Coffee silverskin as nutraceutical ingredient inyogurt: its effect on functional properties and its bioaccessibility.

(Article begins on next page)

07 January 2023

UNIVERSITÀ DEGLI STUDI DI TORINO

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

Marta Bertolino,[*a] Letricia Barbosa-Pereira,[a] Daniela Ghirardello,[a] Cristian Botta,[a] Luca Rolle,[a] Alessandro Guglielmetti,[a] Stefania Borotto Dalla Vecchia[a] Giuseppe Zeppa[a]

Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy

Corresponding Author Address: Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy

Tel.: +39 011-670-8686; fax: +39 011-670-8549

Email: marta.bertolino@unito.it

Abstract

BACKGROUNDS: Silverskin is the by-products obtained from the coffee roasting processing. It is characterised by a high content of dietary fibre, phenolic compounds and caffeine. The aim of this study was to assess the silveskin 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 the bioactive compounds during the shelf-life of 3 weeks.

RESULTS: The amount and origin of silverskin significantly influenced all the physico-chemical 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 use 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 the bioaccessibility of the resulting yogurt were strongly correlated with the Coffee species and with the percentage added.

1. Introduction

Production of green coffee beans in 2016 was 9 million tons, of which 63% come 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 the circular economy principles, some researcher are looking for the utilisation of 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. Due to 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 the digestion: polyphenols liberated can be diffuse in the small intestine or metabolised by the microflora present in the intestine.[9]

CS is also characterised 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 performances and reducing the problems associated with Parkinson's disease.[11]

Recently, CS such as or its phenolic extracts have been combined to a coffee blend and bakery products such as bread, cake 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 compounds content to create a healthier food. Furthermore, the final products were subjected to an *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.

2. Materials and Methods

2.1. Materials

CS samples from Arabica and Robusta species were obtained from Caffé Vergnano S.p.A. (Italy) and were grinded 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).

2.2. Yogurt production

Yogurt was produced using sterilized cow 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 got to 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 into single pots to make 100 g of 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 one week until the end of storage (21 days). The yogurt production was carried out in duplicate.

2.3. Physico-chemical 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.

2.4. Count of starter bacteria in fortified yogurts

These analyses were fulfilled to assess the effects of the CS use on the 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.

2.5. Texture analysis of yogurts

The texture properties of samples were evaluated on a Texture Analyser TA-XT plus (Stable Micro Systems Ltd., 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, Godalming, 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.

2.6. In vitro simulated gastrointestinal digestion (SGD)

SGD was performed according to a standardised static *in vitro* method for food as described by Minekus et al.,[18] which includes the following three stages: oral, gastric, and small-intestinal digestion. After the complete digestion, pH was adjusted to 5.4, and the samples were right after put in ice to lessen enzymatic activity. The samples were immediately 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.

2.7. Bioactive-compound extraction from yogurts before digestion

To allow comparison with data obtained from the *in vitro* digestion process, aqueous bioactive compound extraction was carry out with 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 cellulose acetate membrane filter (VWR, Milan, Italy) and stored at −20 °C for further analysis.

2.8. 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 20–100 mg L^{-1}) and expressed as Gallic 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 mg of gallic acid equivalents (GAE) per gram of a sample. All determinations were made in triplicate for each sample and time of storage.

.

2.9.Analysis of total antioxidant activity

The antioxidant activity of samples was determined by the DPPH (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 µM), and the results expressed as µmol of Trolox equivalents (TE) per gram of a sample. All determinations were performed in triplicate for each sample and point time of storage.

2.10.   HPLC-DAD analysis

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

The compounds 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 the gradient elution conditions were the ones 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).

2.11. Data analysis

To highlight the effect of CS fortification and time of storage the results were analysed by two different one-way analysis of variance. The statically differences were determined using the Duncan's test where a significance level of $p < 0.05$ was used. All the analysis were performed using the IBM SPSS Statistics software for Windows (version 24.0, Armonk, NY, USA).

3. Results

3.1.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.

The same trend of TDF were observed for the TPC and RSA assay, 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.

3.2. Overall composition of yogurt

Table 2 shows these data. The global yogurts 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 determined also 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 where 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.

3.3. Physico-chemical characteristics of yogurt

Syneresis, titrable 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 the 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). 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), while 4% Arabica yogurt yielded the highest (0.13 units). The mean debasement was 0.09 units, lower than the one observed in other research where by-product powders were used into 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.

