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Coffee Pulp Waste as a Functional Ingredient: Effect on Salty Cookies Quality

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Abstract Dry coffee pulp obtained from the residue of the wet processing of Coffea arabica was used as a source of antioxidant dietary fibre to develop "high in fibre" salty cookies. Total phenolic content and antioxidant capacity (ABTS and ORAC) were quantified in cookies and physiological extracts from an in vitro digestion. Enriched cookies' phenolic content (94.42 ± 1.00 mg GAE/30g) and ABTS antioxidant capacity (1168.58 ± 23.50 µmol TE/30g) were found to be significantly higher than those of control cookies, ORAC antioxidant capacity remained similar. Physiological extracts after in vitro digestion showed higher values of total phenolic content (191.53 ± 9.29 mg GAE/30g) and antioxidant capacity (5617.49 ± 211.87 µmol TE/portion in ABTS and 3362.60 ±262.58 µmol TE/portion in ORAC), which were also superior to the antioxidant characteristics of the physiological extracts of control cookies. Cookies were evaluated by consumers who rated the degree of liking on tasting the samples under blind and informed conditions. Results indicated that label had effect on consumers' hedonic perception. Formulation of cookies with dry coffee pulp was successful; an acceptable product with functional properties was obtained.

Keywords: *coffee by-products, dietary fibre, antioxidant capacity, sensory acceptance, cookies*

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1. Introduction

With an annual production of 9.5 million tons [1], green coffee is amongst the most traded commodities in the world, and key to the economy for many producing countries. One of the most relevant species is *Coffea arabica*, which comprises 70% of the whole production [2].

In order to obtain green coffee, *C. arabica* berries are processed via wet methods [3], generating the by-product known as pulp. For every 2 tons of berry processed, 1 ton of coffee pulp is obtained [4]. Applications have been reported by different authors, including solid-state fermentation for the production of ethanol [5] and enzymes [6], obtainment of bioactive phytochemicals [7] and substitution of 20-25% of animal feed [8]. However, there is few evidence of the employment of coffee pulp for human nutrition.

Most of the uses mentioned above are built upon coffee pulp's considerable content of carbohydrates, proteins and minerals, as well as tannins, polyphenols and caffeine [7]. Phenolic compounds such as tannins, chlorogenic acid and caffeic acid are known to have beneficial effects on human health. In fact, it is known that foods rich in phenolic compounds reduce the incidence of several human diseases such as cardiovascular diseases, colon cancer, liver disorders and diabetes [9].

In fruits and vegetables, phenolic compounds tend to occur in association with dietary fibre, which has positive effects on human health itself [10].

Part of dietary fibre's functional properties can be attributed to the existence of phenolic compounds within it [11]. This is related to the incipient concept of "antioxidant dietary fibre", developed by [12]. The author has explained that antioxidant dietary fibre is dietary fibre rich in phenolic compounds, which possesses the physiological effects of both components. [13] claimed that transportation of antioxidants through the gastrointestinal tract is actually one of the fundamental functions of dietary fibre.

Polyphenols in food will bring positive effects on human health if they are bioavailable. Determination of bioavailability involves *in vivo* assays, thus depending on variables such as physiological state and presence of other nutrients in the bloodstream. Bioaccessibility is a concept that refers to the amount of nutrient that is left available for intestinal absorption after its release from the food matrix during digestion, and it can be determined *in vitro* [14].

Recently, interest in consuming foods with potential benefits for human health has been growing. This has triggered the appearance and propagation of functional foods, which are enriched with ingredients that may promote human health (functional ingredients), as well as improving the technological functionality of foods. Some of the ingredients that fall into this category are carotenoids, dietary fibre, phenolic compounds such as flavonoids, and glucosinolates [11] and they can be frequently found simultaneously in fruits and vegetables and their by-products. In this context, bakery products have been widely used as foods with fruit and vegetable by-product incorporation [15].

The aim of this investigation was to use coffee pulp waste as a new functional ingredient in a bakery product of massive consumption on which to evaluate general acceptability as well as potential functional properties, including fibre content and polyphenol bioaccessibility.

2. Materials and Methods

2.1. Coffee Pulp Waste

The coffee pulp waste was obtained from the plantation of Coope Unión R.L. in Tres Ríos, Cartago, Costa Rica. After the extraction of the coffee beans, the pulp obtained was stored at -18 °C. Before its use, the pulp was left at room temperature to defrost and milled in a screw grinder (Kramer-Grebe). Subsequently, a pressing stage in a hydropress (3 bar, 8 min) yielded a solid residue which was later dried at 50 °C in an air cabin drier until a moisture content below 10%. This solid residue, hereafter identified as dry coffee pulp (DCP), was finally ground in a laboratory mill (Retsch ZM 200) with a 1 mm sieve for further analyses.

