these health benefits, the fibre intake should increase to 30 g d^{-1}. The polyphenol compounds identified in CS are chlorogenic acids – among which 5-caffeoylquinic acid (5-CQA) is the most abundant – at a concentration similar to that in coffee brew and roasted coffee, with antioxidant capacity similar to that of dark chocolate, oregano, rosemary, paprika and black pepper.[6] The antioxidant capacity of CS is also correlated with the production of melanoidins during the roasting process.[7] Antioxidants, from a medical point of view, can help to reduce oxidative stress, which can cause cancer, cardiovascular diseases, type 2 diabetes, and Alzheimer's and Parkinson's diseases.[8] Therefore, recommended polyphenol dietary intake could be as high as 1 g d^{-1}.[8] Nonetheless, the bioavailability of polyphenols is linked to enzymatic action during digestion: polyphenols liberated can be diffuse in the small intestine or metabolized by the microflora present in the intestine.[9]

CS is also characterized by a caffeine content of 4.44–10.00 mg g^{-1}, as reported by Bresciani *et al.* and Barbosa-Pereira *et al.*[6,10] Caffeine, from a medical point of view, can have a positive effect by stimulating the central nervous system, thus improving physical and cognitive performance and reducing the problems associated with Parkinson's disease.[11]

Recently, CS itself or its phenolic extracts have been combined to produce a coffee blend and bakery products such as bread, cake

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Table 1. Chemical composition, total phenolic content (TPC) and DPPH radical-scavenging activity (RSA) of coffee silverskin (CS) and results of variance analysis

Composition	Coffee silverskin varietals		Significance
	Arabica	Robusta	
Humidity (g kg^{-1})	0.69 ± 0.00	0.70 ± 0.02	ns
Protein (g kg^{-1} dw)	1.98 ± 0.01a	2.02 ± 0.00b	***
Total fat (g kg^{-1} dw)	0.34 ± 0.01b	0.25 ± 0.01a	***
Carbohydrates (g kg^{-1} dw)	1.12 ± 0.01a	1.18 ± 0.01b	***
Ash(g kg^{-1} dw)	0.83 ± 0.03	0.76 ± 0.03	ns
Total dietary fibre (g kg^{-1} dw)	6.85 ± 0.21b	6.08 ± 0.28a	**
Soluble dietary fibre (g kg^{-1} dw)	1.26 ± 0.03b	0.84 ± 0.04a	***
Insoluble dietary fibre (g kg^{-1} dw)	5.29 ± 0.03b	5.20 ± 0.05a	*
TPC (GAE mg g^{-1} dw)	11.95 ± 0.20b	10.80 ± 0.50a	**
RSA (TE μg^{-1} dw)	47.74 ± 0.60b	41.61 ± 1.30a	**

dw, dry weight; TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; GAE, gallic acid equivalent; TE, Trolox equivalent.
Means followed by different letters are significantly different at $P < 0.05$.
Significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.
Data are expressed as mean ± SD ($n = 6$).

or biscuits, or used alone to create a new beverage to analyse the outcomes in terms of a potential source of antioxidants and dietary fibre on the products.[9,12–15]

Therefore, the focus of this work was to implement CS in a proteinaceous food such as yogurt to increase its dietary fibre and phenolic compound content to create a healthier food. Furthermore, the final products were subjected to *in vitro* digestion to analyse the bioaccessibility of phenolic compounds, 5-caffeoylquinic acid and caffeine for intestinal absorption as well as the antioxidant capacity of functional products after ingestion and complete gastrointestinal digestion.

MATERIALS AND METHODS

Materials

CS samples from Arabica and Robusta species were obtained from Caffé Vergnano SpA (Italy) and were ground and sifted to obtain a powder of 80 μm using a Retsch ZM 200 mill (Retsch Gmbh, Haan, Germany). The powders were maintained at 4 °C until analysis.

All the reagents, standards, solvents, enzymes and bile salts were acquired from Sigma-Aldrich (St Louis, MO, USA).

Yogurt production

Yogurt was produced using cow's sterilized whole milk. Milk (20 L) was heated to 42 °C, and then inoculated with yogurt starter culture YO-MIX 401 (Santamaria, Burago di Molgora, Italy). Incubation was achieved until pH reached 4.8. After that, the fermentation was stopped and the coagulum broken and cooled. To achieve dispersion of the CS in the final product, pulp was prepared from 30% of CS and 70% of sterilized milk. The pulp (0, 7, 14 or 21 g) was added to single pots to make 100 g yogurt and was gently stirred to obtain a final CS percentage of 0% (control), 2%, 4% or 6%. The yogurts were kept at 4 °C and analysed at day 1 and after 1 week until the end of storage (21 days). Yogurt production was carried out in duplicate.

Physicochemical composition of CS and yogurts

Moisture, fat, protein, ash, carbohydrate and dietary fibre (TDT, IDF and SDF) were determined using the methods of Bertolino *et al.*[16] Nutritional analyses of samples were performed at day 1.

For each sample and time point of storage on yogurt samples, the pH, titratable acidity and syneresis were determined and expressed as reported by Marchiani *et al.*[17]

The analyses were run in triplicate.