3.4. Survival of starter bacteria

As shown in Figure 1, the use of CS into yogurt did not affect the starter survival; at the end of storage, each strains 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 (Figure 1 A & B).

Viability of *L. bulgaricus* decreased during refrigerated storage (Figure 1 C & D), but only in the yogurt supplemented with Robusta CS, the difference was 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: the 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 in Marchiani et al.,[17] and do Espírito Santo et al.[21]

3.5. Texture analysis of yogurts

The texture profiles of the different yogurts are given in Table 4. During the cold storage, firmness values increased due to gel shrinking caused by syneresis and pH reduction as discussed in subsection 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 whit added fibre by Sah et al.,[20] and Hashim et al.[23]

3.6. 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 statistically significant increase ($p < 0.001$) in TPC in control yogurt during the first week of storage was linked with bacterial modification of some compounds that reacted with the 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 shown 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.

3.7. Effects of digestion on bioactive compounds

Figure 2 illustrates the free radical–scavenging activity, total phenolic content as well as caffeine and 5-CQA contents of the functional yogurts subjected or not subjected (before digestion) to the *in vitro* digestion. In general, *in vitro* digestion improved the TPC by a mean value of 12% without a 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 the 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.

4. 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 physico-chemical 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.

5. Conflicts of interest

There are no conflicts to declare.

6. References

1   ICO (2017). http://www.ico.org/trade_statistics.asp. Accessed 24.04.2017.

2   Behrouzian F, Amini AM, Alghooneh A, Razavi SM. Characterization of dietary fiber from coffee silverskin: An optimization study using response surface methodology. Bioact. Carbohydr. Dietary Fibre. 2016 Oct; 8(2): 58–64.

3   Borrelli RC, Esposito F, Napolitano A, Ritieni A, Fogliano V. Characterization of a new potential functional ingredient:coffee silverskin. J. Agr. Food Chem. 2004 Mar; 52(5): 1338-43. https:// doi.org/10.1021/jf034974x

4   Napolitano A, Fogliano V, Tafuri A, Ritieni A. Natural Occurrence of Ochratoxin A and Antioxidant Activities of Green and Roasted Coffees and Corresponding By products. J. Agr. Food Chem. 2007 Dec; 55(25): 10499–504. https:// doi.org/ 10.1021/jf071959+

5   Narita Y, Inouye K. Review on utilization and composition of coffee silverskin. Food Res. Int. 2014 Jul; 61: 16–22. https:// doi.org/ 10.1016/j.foodres.2014.01.023

6   Bresciani L, Calani L, Bruni R, Brighenti F, Del Rio D. Phenolic composition, caffeine content and antioxidant capacity of coffee silverskin. Food Res. Int. 2014 Jul; 61: 196–201. https:// doi.org/ 10.1016/j.foodres.2013.10.047

7   Esquivel P, Jiménez VM. Functional properties of coffee and coffee by-products. Food Res. Int. 2012 May; 46(2): 488–95. https:// doi.org/ 10.1016/j.foodres.2011.05.028

8   Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr. 2005 Jan; 81(1Suppl): 215S–17S. https:// doi.org/ 10.1093/ajcn/81.1.215S

9   Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr. 2004 May; 79(5): 727–47. https://doi.org/10.1093/ajcn/79.5.727

10   Barbosa-Pereira L, Guglielmetti A, Zeppa G. Pulsed electric field assisted extraction of bioactive compounds from cocoa bean shell and coffee silverskin. Food Bioproc. Tech. 2018 Apr;11(4): 818-35. https://doi.org/10.1007/s11947-017-2045-6

11   Glade MJ. Caffeine — Not just a stimulant. Nutrition 2010 Oct; 26(10): 932–938. https://doi.org/10.1016/j.nut.2010.08.004

12   Ateş G, Elmaci Y. Coffee silverskin as fat replacer in cake formulations and its effect on physical, chemical and sensory attributes of cakes. LWT-Food Sci. Technol. 2018 Apr; 90: 519-25. https://doi.org/10.1016/j.lwt.2018.01.003

13   Martinez-Saez N, Ullate M, Martin-Cabrejas MA, Martorell P, Genovés S, Ramon D, et al. A novel antioxidant beverage for body weight control based on coffee silverskin. Food Chem. 2014 May; 150: 227–34. https://doi.org/10.1016/j.foodchem.2013.10.100

14   Pourfarzad A, Mahdavian-Mehr H, Sedaghat N. Coffee silverskin as a source of dietary fiber in bread-making: Optimization of chemical treatment using response surface methodology. LWT-Food Sci. Technol. 2013 Mar; 50(2): 599–606. https://doi.org/10.1016/j.lwt.2012.08.001.