2.2. Cookie Formulations

DCP was used as an ingredient in the elaboration of enriched salty cookies. Two cookies (enriched and control) were formulated as shown in Table 1. Basic ingredients were obtained from the local market, soy lecithin from ADM (USA), Coffee aroma by L&G S.A (Uruguay). All ingredients were mixed and knead for the preparation of the dough, which was rolled out to a thickness of 0.4 cm and cut in the shape of discs of 4 cm of diameter. The cookies were baked in a convection oven (James, Uruguay) at 180 °C during 15 min.

Table 1. Formulation of control cookies and enriched salty cookies

Ingredient (%)	Control cookies	Enriched cookies
Wheat flour	59.58	53.58
Water	28.37	28.37
Vegetable oil	10.15	10.15
Dry coffee pulp	0	6.00
Baking powder	1.06	1.06
Salt	0.39	0.39
Soy lecithin	0.35	0.35
Coffee aroma	0.10	0.10

2.3. Proximate Composition

Proximate composition analyses were carried out on DCP, control and enriched cookies. Determination of moisture content was made by gravimetric analysis in a convection oven (Labotec group corp.) at 105°C until constant weight. Fat content was obtained following [16]. Protein and ash were quantified with [17] AOAC methods 984.13 and 985.29 respectively. Ash was determined in a muffle furnace (Labotec group corp.) as detailed in [18]. Total carbohydrate content was estimated by difference.

2.4. Antioxidant Extraction from Cookies

The procedure described by [19], was followed to accomplish the extraction of antioxidants from the cookie samples. These samples were extracted first with 40 mL of methanol/water (50:50, v/v) at room temperature with continuous agitation during 60 min. Afterwards, samples were centrifuged at 2500 g for 15 min. The supernatant was recovered. The solid residue was added 40 mL of acetone/water (70:30, v/v) and extracted and centrifuged in the same conditions as before. The supernatant was combined with the first one, and the total volume was taken to 100 mL with distilled water. This extract was later used to quantify antioxidant capacity (ABTS) and total phenolic content.

2.5. Total Phenolic Content

Total phenolic content was quantified using the methodology described by [20]. Gallic acid was used as an external standard (r^2=0.998). Folin-Ciocalteu reagent was diluted with distilled water (1:10, v/v) right before use. 2.5 mL of it were added to 500 µL of the extract (chemical extracts and lyophilised samples reconstituted with distilled water); they were mixed and left for 2 min. Afterwards, 2 mL of sodium carbonate (0,7 M) were added and the samples were left in darkness. After 30 min, absorbance was measured at 760 nm in a Shimadzu 1800 UV-visible spectrophotometer. Results are expressed as gallic acid equivalents (GAE/g sample).

2.6. Antioxidant Capacity

Antioxidant capacity was measured by two different methods: an electron transfer based assay (ABTS) and a hydrogen atom transfer based assay (ORAC).

ABTS was quantified based on [21]. ABTS radical stock solution (2.5 mM) was prepared and added potassium persulfate (140 mM) 16 hours prior to use in order to activate the radical. Right before use, the stock solution was diluted with ethanol:water (50:50, v/v) until the absorbance value at 734 nm was 0.700 ± 0.020. Aliquots of 30 µL of the extracts (chemical extracts and lyophilised samples reconstituted with ethanol:water (50:50, v/v)) were combined with 3 mL of the ABTS solution. After a resting time of 30 min in darkness, absorbance was measured at 734 nm in a Shimadzu 1800 UV-visible spectrophotometer. Trolox was used as a standard for the calibration curve and the ABTS scavenging activity

was reported as Trolox Equivalents (TE). Results are expressed in µmol Trolox Equivalent (TE/g sample).

Hydrophilic oxygen radical absorbance capacity (ORAC) assay was conducted combining the methods of [22,23,24]. The reaction was carried out in phosphate buffer (75 mM, pH 7.4). The extracts were placed in plates in a Biotek Spectrofluorometer, where they were put together with fluorescein (82 nM) and preincubated at 37 °C for 30 min. Past this time, AAPH (2,2'-azobis(2-amidino-propane) dihydrochloride) solution (145 mM) was added. The plates were agitated for 15 s, after which fluorescence was recorded every minute during 45 min with 485 nm excitation and 530 nm emission filters. Results were expressed in µmol TE/g sample.