Table 2. Chemical composition of yogurts with 0% (control), 2%, 4% and 6% of coffee silverskin (CS) and results of variance analysis

Composition	0% (control)	Coffee silverskin varietals						Significance
		Arabica			Robusta			
		2% CS	4% CS	6% CS	2% CS	4% CS	6%CS	
Humidity (g kg^{-1})	8.43 ± 0.02d	8.21 ± 0.03c	8.08 ± 0.03b	7.97 ± 0.04a	8.21 ± 0.03c	8.08 ± 0.03b	7.95 ± 0.04a	***
Protein (g kg^{-1} dw)	2.23 ± 0.09	2.10 ± 0.09	2.11 ± 0.08	2.12 ± 0.08	2.10 ± 0.09	2.11 ± 0.08	2.12 ± 0.10	ns
Total fat (g kg^{-1} dw)	2.62 ± 0.12c	2.60 ± 0.11b	2.08 ± 0.10a,b	1.93 ± 0.09a	2.25 ± 0.11b	2.07 ± 0.10a	1.91 ± 0.09a	***
Carbohydrates (g kg^{-1} dw)	4.80 ± 0.08d	4.13 ± 0.08c	3.80 ± 0.07b	3.52 ± 0.06a	4.13 ± 0.08c	3.82 ± 0.07b	3.54 ± 0.07a	***
Ash (g kg^{-1} dw)	0.53 ± 0.01a	0.53 ± 0.01a	0.57 ± 0.02b,c	0.59 ± 0.03c	0.53 ± 0.01a	0.55 ± 0.02a,b	0.57 ± 0.03b,c	***
Total dietary fibre (g kg^{-1} dw)	– ± – a	0.71 ± 0.03b	1.33 ± 0.06d	1.87 ± 0.09f	0.63 ± 0.04b	1.18 ± 0.07c	1.65 ± 0.10e	***
Soluble dietary fibre (g kg^{-1} dw)	– ± – a	0.13 ± 0.00c	0.24 ± 0.01f	0.34 ± 0.01g	0.09 ± 0.00b	0.16 ± 0.01d	0.23 ± 0.01e	***
Insoluble dietary fibre (g kg^{-1} dw)	– ± – a	0.55 ± 0.01b	1.03 ± 0.02c	1.44 ± 0.03d	0.54 ± 0.01b	1.01 ± 0.02c	1.42 ± 0.03d	***

dw, dry weight; TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre.
Means followed by different letters are significantly different at $P < 0.05$.
Significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.
Data are expressed as mean ± SD ($n = 6$).

Table 3. syneresis (expressed as % of whey removed), acidity (express as lactic acid %) and pH of yogurts with 0% (control), 2%, 4% and 6% of coffee silverskin (CS) during 21 days of storage at 4 °C and results of variance analysis

Parameter	CS species	CS %	Storage period (days) 1	7	14	21	Significance
Syneresis	Control	0	a19.54 ± 0.37A	a25.45 ± 0.88B	a45.20 ± 0.57C	a48.50 ± 0.21D	***
		2	d28.29 ± 0.02A	b30.13 ± 1.27A	b,c49.55 ± 0.28B	c53.23 ± 0.81C	***
	Arabica	4	c,d28.18 ± 1.59A	b,c31.92 ± 1.25A	c50.92 ± 0.88B	b,c52.45 ± 3.04B	***
		6	b,c26.60 ± 1.09A	c33.03 ± 2.5B	a43.28 ± 0.68C	a,b,c51.67 ± 1.45D	***
		2	c29.47 ± 0.94A	b29.29 ± 0.02A	c51.27 ± 1.24B	a,b,c51.32 ± 0.60B	***
	Robusta	4	b25.86 ± 0.09A	b30.20 ± 0.10B	b48.15 ± 1.98C	a,b49.73 ± 2.08C	***
		6	d29.20 ± 0.89A	b,c30.74 ± 1.51A	a43.73 ± 1.74B	a49.17 ± 0.39C	***
Significance			***	***	***	*	
Acidity	Control	0	0.99 ± 0.04	d1.05 ± 0.02	d1.05 ± 0.00	1.13 ± 0.04	NS
		2	1.05 ± 0.07A	b,c1.02 ± 0.00A	c,d1.04 ± 0.00A	1.18 ± 0.03B	*
	Arabica	4	0.99 ± 0.01A	a 0.99 ± 0.01A	b1.01 ± 0.01A	1.04 ± 0.00B	*
		6	1.01 ± 0.04A	a0.99 ± 0.01A	a0.99 ± 0.01A	1.10 ± 0.00B	*
		2	1.04 ± 0.00A	c,d1.04 ± 0.01A	d1.05 ± 0.00A	1.10 ± 0.00B	**
	Robusta	4	0.99 ± 0.01A	b,c,d1.03 ± 0.00A	c,d1.04 ± 0.01A	1.12± 0.04B	*
		6	0.96 ± 0.01A	a,b1.01 ± 0.00A	c1.04 ± 0.01A	1.20 ± 1.05B	*
Significance			ns	***	ns		
pH	Control	0	a4.21 ± 0.01B	a4.22 ± 0.01B	a4.17 ± 0.01A	a4.17 ± 0.02A	*
		2	b,c4.35 ± 0.04B	b4.29 ± 0.00A,B	b4.26 ± 0.01B	b4.24 ± 0.01B	*
	Arabica	4	d,e4.44 ± 0.01C	c4.39 ± 0.00B	c4.37 ± 0.01B	c4.31 ± 0.02A	**
		6	f4.52 ± 0.09	d4.48 ± 0.03	e4.48 ± 0.02	e4.45 ± 0.01	NS
		2	b4.31 ± 0.00C	b4.30 ± 0.00C	b4.26 ± 0.02B	b4.22 ± 0.01A	**
	Robusta	4	c,d4.40 ± 0.01B	c4.39 ± 0.02B	c4.35 ± 0.03A,B	c4.32 ± 0.01A	*
		6	e,f4.52 ± 0.01B	d4.49 ± 0.02B	d4.44 ± 0,00A	d4.41 ± 0.00A	**
Significance			***	***	***	***	