15   Ribeiro VS, Leitão AE, Ramalho JC, Lidon FC. Chemical characterization and antioxidant properties of a new coffee blend with cocoa, coffee silverskin and green coffee minimally processed. Food Res. Int. 2014 Jul; 61: 39–47. https://doi.org/10.1016/j.foodres.2014.05.003

16   Bertolino M, Belviso S, Dal Bello B, Ghirardello D, Giordano M, Rolle L, et al. Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt. LWT-Food Sci. Technol. 2015 Oct; 63(2):1145–54. https://doi.org/10.1016/j.lwt.2015.03.113

17   Marchiani R, Bertolino M, Belviso S, Giordano M, Ghirardello D, Torri L, et al. Yogurt Enrichment with Grape Pomace: Effect of Grape Cultivar on Physicochemical, Microbiological and Sensory Properties. J. Food Quality. 2016 Apr; 39(2): 77–89. https://doi.org/10.1111/jfq.12181

18   Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, ,et al. A standardised static in vitro digestion method suitable for food – an international consensus. Food & Function. 2014 Jun; 5(6): 1113-24. DOI: 10.1039/c3fo60702j.

19 Tseng A,Zhao Y. Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. Food Chem. 2013 May; 138(1),

356–65. https://doi.org/10.1039/c3fo60702j

20 Sah BN, Vasiljevic T, McKechnie S, Donkor ON. Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. LWT-Food Sci. Technol. 2016 Jan; 65: 978–86. https://doi.org/10.1016/j.lwt.2015.09.027

21 do Espírito Santo AP, Perego P, Converti A, Oliveira MN. Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts. LWT-Food Sci. Technol. 2012 Jul; 47(2): 393–99. https://doi.org/10.1016/j.lwt.2012.01.038

22 Garcia-Serna E, Martinez-Saez N, Mesias M, Morales FJ, Castillo MD. Use of Coffee Silverskin and Stevia to Improve the Formulation of Biscuits. Pol. J. Food Nutri. Sci. 2014; 64(4): 243–51. https://doi.org/10.2478/pjfns-2013-0024

23 Hashim IB, Khalil AH, Afifi HS. Quality characteristics and consumer acceptance of yogurt fortified with date fiber. J. Dairy Sci. 2009 Nov; 92(11): 5403–07. https://doi.org/10.3168/jds.2009-2234

24 Corredig M, Sharafbafi N, Kristo E. Polysaccharide–protein interactions in dairy matrices, control and design of structures. Food Hydrocolloids. 2011 Dec; 25(8):1833–41. https://doi.org/10.1016/j.foodhyd.2011.05.014

25 Karaaslan M, Ozden M, Vardin H, Turkoglu H. Phenolic fortification of yogurt using grape and callus extracts. LWT-Food Sci. Technol. 2011 May; 44(4):1065–72. https://doi.org/10.1016/j.lwt.2010.12.009

26 Hsu CL, Hurang SL, Chen W, Weng YM, Tseng CY. Qualities and antioxidant properties of bread as affected by the incorporation of yam flour in the formulation. Int. J. Food Sci. Tech. 2004 Feb; 39(2): 231-238. https://doi.org/10.1046/j.0950-5423.2003.00770.x

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

Abbreviations: dw = dry weight; TDF = total dietary fibre; SDF = soluble dietary fibre; IDF = insoluble dietary fibre; GAE = gallic acid equivalent and TE = trolox equivalent.
Means followed by different letters 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).