2.7. In Vitro Digestion

An in vitro digestion was carried out on the enriched cookie and control cookie, following the method proposed by [25]. All incubations were performed in closed Erlenmeyer flasks of 50 mL, in a shaking water bath at 37°C and 200 rpm. All volumes were adjusted with phosphate buffer saline (PBS) 10 mM, pH 6.9. For the first step, incubation volume was 10.43 mL, with α-amylase (90 units/mL, 0.43 mL). The incubation time was 5 min. For the gastric step, pepsin was added and pH adjusted to 2.0 with HCl 1M. Volume was adjusted to 22.73 mL with PBS; reaction time was 90 min. In the last step, pancreatin and bile were added and pH was adjusted to 7.0 with NaHCO3 0.1 M. Volume was completed to 30.09 mL and the sample was incubated for 150 min. When all the steps were finished, enzymatic reactions were stopped in a water bath at 90°C for 10 min. Samples were centrifuged at 10000 rpm for 10 min. After separating the supernatant from solid residue, samples were frozen at -80°C and lyophilized.

2.8. Consumer Tests

Consumer tests were performed with the aim of determining acceptability of cookies as well as the effect of expectations created by the label. Two sessions were carried out by students and workers of Universidad Católica del Uruguay whose ages ranged between 18-60 years old. All of them were regular consumers of salty cookies. During a first session, 103 consumers (46% men and 54% women) were asked to indicate acceptability of the enriched and control cookies without being given any information about the products. The cookies were served to consumers on plastic plates, coded using random three-digit numbers. Overall acceptability was evaluated with a nine-point hedonic scale, ranging from 1 (“I dislike extremely”) to 9 (“I like extremely”). The blind test included 19 descriptors to be checked in the mode of a check-all-that-apply (CATA) question, that is, consumers had to indicate all of the 19 terms that appropriately described the cookie sample. In a second session, 1 month later, 103 consumers (42% men and 58% women), were given enriched and control cookies together with their corresponding labels. With identical scales as before, they were requested to mark acceptability of both cookies taking into consideration the information contained in the label.

2.9. Cookies’ Packaging

To communicate the characteristics of cookies to consumers, an image of their packaging was designed. These images contained nutritional claims associated with the product in question. For enriched cookies, the label read “with natural antioxidants” and “high in fibre”. The labels differed from that of other products available in the Uruguayan market so as to avoid any external influence on consumer responses. Images were designed by a professional graphic designer (Figure 1).

Figure 1. Information provided to the consumers during the informed test about (a) Control cookies and (b) Enriched cookies

2.10. Statistical Analysis

All results are expressed as mean ± standard deviation. Differences in acceptability of both types of cookies was determined using Student's t-test. In order to determine the differences in proximate composition and antioxidant properties, an analysis of variance (ANOVA) was performed. Cochran's Q test was used to determine significant differences in CATA descriptors between samples. XLSTAT Version 2018 (Addinsoft 2018, Paris) was used.

3. Results and Discussion

3.1. Proximate Composition

Proximate composition (Table 2) revealed a high content of dietary fibre for DCP. Contents of fat, ash, protein and carbohydrates were consistent with those reported by [26] and [27], for similar residues of Coffea arabica. DCP was used as source of dietary fibre for cookie formulations. Similar uses have also been given to the by-products of the processing of mango [28], potato [29], orange [30] and other fruit and vegetable by-products in general [15].

Table 2. Composition of dry coffee pulp in g/100 g dwb

	Dry coffee pulp
Fat	2.96 ± 0.15
Ash	8.10 ± 0.19
Dietary Fibre	56.65 ± 2.08
Protein	11.52 ± 0.28
Carbohydrates	20.77 *

* Carbohydrates were determined by difference.
dwb: dry weight basis Mean ± SD; n =3.

The addition of DCP to the formulation of the cookies produce a significant increase in fat, ash and dietary fibre contents, while moisture decreased and protein content remained similar (Table 3). [31], produced biscuits enriched with fibre from spent coffee grounds, and they found that this addition had a positive effect on the nutritional composition of the biscuits, increasing fat content as well.

The enriched cookies elaborated with dry coffee pulp can be considered "high in fibre" according to [32] (1.8 g of dietary fibre per 30 g).

Table 3. Proximate composition of control and enriched cookies in g/30g (a portion is equivalent to 30 g).

	Control cookies	Enriched cookies
Moisture	1.38 ± 0.04 ^{a}	1.17 ± 0.04 ^{b}
Fat	4.13 ± 0.02 ^{a}	4.46 ± 0.02 ^{b}
Ash	0.46 ± 0.02 ^{a}	0.67 ± 0.03 ^{b}
Dietary Fibre	1.76 ± 0.17 ^{a}	2.94 ± 0.11 ^{b}
Protein	2.58 ± 0.02 ^{a}	2.55 ± 0.16 ^{a}
Carbohydrates	19.52 *	18.20 *

Different letters indicate a significant difference ($p<0.05$) between control and enriched cookies. Mean ± SD; n =2.