CS, coffee silverskin; dw, dry weight.
Means followed by different upper-case letters in same row within each concentration are significantly different at $P < 0.05$; means preceded by different lower-case letters in the same column within each storage time are significantly different at $P < 0.05$.
Significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.
Data are expressed as mean ± SD ($n = 6$).

Count of starter bacteria in fortified yogurts

These analyses were fulfilled to assess the effects of CS use on starter viability. Lactobacilli and streptococci were counted according to the methods of Bertolino *et al.*[16] For each sample and time point of storage, three analyses were carried out.

Texture analysis of yogurts

The texture properties of samples were evaluated on a Texture Analyser TA-XT plus (Stable Micro Systems Ltd, Godalming. UK) equipped with a 5 kg load cell and a back-extrusion cell with a compression disc (35 mm diameter). The samples were spilled into the container (50 mm internal diameter and 75 mm height), and one-cycle analysis was done at a constant velocity of 1 mm s^{-1}, to a sample penetration of 25 mm, and then returned. The Texture Export Exceed software rel. 2.54 (Stable Micro Systems, UK) was used for construction of the force–time curve, and the firmness (area acquired during the probe penetration in the samples, mJ) and adhesiveness (area acquired during the probe returning to the trigger point, mJ) were measured. For each sample and time point of storage, three analyses were carried out.

In vitro simulated gastrointestinal digestion (SGD)

SGD was performed according to a standardized static *in vitro* method for food as described by Minekus *et al.*,[18] which included the following three stages: oral, gastric and small-intestinal digestion. After complete digestion, pH was adjusted to 5.4, and the samples were immediately placed in ice to lessen enzymatic activity and centrifuged at 12 500 × *g* for 10 min at 4 °C. The supernatants were filtered through a 0.22 µm cellulose acetate membrane filter (VWR, Milan, Italy) and stored at −20 °C for further analysis. Two types of control tests were performed. In the first control test (the matrix control), yogurt was replaced by distilled water, and the obtained product was subjected to a digestion process to evaluate the matrix effect on digestion of bioactive compounds present in CS. The second control was set up by replacing the enzymes and bile salts with distilled water to evaluate the effects of enzymes on digestion of bioactive compounds present in CS. The results obtained from the two controls were subtracted from the results on fortified digested yogurt. For each sample and time point of storage, three analyses were carried out.

Bioactive-compound extraction from yogurts before digestion

To allow comparison with data obtained from the *in vitro* digestion process, aqueous bioactive compound extraction was carried out under the same conditions of temperature/time and weight/volume used in the digestive process. Briefly, each yogurt (5 g) was diluted with water (40 mL) and shaken for 242 min at 37 °C. After that, the samples were centrifuged (12 500 × *g* for 10 min at 4 °C), and the supernatant was passed through a 0.22 µm