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 ± 0.02 d	8.21 ± 0.03 c	8.08 ± 0.03 b	7.97 ± 0.04 a	8.21 ± 0.03 c	8.08 ± 0.03 b	7.95 ± 0.04 a	***
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 ± 0.12 c	2.60 ± 0.11 b	2.08 ± 0.10 a,b	1.93 ± 0.09 a	2.25 ± 0.11 b	2.07 ± 0.10 a	1.91 ± 0.09 a	***
Carbohydrates (g kg^{-1} dw)	4.80 ± 0.08 d	4.13 ± 0.08 c	3.80 ± 0.07 b	3.52 ± 0.06 a	4.13 ± 0.08 c	3.82 ± 0.07 b	3.54 ± 0.07 a	***
Ash (g kg^{-1} dw)	0.53 ± 0.01 a	0.53 ± 0.01 a	0.57 ± 0.02 b,c	0.59 ± 0.03 c	0.53 ± 0.01 a	0.55 ± 0.02 a,b	0.57 ± 0.03 b,c	***
Total dietary fibre (g kg^{-1} dw)	- ± - a	0.71 ± 0.03 b	1.33 ± 0.06 d	1.87 ± 0.09 f	0.63 ± 0.04 b	1.18 ± 0.07 c	1.65 ± 0.10 e	***
Soluble dietary fibre (g kg^{-1} dw)	- ± - a	0.13 ± 0.00 c	0.24 ± 0.01 f	0.34 ± 0.01 g	0.09 ± 0.00 b	0.16 ± 0.01 d	0.23 ± 0.01 e	***
Insoluble dietary fibre (g kg^{-1} dw)	- ± - a	0.55 ± 0.01 b	1.03 ± 0.02 c	1.44 ± 0.03 d	0.54 ± 0.01 b	1.01 ± 0.02 c	1.42 ± 0.03 d	***

Abbreviations: dw = dry weight; TDF = total dietary fibre; SDF = soluble dietary fibre; IDF = insoluble dietary fibre.
Means followed by different letters 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).

Table 3: syneresis (express 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)				Significance
			1	7	14	21	
Syneresis	Control	0	a 19.54 ± 0.37 A	a 25.45 ± 0.88 B	a 45.20 ± 0.57 C	a 48.50 ± 0.21 D	***
		2	d 28.29 ± 0.02 A	b 30.13 ± 1.27 A	b,c 49.55 ± 0.28 B	c 53.23 ± 0.81 C	***
	Arabica	4	c,d 28.18 ± 1.59 A	b,c 31.92 ± 1.25 A	c 50.92 ± 0.88 B	b,c 52.45 ± 3.04 B	***
		6	b,c 26.60 ± 1.09 A	c 33.03 ± 2.5 B	a 43.28 ± 0.68 C	a,b,c 51.67 ± 1.45 D	***
		2	c 29.47 ± 0.94 A	b 29.29 ± 0.02 A	c 51.27 ± 1.24 B	a,b,c 51.32 ± 0.60 B	***
	Robusta	4	b 25.86 ± 0.09 A	b 30.20 ± 0.10 B	b 48.15 ± 1.98 C	a,b 49.73 ± 2.08 C	***
		6	d 29.20 ± 0.89 A	b,c 30.74 ± 1.51 A	a 43.73 ± 1.74 B	a 49.17 ± 0.39 C	***
Significance			***	***	***	*	
Acidity	Control	0	0.99 ± 0.04	d 1.05 ± 0.02	d 1.05 ± 0.00	1.13 ± 0.04	NS
		2	1.05 ± 0.07 A	b,c 1.02 ± 0.00 A	c,d 1.04 ± 0.00 A	1.18 ± 0.03 B	*
	Arabica	4	0.99 ± 0.01 A	a 0.99 ± 0.01 A	b 1.01 ± 0.01 A	1.04 ± 0.00 B	*
		6	1.01 ± 0.04 A	a 0.99 ± 0.01 A	a 0.99 ± 0.01 A	1.10 ± 0.00 B	*
		2	1.04 ± 0.00 A	c,d 1.04 ± 0.01 A	d 1.05 ± 0.00 A	1.10 ± 0.00 B	**
	Robusta	4	0.99 ± 0.01 A	b,c,d 1.03 ± 0.00 A	c,d 1.04 ± 0.01 A	1.12 ± 0.04 B	*
		6	0.96 ± 0.01 A	a,b 1.01 ± 0.00 A	c 1.04 ± 0.01 A	1.20 ± 1.05 B	*
Significance			ns	**	***	ns	
pH	Control	0	a 4.21 ± 0.01 B	a 4.22 ± 0.01 B	a 4.17 ± 0.01 A	a 4.17 ± 0.02 A	*
		2	b,c 4.35 ± 0.04 B	b 4.29 ± 0.00 A,B	b 4.26 ± 0.01 B	b 4.24 ± 0.01 B	*
	Arabica	4	d,e 4.44 ± 0.01 C	c 4.39 ± 0.00 B	c 4.37 ± 0.01 B	c 4.31 ± 0.02 A	**
		6	f 4.52 ± 0.09	d 4.48 ± 0.03	e 4.48 ± 0.02	e 4.45 ± 0.01	NS
		2	b 4.31 ± 0.00 C	b 4.30 ± 0.00 C	b 4.26 ± 0.02 B	b 4.22 ± 0.01 A	**
	Robusta	4	c,d 4.40 ± 0.01 B	c 4.39 ± 0.02 B	c 4.35 ± 0.03 A,B	c 4.32 ± 0.01 A	*
		6	e,f 4.52 ± 0.01 B	d 4.49 ± 0.02 B	d 4.44 ± 0,00 A	d 4.41 ± 0.00 A	**
Significance			***	***	***	***	