3.2. Total Phenolic Content and Antioxidant Capacity of Cookies and Physiological Extracts

The presence of DCP as a functional ingredient increases total phenolic content and antioxidant capacity of cookies and physiological extracts (Figure 2). Total phenolic content of enriched cookies improved 6 times in comparison with control cookies. Likewise, the physiological extract of enriched cookies has a phenolic content 2.7 times higher than the value found for the physiological extract of control cookies.

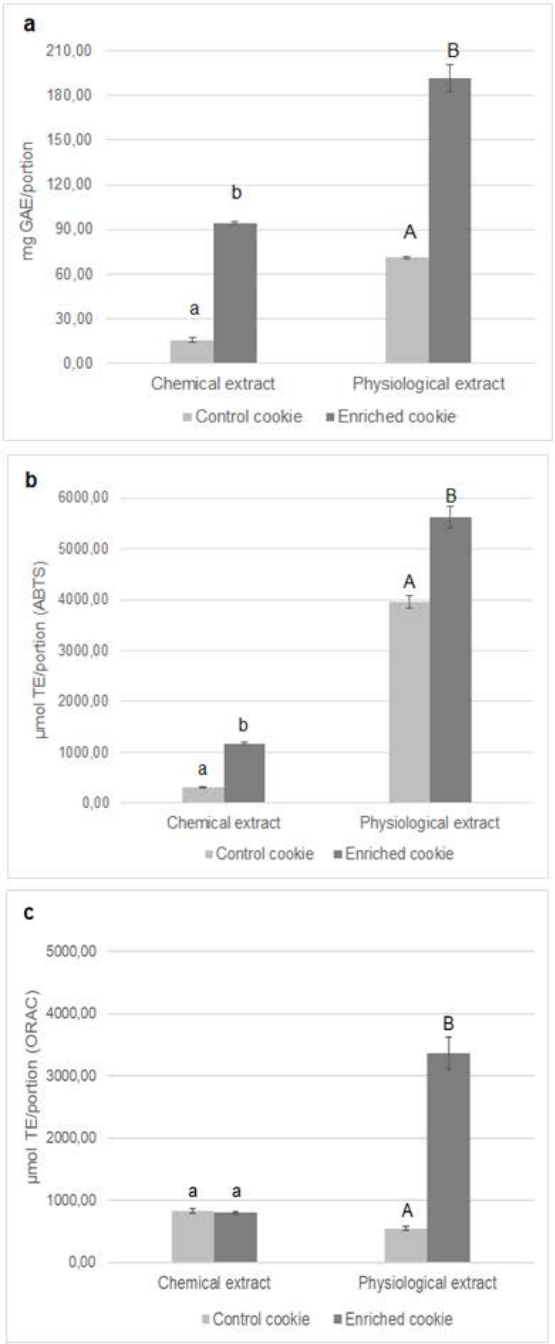

Figure 2. (a) Total phenolic content, (b) ABTS Antioxidant Capacity and (c) ORAC Antioxidant Capacity of control and enriched cookies per portion of to 30 g. Different lowercase letters indicate significant differences ($p<0.05$) between cookies. Different capital letters indicate significant differences ($p<0.05$) between physiological extracts. Lines correspond to SD, n=3

A similar tendency can be observed for antioxidant capacity; while the improvement of ABTS antioxidant capacity is of 3.7 times in cookies and 1.4 times in the physiological extract after the addition of DCP, ORAC showed different results. There was no significant difference between antioxidant capacity of control and enriched cookies. However, in physiological extracts the improvement was of 6.2 times after the addition of the new functional ingredient.

These results confirm that coffee pulp has high phenolic content, as expressed by [33]. [34], also pointed that there is a considerable content of polyphenols as well as antioxidant activity in coffee pulp. There is also an increase in both total phenolic content and antioxidant capacity of enriched cookies after the digestion process, as has also been observed by [35,36,37,38]. Phenolic content increased 2 times for enriched cookies, which leads to affirm that there is a great amount of hydrolysable (not assessed with chemical extraction methods) polyphenols associated with dietary fibre that are release during digestion. [11] explain that direct solubilization of the food in the intestinal fluids (at physiological conditions: 37°C, pH 1-7.5) and the action of the digestive enzymes (which are responsible for the hydrolysis of proteins, carbohydrates and lipids) cause the release of phenolic compounds from the food matrix. Thus, hydrolysis of non-extractable polyphenols occurs, rendering a greater content of bioavailable polyphenols [38]. An additional quantity of non-extractable polyphenols could be released and made available during colonic fermentation [39].

The antioxidant capacity of non-extractable phenolics is greater than extractable polyphenols [38]. Antioxidant capacity increases with the digestion process (4.8 and 4.2 times as obtained from ABTS and ORAC assays respectively). [31] concluded that the addition of fibre from spent coffee grounds to biscuits was responsible for an increase in their content of dietary fibre and in the antioxidant capacity after in vitro digestion and higher bioavailability of phenolic compounds.

Molecular interactions between polyphenols and dietary fibre could interfere with the bioaccessibility and bioavailability of polyphenols, so that phenolic content is not necessarily related to functional properties [40]. [41] elaborated water cookies with red beetroot with phenolic content up to 75 mg GAE/30 g and ABTS antioxidant capacity below 450 µmol TE/30 g; [42], produced snacks enriched with powdered tomato with phenolic content of 72 mg GAE/30 g; [43], enriched bread with plantago seeds and husks and obtained products with total phenolic content below 20 mg GAE/30 g and ABTS antioxidant capacity below 4000 µmol TE/30 g. Considering these results as reference, it would appear that salty cookies enriched with DCP have a potential antioxidant capacity which could translate into a benefit for health. Nevertheless, in vivo analyses are needed to be conducted in order to confirm health beneficial and allow these enriched cookies to be considered functional foods.

3.3. Consumer Tests

Neither the blind nor informed tests showed significant difference in acceptability between control and enriched cookies (Table 4). [44], found that fibre enrichment (especially of cookies) tends to lower consumer acceptability. When comparing acceptability of cookies between blind and informed tests, a significant difference can be found for enriched cookies, thus suggesting that the presence of fibre is perceived as a positive attribute that influences consumer acceptability. In a similar way, [45], reported that overall acceptance of muffins containing whole meal increases significantly in comparison with all-wheat flour muffins when ingredients´ information is provided to consumer.

Table 4. Acceptability of cookies in the blind and informed condition

	Blind condition	Informed condition
Control cookies	6.41 ^{aA}	6.80 ^{aA}
Enriched cookies	6.11 ^{aA}	6.62 ^{bA}

Different lowercase letters indicate a significant difference ($p<0.05$) between tests (blind and informed).
Different capital letters indicate a significant difference ($p<0.05$) between cookies (control and enriched).

Acceptability of the enriched cookies (in informed and uninformed consumers) reached a mean above 6 value by which, the product can be considered acceptable and may be commercialized. In fact, 75% of all informed panelists, as opposed to 68% in the blind test, gave the enriched cookie an acceptability value equal or higher than 6. According to [46], although consumer acceptability is mainly affected by the product's sensory characteristics, extrinsic factors (non-sensory) such as health benefits influence acceptance. In accordance with (47), these authors conclude that health claims of foods enriched with dietary fibre and antioxidants generate an impact on consumers who are health-conscious or show interest in health issues.

Table 5. Frequency of use (%) of descriptors included in CATA question

	Control Cookies	Enriched Cookies
Crunchy	98	96
Off-flavour	1	0
Delicious	57	44
Poor taste	36	32
Healthy	25	46
Good quality	33	26
Intense flavour	7	8
Fibrous	7	21
Bitter	3	10
Tasty	23	25
Non-characteristic flavour	7	14
Crambly	5	4
Aftertaste	8	14
Intense flavour	2	2
Soft	1	0
Hard	33	57
Dry	43	43
Rough	10	7
Floury	21	13

Descriptors in bold showed a significant difference ($p<0.05$) between control and enriched cookies.

According to CATA question analysis, both cookies, the enriched and the control, were found to be crunchy, which tends to be a desirable characteristic in salty cookies. Enriched salty cookies, were crunchier than control cookies. Nevertheless, significant differences were observed in the frequency of use of 4 of the 19 terms evaluated (Table 5).

Enriched cookies were perceived as healthier than control cookies (even without informing consumers), a characteristic related to the presence of fibre.

4. Conclusions

It is possible to incorporate coffee pulp waste as a new functional ingredient in human diet through a product with good acceptability. Dry coffee pulp in cookies will enhance their functional qualities, as fibre content and antioxidant capacity. Antioxidant capacity was mainly due to the hydrolysable fraction, explained by the bioactive compounds bonded to the fibre. The bioaccessibility of antioxidants improve during the gastrointestinal digestion of the enriched cookies. The use of this coffee by-product promises environmental sustainability with a new paradigm in which there are no more wastes but new ingredients with functional properties that still long to be exposed.

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