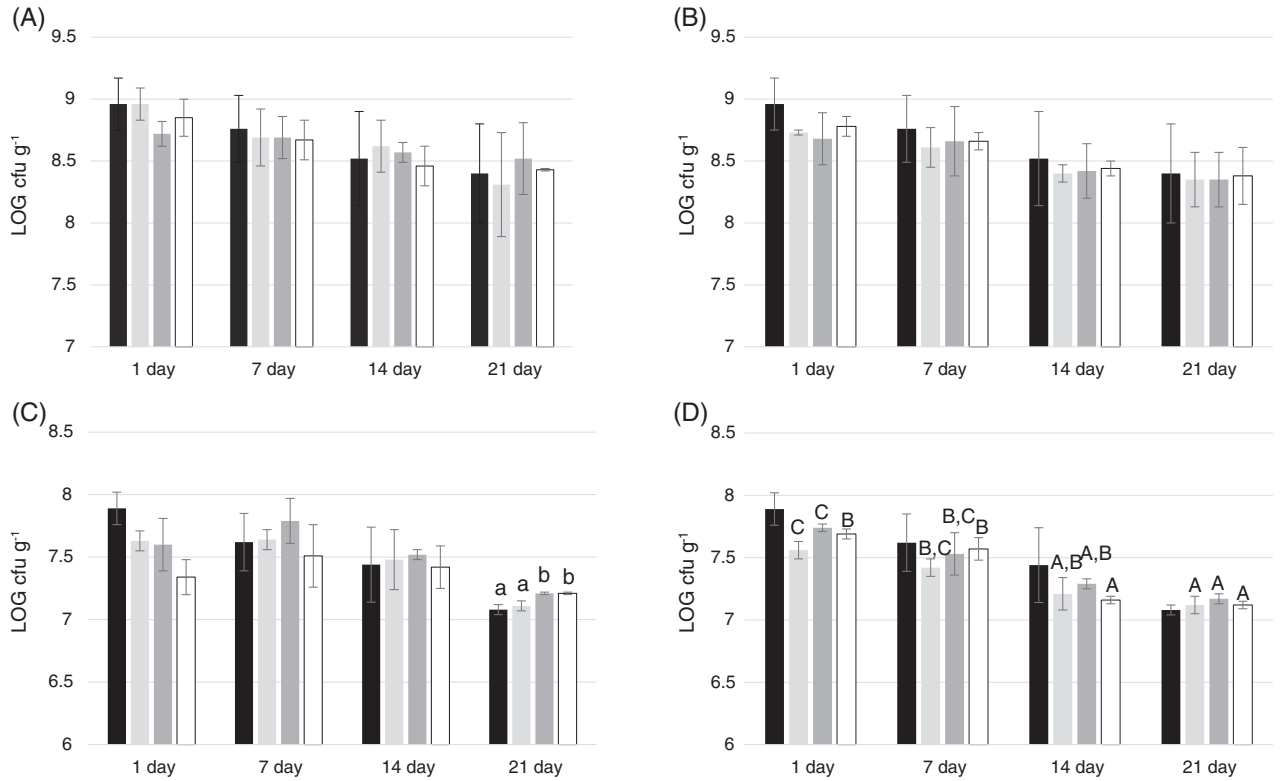

Figure 1. *Streptococcus thermophilus* (A) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (C) counts in yogurts with 0% (control) and 2%, 4%, 6% of Arabica coffee silverskin during 21 days of storage at 4 °C. *Streptococcus thermophilus* (B) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (D) counts in yogurts with 0% (control) and 2%, 4%, 6% of Robusta coffee silverskin during 21 days of storage at 4 °C. 0%, control (black histogram) and 2% (light grey), 4%, (dark grey) and 6% (white) silverskin addition. Histograms with different lower-case letters at the same storage time were significantly different at $P < 0.05$. Histograms with different upper-case letters were significantly different at $P < 0.05$ during the storage time.

cellulose acetate membrane filter (VWR, Milan, Italy) and stored at −20 °C for further analysis.

Analysis of total phenolic content

The concentration of total phenolic compounds (TPC) was evaluated according to the Folin–Ciocalteu colorimetric method, detailed in Barbosa-Pereira *et al.*[10]

The TPC was determined by comparison against the standard curve of gallic acid (ranging from 20–100 mg L^{-1}) and expressed as gallic acid equivalents (GAE) per gram of sample. The TPC was calculated based on the standard curve of gallic acid (20–100 mg L^{-1}) and was expressed as milligrams of gallic acid equivalents (GAE) per gram of a sample. All determinations were made in triplicate for each sample and time of storage.

Analysis of total antioxidant activity

The antioxidant activity of samples was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical-scavenging method detailed in Barbosa-Pereira *et al.*[10]

For each sample, the radical-scavenging activity (RSA) was determined from the linear regression curve of Trolox (12.5–300 µmol L^{-1}), and the results were expressed as micromoles of Trolox equivalents (TE) per gram of sample. All determinations were performed in triplicate for each sample and point time of storage.

Analysis by high-performance liquid chromatography (HPLC) with diode array detection

Chromatographic analysis of functional yogurts (before and after completed digestion) was performed on an HPLC-PDA Thermo-Finnigan Spectra System (Thermo-Finnigan, Waltham, MA, USA).

Compound separation was performed with a reverse-phase Kinetex Phenyl-Hexyl C18 column (150 × 4.6 mm internal diameter and 5 µm particle size) (Phenomenex, Castel Maggiore, Italy) thermostated at 35 °C. The mobile phase and gradient elution conditions were those used by Barbosa-Pereira *et al.*[10] Monitoring and quantification of 5-CQA and caffeine were carried out at 325 and 273 nm, respectively, using the external standard method calibration curves constructed under the same chromatographic conditions ($R^2 = 0.9993$ for 5-CQA and $R^2 = 0.9960$ for caffeine).

Data analysis

To highlight the effect of CS fortification and time of storage the results were analysed by two different one-way analyses of variance. Statistical differences were determined using Duncan's test with significance level of $P < 0.05$. All the analysis were performed using IBM SPSS Statistics software for Windows (Version 24.0, Armonk, NY, USA).

RESULTS

CS chemical composition

Data are shown in Table 1. As already underlined by Napolitano *et al.*,[4] TDF was the major component, and 81% of it on average was represented by IDF, with significant differences between the species: Robusta CS had lower TDF content characterised by higher IDF content in comparison with Arabica CS.