Abbreviations: CS= coffee silverskin; dw = dry weight.

Means followed by different capital letters in same row within each concentration were significantly different at $p < 0.05$; mean forerun by different lowcase 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).

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)				Significance
			1	7	14	21	
Firmness (mJ)	Control	0	b 4.49 ± 0.15	d,e 5.17 ± 0.41	d 5.72 ± 0.05	5.66 ± 0.35	NS
		2	a 3.99 ± 0.07 A	c,d,e 4.98 ± 0.00 B	c,d 5.38 ± 0.13 C	5.50 ± 0.42 C	**
	Arabica	4	a 4.00 ± 0.1 A	b,c,d 4.76 ± 0.17 B	c,d 5.44 ± 0.22 C	5.37 ± 0.33 B,C	**
		6	b 4.44 ± 0.08 A	e 5.39 ± 0.08 B	b,c 5.21 ± 0.03 B	5.27 ± 0.17 B	**
		2	a 4.05 ± 0.07 A	b,c 4.63 ± 0.15 B	a,b,c 5.12 ± 0.12 C	5.46 ± 0.25 C	**
	Robusta	4	a 4.10 ± 0.15 A	a 4.15 ± 0.02 A	a 4.81 ± 0.09 B	5.10 ± 0.09 C	***
		6	a 4.01 ± 0.25 A	a,b 4.47 ± 0.06 A,B	a,b 4.95 ± 0.22 B	5.04 ± 0.3 B	*
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 ± 0.11 A	-2.26 ± 0.11 A	b,c -2.98 ± 0.27 B	-2.97 ± 0.35 B	*
		6	-2.75 ± 0.26	-3.06 ± 0.15	b,c -2.81 ± 0.00	-2.86 ± 0.14	NS
		2	-2.15 ± 0.09 A	-2.05 ± 0.13 A	a,b -2.58 ± 0.20 A,B	-2.94 ± 0.35 B	*
	Robusta	4	-2.30 ± 0.23 B	-1.65 ± 0.11 A	a -2.32 ± 0.17 B	-2.64 ± 0.13 B	*
		6	-2.16 ± 0.25	-2.09 ± 0.11	a,b -2.59 ± 0.12	-2.84 ± 0.47	NS
Significance			ns	ns	*	ns	

Abbreviations: CS= coffee silverskin.

Means followed by different capital letters in same row within each concentration were significantly different at $p < 0.05$; mean forerun by different lowcase 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).