Table 4. Firmness and adhesiveness of yogurts with 0% (control), 2%, 4% and 6% of coffee silverskin (CS) during 21 days of storage at 4 °C and results of variance analysis

Parameter	CS species	CS %	Storage period (days) 1	7	14	21	Significance
Firmness (mJ)	Control	0	b4.49 ± 0.15	d,e5.17 ± 0.41	d5.72 ± 0.05	5.66 ± 0.35	NS
		2	a3.99 ± 0.07A	c,d,e4.98 ± 0.00B	c,d5.38 ± 0.13C	5.50 ± 0.42C	**
	Arabica	4	a4.00 ± 0.1A	b,c,d4.76 ± 0.17B	c,d5.44 ± 0.22C	5.37 ± 0.33B,C	**
		6	b4.44 ± 0.08A	e5.39 ± 0.08B	b,c5.21 ± 0.03B	5.27 ± 0.17B	**
		2	a4.05 ± 0.07A	b,c4.63 ± 0.15B	a,b,c5.12 ± ± 0.12C	5.46 ± 0.25C	**
	Robusta	4	a4.10 ± 0.15A	a4.15 ± 0.02A	a4.81 ± 0.09B	5.10 ± 0.09C	***
		6	a4.01 ± 0.25A	a,b4.47 ± 0.06A,B	a,b4.95 ± 0.22B	5.04 ± 0.3B	*
Significance			*	**	**	ns	
Adhesiveness (mJ)	Control	0	−2.58 ± 0.25	−2.20 ± 1.45	c−3.19 ± 0.11	−3.07 ± 0.25	NS
		2	−2.02 ± 0.06	−2.36 ± 0.07	b,c−2.79 ± 0.10	−2.99 ± 1.44	NS
	Arabica	4	−2.10 ± 0.11A	−2.26 ± 0.11A	b,c−2.98 ± 0.27B	−2.97 ± 0.35B	*
		6	−2.75 ± 0.26	−3.06 ± 0.15	b,c−2.81 ± 0.00	−2.86 ± 0.14	NS
		2	−2.15 ± 0.09A	−2.05 ± 0.13A	a,b−2.58 ± 0.20A,B	−2.94 ± 0.35B	*
	Robusta	4	−2.30 ± 0.23B	−1.65 ± 0.11A	a−2.32 ± 0.17B	−2.64 ± 0.13B	*
		6	−2.16 ± 0.25	−2.09 ± 0.11	a,b−2.59 ± 0.12	−2.84 ± 0.47	NS
Significance			ns	ns	*	ns	

CS, coffee silverskin.
Means followed by different upper-case letters in same row within each concentration are significantly different at $P < 0.05$; means preceded by different lower-case letters in the same column within each storage time are significantly different at $P < 0.05$.
Significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.
Data are expressed as mean ± SD ($n = 6$).

The same trends of TDF were observed for the TPC and RSA assays, where the highest values were measured in Arabica CS.

The TPC results for Arabica samples were higher than those obtained by Barbosa-Pereira *et al.*,[10] under control conditions, whereas results on Robusta TPC were in agreement with their data. The RSA results for Arabica CS samples were in accordance with those obtained by Barbosa-Pereira *et al.*,[10] while those of Robusta CS were lower.

Overall composition of yogurt

Table 2 shows these data. The global yogurt composition was statistically different ($P < 0.001$). In particular, a mean linear decrease in humidity of 2.6%, 4.1%, and 5.7% for the 2%, 4%, and 6% CS supplementation respectively was observed, but without differences between the coffee species. This decrease was in line with those obtained by Bertolino *et al.*,[16] and Marchiani *et al.*,[17] who added hazelnut skin and grape pomace to yogurt. The CS addition also determined a decrease in the concentrations of lipids and carbohydrates and an increase in ash content.

As for TDF, the use of CS was associated with its concentration in yogurts; furthermore, TDF content enlarged by mean values of 0.67, 1.25, and 1.76 g kg^{-1} of sample in yogurt with 2%, 4%, and 6% CS, respectively. Between the coffee species, the highest concentration was noticed in the yogurt in which Arabica CS was used but with a statistically significant difference only for 4% and 6% CS addition. Similar data, showing upregulation of TDF in yogurt owing to added fibre, were obtained by Bertolino *et al.*, and Tseng and Zhao.[16,19]

Between the two species, no differences were found in the concentration of IDF, whereas differences were observed in the SDF concentration, as expected, owing to the dietary-fibre composition of the raw material.

Physicochemical characteristics of yogurt

Syneresis, titratable acidity and pH of yogurts are presented in Table 3. As for syneresis, the use of CS determined a higher whey separation at each storage time points. The reason was the reorganisation of the matrix due to the high concentration of IDF in the CS and its incompatibility with milk proteins.[16,17,20]

The incorporation of CS had a statistically significant effect on titrable acidity during the storage period. The 6% Robusta-fortified yogurt exhibited the highest increase (0.13 units) and the 4% Arabica-fortified yogurt manifested the lowest (0.05 units). A similar trend was reported by do Espírito Santo *et al.*[21] for yogurt where passion fruit peel powder was used. The pH of yogurts dropped during storage independently of CS. Among the fortified products, 6% Arabica yogurt manifested during storage the lowest pH reduction (0.07 units), whereas 4% Arabica yogurt yielded the highest (0.13 units). The mean debasement was 0.09 units – lower than that observed in other research where by-product powders were used in the same matrix.[16,17,19,20,22] Moreover, significant differences in pH ($P < 0.001$) among the percentages of CS added and between the types of CS (only at 6%) were detected at all storage time points: increasing the CS fortification resulted in higher pH as compared with the control. The same pH increase was highlighted by Hashim *et al.*,[23] for a yogurt where date fibre was used.

Survival of starter bacteria

As shown in Fig. 1, the use of CS in yogurt did not affect the starter survival; at the end of storage, each strain had a population (CFU g^{-1}) higher than that required for the sum of the two strains by the Codex Alimentarius (10^7 CFU g^{-1}).

During the whole storage, *S. thermophilus* counts were statistically stable and ranged on average from 8.83 to 8.39 Log CFU g^{-1} without a difference between the two coffee species (Fig. 1A,B).

Table 5. DPPH radical-scavenging activity (RSA), total phenolic content (TPC), caffeine and 5-caffeoilquinic acid (5-CQA) content of yogurts with 0% (control), 2%, 4% and 6% of coffee silverskin (CS) during 21 days of storage at 4 °C and results of variance analysis

Parameter	CS species	CS %	Storage period (days) 1	7	14	21	Significance
RSA (μmol TE g^{-1} yogurt)	Control	0	a0.16 ± 0,00B	a0.06 ± 0.02A	a0.06 ± ± 0,00A	a0.06 ± 0.02A	***
		2	b0.44 ± 0.02	b0.42 ± 0.06	b0.43 ± 0.06	b0.38 ± 0.08	NS
	Arabica	4	d0.70 ± 0.02	c0.68 ± 0.11	d0.67 ± 0.02	d0.70 ± 0.04	NS
		6	f0.91 ± 0.03	d0.99 ± 0.06	f0.88 ± 0.09	e0.93 ± 0.01	NS
		2	b0.46 ± 0.01	b0.47 ± 0.05	b0.39 ± 0.01	b0.38 ± 0.04	NS
	Robusta	4	c0.63 ± 0.05	c0.68 ± 0.03	c0.58 ± 0.00	c0.57 ± 0.04	NS
		6	e0.83 ± 0.03A,B	d0.94 ± 0.03C	e0.76 ± 0.01A	e0.87 ± 0.07B,C	**
Significance			***	***	***		
TPC (GAE mg g^{-1} yogurt)	Control	0	a0.07 ± 0.01A	a0.12 ± 0.01B	a0.07 ± 0.01A	a0.06 ± 0.01A	***
		2	b0.18 ± 0.04	b0.23 ± 0.04	b0.21 ± 0,00	b0.17 ± 0.02	NS
	Arabica	4	c0.25 ± 0.01A	c0.32 ± 0.04B	c0.26 ± 0.00A	c0.23 ± 0.02A	**
		6	d0.31 ± 0.00A	d0.40 ± 0.04B	d0.31 ± 0.01A	d0.35 ± 0.03A	**
		2	b0.18 ± 0.02	b0.25 ± 0.03	b0.19 ± 0,00	b0.19 ± 0.04	NS
	Robusta	4	d0.31 ± 0.05	c,d0.37 ± 0.05	c0.26 ± 0.05	c0.28 ± 0.03	NS
		6	d0.35 ± 0.01	d0.42 ± 0.07	d0.38 ± 0.01	d0.38 ± 0.04	NS
Significance			***	***	***	***	
Caffeine (mg g^{-1} yogurt)	Control	0	a- ± -	a- ± -	a- ± -	a- ± -	
		2	b1.73 ± 0.04A	b2.06 ± 0.04B	b2.15 ± 0.01C	c2.37 ± 0.05D	***
	Arabica	4	c2.56 ± 0.03A	d3.33 ± 0.05B	d3.88 ± 0.01C	d3.88 ± 0.03C	***
		6	e3.35 ± 0.02A	f4.11 ± 0.08B	f4.86 ± 0.06D	4.70 ± 0.12C	***
		2	c2.03 ± 0.04A	c2.57 ± 0.05C	c3.09 ± 0.04D	b2.14 ± 0.01B	***
	Robusta	4	d3.03 ± 0.03A	e3.80 ± 0.08B	e4.41 ± 0.17C	e4.45 ± 0.03C	***
		6	f3.70 ± 0.08A	g4.36 ± 0.07B	g5.05 ± 0.06C	f5.15 ± 0.14C	***
Significance			***	***	***	***	
5-CQA (mg g^{-1} yogurt)	Control	0	a– ± -	a– ± –	a– ± –	a– ± –	
		2	d0.17 ± 0.01A,B	d0.19 ± 0.01C	d0.18 ± 0.00B,C	e0.17 ± 0.01A	*
	Arabica	4	e0.33 ± 0.00A	e0.38 ± 0.01B	e0.40 ± 0.01C	f0.38 ± 0.00B	***
		6	f0.55 ± 0.01A	f0.67 ± 0.01C	f0.70 ± 0.02D	g0.62 ± 0.01B	***
		2	b0.05 ± 0.00B	b0.06 ± 0.00C	b0.07 ± 0.00D	b0.04 ± 0.00A	***
	Robusta	4	b0.05 ± 0.00A	c0.08 ± 0.00B	c0.10± 0.00C	d0.08 ± 0.00B	***
		6	c0.07 ± 0.01A	c0.08 ± 0.00A	c0.09 ± 0.00B,C	c0.07 ± 0.00A	**
Significance			***	***	***	***	

CS, coffee silverskin; GAE, gallic acid equivalent; TE, Trolox equivalent.
Means followed by different upper-case letters in the same row within each concentration are significantly different at $P < 0.05$; means preceded by different lower-case letters in same column within each storage time were significantly different at $P < 0.05$.
Significance:
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.
Data are expressed as mean ± SD ($n = 6$).

Viability of *L. bulgaricus* decreased during refrigerated storage (Fig. 1C,D), but only in the yogurt supplemented with Robusta CS was the difference statistically significant. In particular, the counts decreased by less than 1 CFU g^{-1}. Concerning the Arabica CS-supplemented yogurts, at the end of shelf-life there was a statistically significant difference among the samples: increasing CS fortification was associated with a higher count of *L. bulgaricus*. The larger effect of fibre addition on lactobacilli compared to streptococci has already been observed by Marchiani *et al.*[17] and do Espírito Santo *et al.*[21]

Texture analysis of yogurts

The texture profiles of the different yogurts are given in Table 4. During cold storage, firmness values increased due to gel shrinking caused by syneresis and pH reduction, as discussed in Section 3.3. This increase was statistically significant in the fortified samples with mean values of 26.6%, 22.6% and 18.1% for the 2%, 4% and 6% CS supplementation, respectively. In a comparison of the yogurts by CS origin, Arabica yogurts needed a greater force to be compressed than Robusta yogurts did, as a consequence of the higher syneresis value. Regarding the CS percentage addition, at each time point of sampling the control yogurt showed higher firmness than did the CS-supplemented samples. These observations may be supported by the fact that by reducing the fat content and by increasing fibre addition it is possible to reduce the network of proteins.[24]

Furthermore, fortified yogurt, after 14 days of storage, showed a lower firmness value compared to the control owing to the weak network as already observed in yogurt with added fibre by Sah *et al.*[20] and Hashim *et al.*[23]

Bioactive compounds of yogurt

Table 5 presents the free radical-scavenging activity, total phenolic content, and caffeine and 5-CQA contents of the yogurts. The

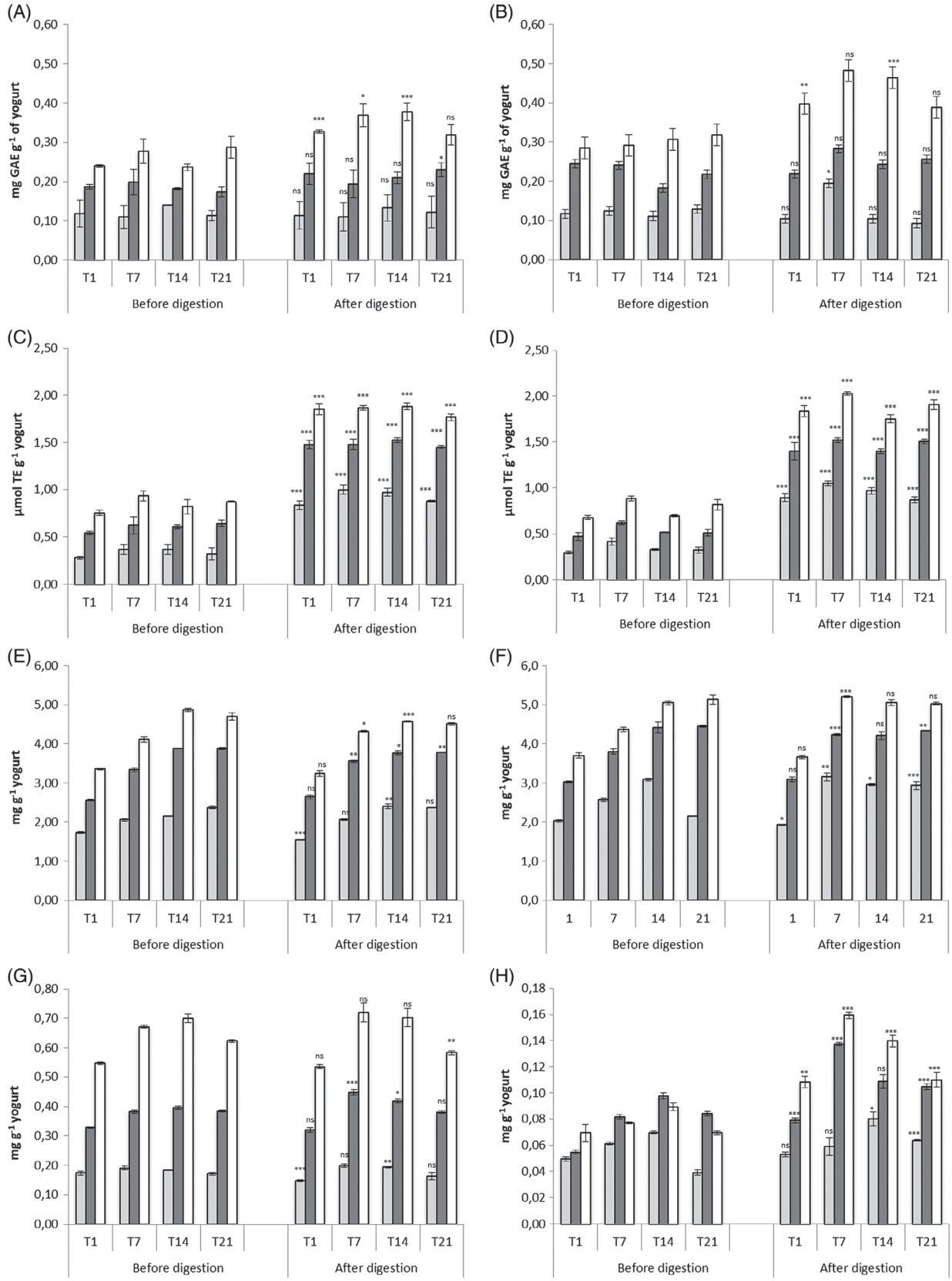

Figure 2. Total phenolic content (A), DPPH radical-scavenging activity (C), caffeine (E) and 5-caffeoylquinic acid (G) content of yogurts added with 2%, 4% and 6% of Arabica coffee silverskin during 21 days of storage at 4 °C before and after the *in vitro* digestion. Total phenolic content (B), DPPH radical-scavenging activity (D), caffeine (F) and 5-caffeoylquinic acid (H) content of yogurts added with 2%, 4% and 6% of Robusta coffee silverskin during 21 days of storage at 4 °C before and after *in vitro* digestion. 2% (light grey), 4%, (dark grey) and 6% (white) silverskin species fortification. Abbreviations: GAE, gallic acid equivalent; TE, Trolox equivalent. Significance between before vs after digestion: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

statistically significant increase ($P < 0.001$) in TPC in control yogurt during the first week of storage was linked to bacterial modification of some compounds that reacted with Folin–Ciocalteu reagent. At each storage time point, CS-added yogurts showed statistically significant differences ($P < 0.001$), and an increase was highlighted during the first week of storage among the samples. Between the coffee species, Robusta showed the highest concentration.

During storage, RSA of the yogurt increased during the first week but decreased afterwards. This decrease is in agreement with the results obtained by Tseng and Zhao,[19] who fortified yogurt with grape pomace, and with the data obtained by Karaaslan *et al.*,[25] who evaluated different grape berries and a callus extract.

During the storage period, caffeine concentration dropped significantly ($P < 0.001$) in all fortified yogurts because of the bacterial activity that determined a simplification of the matrix and/or with solubilisation and transfer of caffeine from CS into whey. The 4% fortified yogurt (34% for Arabica and 32% for Robusta samples) shown the highest increase, whereas the highest concentration was observed in 6% fortified yogurt.

The 5-CQA concentration during the storage period increased significantly until the third week. The highest increase was observed in 4% fortified yogurt (21% for Arabica and 38% for Robusta), whereas the highest concentration was shown by 6% fortified yogurt. The decrease observed on day 21 can be due to the transformation of 5-CQA into other compounds owing to the pH decrease during shelf-life. Between the coffee species, Arabica yogurts shown the highest concentration.

Effects of digestion on bioactive compounds

Figure 2 illustrates the free radical-scavenging activity, total phenolic content as well as caffeine and 5-CQA content of the functional yogurts subjected or not subjected (before digestion) to *in vitro* digestion. In general, *in vitro* digestion improved the TPC by a mean value of 12% with no difference between the coffee species but with differences among the percentages of fortification. This increase could be due to hydrolysis of the phenolic compounds from the polysaccharides present in CS and from the protein present in yogurt because of the action of digestive enzymes.

As a consequence of the increased concentration of phenolic compounds in the *in vitro* digestion extracts, RSA of fortified yogurts after *in vitro* digestion was higher for both coffee species at each storage time point than before *in vitro* digestion. This increase could be caused not only by the enzymatic hydrolysis of phenolic compounds that increases their concentration but also by deprotonation of the hydroxyl groups present on the aromatic rings of the phenolic compounds.[26] The mean increase was 61%, with a higher value for Arabica samples than for Robusta yogurts. With the increasing percentage of fortification, a decrease in RSA was observed.

The caffeine content of fortified yogurts showed a lower value (Arabica yogurts) or a slightly higher value (Robusta yogurts; a mean increase of 4%) after *in vitro* digestion than before.

The 5-CQA content of fortified yogurts revealed a higher value for both coffee species after *in vitro* digestion than before. The mean increase was only 0.1% for the Arabica yogurt and 26% for the Robusta yogurt. For both coffee species, with increasing fortification an increase in 5-CQA concentration was observed. This phenomenon could be due to the liberation of phenolic compounds from CS owing to the action of digestive enzymes.

CONCLUSIONS

This study revealed that CS can be utilised to fortify yogurt with bioactive compounds such as dietary fibre, phenolic compounds, chlorogenic acids and caffeine. The percentages of supplementation and the coffee species of CS contributed differently to all the physicochemical parameters and to the functionality of the final product under study. During storage, antioxidant capacity and bioactive-compound concentration increased, and digestion of products can increase their bioaccessibility.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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