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	a 0.16 ± 0,00 B	a 0.06 ± 0.02 A	a 0.06 ± 0,00 A	a 0.06 ± 0.02 A	***
		2	b 0.44 ± 0.02	b 0.42 ± 0.06	b 0.43 ± 0.06	b 0.38 ± 0.08	NS
	Arabica	4	d 0.70 ± 0.02	c 0.68 ± 0.11	d 0.67 ± 0.02	d 0.70 ± 0.04	NS
		6	f 0.91 ± 0.03	d 0.99 ± 0.06	f 0.88 ± 0.09	e 0.93 ± 0.01	NS
		2	b 0.46 ± 0.01	b 0.47 ± 0.05	b 0.39 ± 0.01	b 0.38 ± 0.04	NS
	Robusta	4	c 0.63 ± 0.05	c 0.68 ± 0.03	c 0.58 ± 0.00	c 0.57 ± 0.04	NS
		6	e 0.83 ± 0.03 A,B	d 0.94 ± 0.03 C	e 0.76 ± 0.01 A	e 0.87 ± 0.07 B,C	**
Significance			***	***	***		
TPC (GAE mg g^{-1} yogurt)	Control	0	a 0.07 ± 0.01 A	a 0.12 ± 0.01 B	a 0.07 ± 0.01 A	a 0.06 ± 0.01 A	***
		2	b 0.18 ± 0.04	b 0.23 ± 0.04	b 0.21 ± 0,00	b 0.17 ± 0.02	NS
	Arabica	4	c 0.25 ± 0.01 A	c 0.32 ± 0.04 B	c 0.26 ± 0.00 A	c 0.23 ± 0.02 A	**
		6	d 0.31 ± 0.00 A	d 0.40 ± 0.04 B	d 0.31 ± 0.01 A	d 0.35 ± 0.03 A	**
		2	b 0.18 ± 0.02	b 0.25 ± 0.03	b 0.19 ± 0,00	b 0.19 ± 0.04	NS
	Robusta	4	d 0.31 ± 0.05	c,d 0.37 ± 0.05	c 0.26 ± 0.05	c 0.28 ± 0.03	NS
		6	d 0.35 ± 0.01	d 0.42 ± 0.07	d 0.38 ± 0.01	d 0.38 ± 0.04	NS
Significance			***	***	***	***	
Caffeine (mg g^{-1} yogurt)	Control	0	a - ± -	a - ± -	a - ± -	a - ± -	
		2	b 1.73 ± 0.04 A	b 2.06 ± 0.04 B	b 2.15 ± 0.01 C	c 2.37 ± 0.05 D	***
	Arabica	4	c 2.56 ± 0.03 A	d 3.33 ± 0.05 B	d 3.88 ± 0.01 C	d 3.88 ± 0.03 C	***
		6	e 3.35 ± 0.02 A	f 4.11 ± 0.08 B	f 4.86 ± 0.06 D	4.70 ± 0.12 C	***
		2	c 2.03 ± 0.04 A	c 2.57 ± 0.05 C	c 3.09 ± 0.04 D	b 2.14 ± 0.01 B	***
	Robusta	4	d 3.03 ± 0.03 A	e 3.80 ± 0.08 B	e 4.41 ± 0.17 C	e 4.45 ± 0.03 C	***
		6	f 3.70 ± 0.08 A	g 4.36 ± 0.07 B	g 5.05 ± 0.06 C	f 5.15 ± 0.14 C	***
Significance			***	***	***	***	
5-CQA (mg g^{-1} yogurt)	Control	0	a - ± -	a - ± -	a - ± -	a - ± -	
		2	d 0.17 ± 0.01 A,B	d 0.19 ± 0.01 C	d 0.18 ± 0.00 B,C	e 0.17 ± 0.01 A	*
	Arabica	4	e 0.33 ± 0.00 A	e 0.38 ± 0.01 B	e 0.40 ± 0.01 C	f 0.38 ± 0.00 B	***
		6	f 0.55 ± 0.01 A	f 0.67 ± 0.01 C	f 0.70 ± 0.02 D	g 0.62 ± 0.01 B	***
		2	b 0.05 ± 0.00 B	b 0.06 ± 0.00 C	b 0.07 ± 0.00 D	b 0.04 ± 0.00 A	***
	Robusta	4	b 0.05 ± 0.00 A	c 0.08 ± 0.00 B	c 0.10 ± 0.00 C	d 0.08 ± 0.00 B	***
		6	c 0.07 ± 0.01 A	c 0.08 ± 0.00 A	c 0.09 ± 0.00 B,C	c 0.07 ± 0.00 A	**
Significance			***	***	***	***	

Abbreviations: CS= coffee silverskin; GAE = gallic acid equivalent; TE = trolox equivalent.

Means followed by different capital letters in same row within each concentration were significantly different at $p < 0.05$; mean forerun by different lowcase 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.

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.

Legend:

0% - Control (black histogram) and 2% (light grey), 4%, (dark grey), 6% (white) silverskin addition. Histograms with different lowercase letters at the same storage time were significantly different at $p < 0.05$. Histograms with different capital letters were significantly different at $p < 0.05$ during the storage time.

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 the *in vitro* digestion.

Legend:

2% (light grey), 4%, (dark grey), 6% (white) silverskin species fortification. Abbreviation: GAE= gallic acid equivalent; TE = trolox equivalent. Significance among before and after digestion: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant.