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Original article

Synergism and phenolic bioaccessibility during *in vitro* co-digestion of cooked cowpea with orange juice

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Summary Foods are susceptible to matrix interferences during the gastrointestinal transit that can affect bioactive molecules. We proposed *in vitro* co-digestion of cowpea beans and orange juice to assess polyphenols bioaccessibility and synergisms. We performed gastrointestinal simulation combining beans and a fruit beverage, to mimic a common meal in a more realistic set-up than the usual single-food models. Twenty phenolic compounds were released in oral, gastric and intestinal compartments and were identified by HPLC-DAD. Gallic acid, (−)-epicatechin and chlorogenic acid were the most bioaccessible polyphenols. Cooking and solvent extraction of cowpea beans affected their polyphenolic concentrations. After digestion, the bioaccessibility indexes were higher for cowpea (136.11%) and cooked cowpea (744.74%) when compared to orange juice (31.87%) indicating that the thermal treatment enhanced the bioaccessibility of cowpea phenolics. The antioxidant capacity was higher in the end of co-digestion compared to cooked cowpea and orange juice digested alone due to synergistic polyphenol-polyphenol interactions or polyphenol-protein interactions. The combination of orange juice with cooked cowpea in co-digestion promoted high content of some bioaccessible phenolics, with 10 out of 20 compounds showing positive interactions at the intestinal phase, suggesting that food synergisms are not neglectable for beans and citrus polyphenols release during the digestion course.

Keywords Antioxidant capacity, bioactive compounds, gastrointestinal simulation, legumes, *Vigna unguiculata*.

Introduction

The intake of phenolic compounds is related to health benefits such as protective effects against cancer and cardiovascular diseases, antioxidant and anti-inflammatory properties (Singh *et al.*, 2017). The bioactive action of dietary polyphenols depends on their bioaccessibility, that is, the extent to which ingested compounds are released from food matrix and become available for absorption (McClements *et al.*, 2015). Many studies have focused on polyphenols from beans or fruits and have shown that these compounds are affected by enzymatic action during digestion (Hachibamba *et al.*, 2013; Giusti *et al.*, 2019; Lindemann *et al.*, 2021), by thermal processing (Nderitu *et al.*, 2013; Lafarga *et al.*, 2019) and by food matrix interactions (Nair & Augustine, 2018; Lindemann *et al.*, 2021). Generally, a small portion of the phenolic compounds is available for absorption in the gut despite the high quantity of these constituents in the raw material, reinforcing the importance of digestion and bioaccessibility studies (Lindemann *et al.*, 2021).

Orange juice is commonly consumed around the world, especially during meals (Liu *et al.*, 2012). It has been recognised as a source of energy and nutrients promoting health benefits by containing bioactive compounds such as flavonoids (Khan *et al.*, 2014). Another common constituent in meals are legumes such as cowpea (*Vigna unguiculata*) widely consumed in Africa, Latin America and Asia (Gonçalves *et al.*, 2016), which is rich in proteins (17.4–28.3%), and fibres (19.5–35.6%), and a source of phenolic acids, flavonoids, anthocyanins and proanthocyanidins (Carvalho *et al.*, 2012; Nderitu *et al.*, 2013).

Worldwide, a healthy meal is considered as composed of a protein food source, grains and vegetables and is often accompanied by a fruit beverage. It is known that interactions among nutrients and other

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food constituents can improve potential health benefits when compared to their individual actions alone due to food synergy (Jacobs *et al*., 2009). Complex food matrix interactions occur normally during gastrointestinal digestion, affecting bioaccessibility and, thus, bioavailability of food compounds. Those interactions can act on polyphenolics that can have their chemical structures, ionisable ligands and polymerisation status affected by variations in acidic–basic conditions, enzymatic activity and phenolic synergism (Zehfus *et al*., 2021). The *in vitro* digestion model has been proven useful for assessing bioaccessibility of food polyphenols in common bean (Rossi *et al*., 2022), although there is minimal published information on the content of phenolics in the event of co-digestion. In this work, we provide data on polyphenolic synergism and antioxidant properties of orange juice and cowpea polyphenols in the events that precede intestinal absorption using the *in vitro* model of co-digestion. To better study the *in vitro* co-digestion of these two complex food matrices, the effects of cooking and solvent extraction in cowpea were also evaluated.

Material and methods

Chemicals

Enzymes α-Amylase from *Aspergillus oryzae*, pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, DPPH, ABTS, Trolox, Folin–Ciocalteu and standard phenolic compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chromatographic solvents used for HPLC analysis were of HPLC grade.

Samples

Brazilian cowpea (*Vigna unguiculata*) cultivar BRS Aracê, was provided by Embrapa Meio-Norte. It was cultivated in the experimental field of Embrapa Meio-Norte, Teresina, Piauí, Brazil. Orange fruits (*Citrus sinensis*) were obtained at local market in São Miguel do Oeste, Santa Catarina, Brazil.

Sample preparation

Orange juice (OJ) was extracted using an automatic extractor machine (Skymsen, Santa Catarina, Brazil). Raw cowpea (RC) was grounded in an analytical mill (IKA, Staufen, Germany; particle size equivalent to 60 mesh). Cooked cowpea (CC) was prepared by 100 g of cowpea grains and cooking for 45 min in 500 mL of distilled water, without soaking and pressure. After that, the wet mixture was grounded in an analytical mill (as a puree) and samples were taken for the analyses. For the co-digestion experiments, orange juice plus cooked cowpea (CC + OJ) were mixed at 1:1 (w/w) ratio, considering a standard meal mixing ratio and subjected to the *in vitro* digestion procedure. All samples were stored in plastic tubes at −20 °C until the analysis.

In vitro digestion

The gastrointestinal simulation was performed according to Minekus *et al*. (2014) as detailed described in Supporting Information. The digestion of RC, CC and CC + OJ occurred in three phases (oral, gastric and intestinal). The oral phase is optional to liquid samples, so in this study the OJ samples were submitted only to gastric and intestinal phases.

Extraction and determination of total phenolic content

The aqueous extract was obtained according to Lima *et al*. (2004) with modifications. One gram of grounded sample (RC, CC and CC + OJ) was infused in 5 mL of distilled water for 40 min at 40 °C, then samples were centrifuged at 3000 ***g*** for 10 min, the supernatant was taken as an aqueous extract and the precipitate was resubmitted to the same initial extraction, so the second supernatant was combined to the first and stored at −80 °C, at dark.

Methanol extraction was performed according to Laparra *et al*. (2008), 2 g of grounded sample (RC, CC and CC + OJ) were extracted with 5 mL of acidified methanol (1.0 M HCl, 85:15, v/v) for 1 h at 21 °C. The tubes were centrifuged at 3000 ***g*** for 10 min. Then the supernatants were placed in ambar glass and stored at −80 °C.

The total phenolic content (TPC) was determined by the Folin–Ciocalteu method as described by Seraglio *et al*. (2018). Gallic acid was used as standard and total phenolics were expressed as mg of gallic acid equivalents in 100 g of sample.

Determination of antioxidant capacity (AC)

The AC of samples was measured by the ABTS free radical scavenging method and by the ferric reducing antioxidant power method (FRAP).

The ABTS method was determined according to Seraglio *et al*. (2017), Trolox was used as a standard and the results were expressed as μM Trolox in 1 g of sample. FRAP method was performed as described by Seraglio *et al*. (2018), the results were expressed as μmol ferrous sulphate in 1 g of sample, using ferrous sulphate heptahydrate as standard.

Bioaccessibility index (BI)

The BI was used to evaluate the effect of *in vitro* digestion on the phenolic composition of samples

(Ortega *et al*., 2011). The BI was calculated as described in Supporting Information.

Synergism evaluation

The evaluation of synergism in the course of *in vitro* co-digestion CC + OJ was calculated as described previously (Reber *et al*., 2011) by subtracting the sum of the mean antioxidant capacity or the mean concentration of phenolics of the individual samples digested alone from the mean antioxidant capacity or the mean concentration of phenolic resulting from the co-digestion of the samples (CC + OJ).

Quantification of phenolic compounds by HPLC

Chromatographic analysis was carried out on an Agilent liquid chromatograph (St. Clara, CA, USA) equipped with a quaternary pump system (G1311C) and diode array detector (DAD) (G1316A) according to Burin *et al*. (2014). Analytical separation was performed on a pre-column (4.6 mm × 50 mm × 5 μm) and reversed-phase column (4.6 mm × 250 mm × 5 μm) Phenomenex (Torrance, CA, USA). The samples (10 μL) previously filtered were directly injected into the chromatographic system. The mobile phase consisted of water: acetic acid (98:2 v/v) (A) and water: acetonitrile: acetic acid (58:40:2 v/v/v) (B), the flow rate was 0.7 mL min^{-1}. Elution was carried out using the linear gradient: 0–70% B over 55 min, 70–100% B over 15 min, and returning to 0% B for 10 min for samples with cowpea, and for orange juice samples, the elution was carried out as follows: 0–60% B over 55 min, 60–100% B over 15 min, and returning to 0% B for 10 min. Detection was performed at 280 nm for gallic acid, (+)-catechin, tyrosol, 4-hydroxybenzoic acid, (−)-epicatechin, vanillic acid, syringic acid, taxifolin, naringenin, naringin and hesperidin, 320 nm for chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and apigenin and 360 nm for rutin, myricetin, quercetin and kaempferol. The individual phenolic compounds were identified and quantified by comparison of the analyte peaks with their respective calibration curves of standard solutions.

Statistics

The software used was STATISTICA version 7.0 (StatSoft Inc., Tulsa, OK, USA). The results were expressed as means ± standard deviation of three replicates. Significant differences ($P < 0.05$) between results were determined by one-way analysis of variance (ANOVA) and Tukey's test for three or more samples and Student's *T*-test for two samples. Relationship among data was calculated using Pearson correlation (*r*).

Results and discussion

Total phenolic content (TPC) and antioxidant capacity (AC)

The TPC and AC of samples were determined before and after each phase of digestion (Table 1). The TPC of OJ decreased during digestion, resulting in a BI of 31.87%. This is similar to the BI reported for the digestion of orange and other citrus fruits (Quan *et al*., 2018). The AC by FRAP also decreased, which is consistent with a previous report by Mennah-Govela & Bornhorst (2017). This is normally expected in polyphenols digestion but the extent of this decay, however, is variable and depends on compounds bioaccessibility in a given food matrix, and on their sensibility under alkaline pH conditions (Quan *et al*., 2018).

Cooking promoted a significant decrease of TPC and AC values (Table 1). This effect agrees with previous reports (Mtolo *et al*., 2017). It is expected that the thermal processing can cause reactions of phenolics, including leaching of water-soluble phenolics and degradation, breakdown and transformation of phenolics, or generation of complexes with proteins and carbohydrates (Maghsoudlou *et al*., 2019). In addition, there are a positive correlation between TPC and AC, as before digestion (TPC × ABTS: $r = 0.7402$; TPC × FRAP: $r = 0.9114$) as after digestion (TPC × ABTS: $r = 0.5071$; TPC × FRAP: $r = 0.7922$), which is consistent with a previous report Alves *et al*. (2017), so the decrease of TPC after cooking can influence AC decrease.

The TPC of citrus was decreased by 68.1% in the intestinal phase. The same was not observed for the cowpea samples where TPC values and AC were consistently increased during digestion. Previous studies also reported improvements in TPC values and AC after gastrointestinal simulation (Hachibamba *et al*., 2013). Components of the food matrix can provide stability under different pH conditions and the action of enzymes can promote the hydrolysis of macromolecules such as proteins and carbohydrates, allowing the release of phenolics previously conjugated (Thakur *et al*., 2020). The AC values were mostly variable among the digestion phases and food matrices but were noticeably higher in the intestinal phase for the co-digestion sample and the cooked cowpea sample. It is important to emphasise that the AC measurements do not represent a mere sum of the antioxidant potentials of isolated compounds, but also consider their synergistic interactions (Pellegrini *et al*., 2003). In fact, this could explain the discrepancy in the observed AC values, and also the high increases observed in those two particular samples, one subjected to heating and the other to co-digestion with fruit juice rich in

Table 1 Total phenolic content (mg GAE/100 mL or g) and antioxidant capacity of orange juice, raw cowpea, cooked cowpea and cooked cowpea + orange juice before and after each phase of *in vitro* digestion

Sample	Phase of digestion	TPC (mg GAE/100 mL or g)	BI (%)	ABTS	FRAP
Orange juice	Before	$100.95^{aA} \pm 2.47$	$31.87^{A} \pm 0.89$	$3.41^{aA} \pm 0.00$	$7.55^{aA} \pm 0.71$
	Gastric	$46.95^{bA} \pm 2.44$		$1.74^{bA} \pm 0.07$	$4.00^{bA} \pm 1.01$
	Intestinal	$32.17^{cA} \pm 0.42$		$4.54^{cA} \pm 0.12$	$1.58^{cA} \pm 0.01$
Raw cowpea	Before (aqueous extract)	$136.76^{aB} \pm 5.35$	$136.11^{B} \pm 6.87$	$9.90^{aB} \pm 0.54$	$12.88^{aB} \pm 0.02$
	Before (methanolic extract)	$70.42^{bC} \pm 6.27$		$5.82^{bC} \pm 0.39$	$5.59^{bC} \pm 0.99$
	Oral	$220.06^{cA} \pm 0.33$		$6.83^{bA} \pm 0.28$	$6.76^{bcA} \pm 0.06$
	Gastric	$200.08^{dB} \pm 4.88$		$2.55^{cB} \pm 0.18$	$8.08^{cdB} \pm 0.74$
	Intestinal	$186.14^{dB} \pm 11.15$		$6.93^{abA} \pm 1.14$	$9.92^{dB} \pm 0.15$
Cooked cowpea	Before (aqueous extract)	$17.21^{aD} \pm 2.11$	$744.74^{C} \pm 78.64$	$2.59^{aA} \pm 0.48$	$2.99^{aD} \pm 0.01$
	Before (methanolic extract)	$12.32^{aD} \pm 2.92$		$2.13^{aAD} \pm 0.02$	$1.48^{bDE} \pm 0.00$
	Oral	$17.62^{aB} \pm 1.43$		$2.22^{aB} \pm 0.01$	$1.37^{bB} \pm 0.02$
	Gastric	$41.62^{bA} \pm 6.45$		$1.90^{aA} \pm 0.00$	$1.47^{bA} \pm 0.06$
	Intestinal	$128.18^{cC} \pm 2.86$		$14.90^{bB} \pm 1.37$	$5.28^{cC} \pm 0.01$
Cooked cowpea + orange juice	Before (aqueous extract)	$71.09^{aC} \pm 3.04$	$273.79^{D} \pm 23.46$	$1.34^{aD} \pm 0.57$	$8.33^{aA} \pm 1.00$
	Before (methanolic extract)	$39.79^{bE} \pm 1.27$		$2.78^{bA} \pm 0.10$	$7.02^{aA} \pm 1.08$
	Oral	$39.44^{bC} \pm 0.70$		$2.10^{cB} \pm 0.03$	$4.38^{bC} \pm 0.03$
	Gastric	$107.33^{cC} \pm 8.42$		$0.81^{dC} \pm 0.16$	$7.26^{aC} \pm 0.16$
	Intestinal	$194.64^{dB} \pm 6.87$		$12.79^{eC} \pm 2.27$	$4.70^{bC} \pm 0.11$

Values are the mean of three independent determinations ± standard deviation. Different lowercase letters represent significant difference among undigested samples, oral, gastric and intestinal phases for the same sample. Different uppercase letters represent significant difference among samples for each digestion phase or for undigested samples (Tukey's test. $P \leq 0.0$). BI, Bioaccessibility index.

vitamin C and phenolics. This would be the first evidence of synergisms observed in these samples and these could be elicited more likely from polyphenol-polyphenol or polyphenol-protein interactions.

Cowpea protein content is in the range 17.4–28.3% (Carvalho *et al.*, 2012), and we found that they should be interfering with bioaccessibility of cowpea polyphenols, particularly during the gastrointestinal simulation. For example, when considering the passage from the oral to the final phase of digestion, the obtained increases in total phenolic content were of 627% for the digestion of CC and of 393% for the co-digestion samples. Interestedly, the gains in polyphenolic content were more pronounced in the case of CC samples, which suggests that the thermal treatment in cooked samples contributed to protein-phenolic interactions, indicating that the thermal treatment affected positively the bioaccessibility.

Taking the co-digestion sample, BI was greatly decreased in comparison with the CC sample. Unlike this finding, one could expect the opposite in the event of co-digestion with OJ, a phenolic-rich fruit juice. When studying cowpea digestion, it has been previously reported that phenolic-peptide complexes are formed during upper gut digestion of processed cowpea (Apea-Bah *et al.*, 2021). This could explain the reduction in BI value for the co-digestion sample, even in the presence of a virtually higher amount of polyphenols (CC + OJ). We found that not only the phenolics but also proteins of cowpea were involved in food components interactions during *in vitro* co-digestion, and that these could lead to some synergism between phenolics from orange juice and cowpea. Therefore, we evaluated further the effects of *in vitro* digestion on the individual phenolics (Figs S1–S4).

Effects of cooking on phenolic composition

The cowpea samples contained more polar compounds rather than nonpolar, since higher quantity of phenolics was detected in the aqueous extracts (Table S1). It is known that solvent polarity affects the yield of phenolics (Metrouh-Amir *et al.*, 2015). Cooking is a common processing applied to beans, it can, however, affect composition, as we observed for polyphenols in the samples (Table S1). The abundance of starch in cowpea beans was also considered. According to Lindemann *et al.* (2021), the presence of phenolic compounds that inhibits the activity of amylolytic enzymes is more likely to interfere with starch digestibility rather than the opposite, and the strong physical interaction between free phenolics and cell wall polysaccharides are more prone to affect their bioaccessibility. The chromatographic analysis revealed that except for myricetin and naringenin, the concentrations of all other phenolics were negatively impacted by cooking. Decreases in polyphenols concentrations after cooking were also observed for the same cultivar of cowpea by Barros *et al.* (2017). Giusti *et al.* (2019) also reported a strong reduction in the phenolic content of legumes

after cooking. This can be related to the destruction of the compounds, chemical interactions or leaching of water-soluble phenolics (López-Martínez *et al*., 2017); however, after gastrointestinal digestion there may be greater releases of these compounds or synergistic interactions among them.

Effects of *in vitro* digestion on phenolic composition

The changes in concentrations of individual phenolics throughout the *in vitro* digestion are presented in Table 2. Gallic acid, hesperidin and quercetin were the major phenolics detected in OJ after digestion. This sample showed decreases in concentrations of most phenolics at the intestinal phase, except for gallic and chlorogenic acids, (−)-epicatechin, quercetin and taxifolin. Our results agree with Peña-Vázquez *et al*. (2022) that reported a reduction of 25% in the bioaccessibility of phenolics of orange juice. Koehnlein *et al*. (2016) suggested that phenolics are protected against enzymatic action and pH alterations during digestion by solid and complex matrices, and that, in liquids, this protection does not occur.

In CC, we observed losses of polyphenols only in the oral phase, possibly by the interactions and complexes formed between phenolics and macromolecules (Apea-Bah *et al*., 2021; Lindemann *et al*., 2021). Gallic, caffeic and chlorogenic acids and myricetin were the most significant compounds identified before and post digestion of CC. The flavonoids of CC were augmented after the gastric and intestinal phases; however, they did not surpass the pre-digestion levels. Concentrations of phenolic acids, except for ferulic acid, were higher in the intestinal phase when compared to the non-digested sample (BI > 200%). These results agree with Apea-Bah *et al*. (2021) that also evidenced that not all polyphenols are equally released from the cowpea during digestion in the gut.

The combination of CC + OJ showed decreases in concentrations of most polyphenols at the oral phase and increases in their gastric and intestinal concentrations. Naringenin and rutin concentrations decreased throughout the entire digestion. Once again, some phenolic acids had higher levels in the intestinal phase than before the digestion, such as 4-hydroxybenzoic, gallic, and ferulic acids and myricetin. According to Koehnlein *et al*. (2016) the food matrix can show a protective effect on phenolics through digestive conditions. Furthermore, hydrolysis of macromolecules and consequent release of phenolics can occur through the action of enzymes present in digestion media and according to the pH in the different phases of *in vitro* digestion. The higher BI at the end of co-digestion was obtained for the phenolics: ferulic, gallic, syringic and 4-hydroxybenzoic acids. The low bioaccessibility of other flavonoids, as well as the decrease in the BI of the co-digestion sample in relation to the individual matrices, can be explained by the interactions and complexes formed between phenolics and proteins or other macromolecules present in the samples (Apea-Bah *et al*., 2021). Particularly for hesperidin, which is not naturally occurring in beans and was not detected in cowpea samples, we found that after co-digestion its concentrations raised up significantly at the gastric and intestinal phases, rising its BI value up to 52.8%. This implies that matrix interactions or compounds synergism led to favouring this bioaccessible polyphenol. Although to a lower extent, it was possible to verify that the co-digestion of CC + OJ resulted in substantial bioaccessibility for 13 out of the 20 phenolics found in samples, revealing that the co-digestion set-up is relevant in studying bioactive polyphenols in nutrition-health protocols. In order to expand the understanding of the effects of this co-digestion, we further evaluated synergisms by means of antioxidant activity, total phenolic content and individual phenolics.

Synergism

The co-digestion CC + OJ had a positive effect during the course of digestion on TPC values (Table 3), as the calculated differences were positive for all phases of the digestion, it means that the concentration of total phenolics, in this case, was higher for the co-digestion sample in comparison to the sum of TPC of cowpea and orange juice when both were digested alone. The same applies to the AC and to the concentrations of phenolics as determined by HPLC-DAD. Our results revealed that the co-digestion led to unidentified interactions that caused some enhance on the levels of polyphenols at the end of the gastrointestinal simulation and is hereby identified as synergism, given the rise in the total concentration of bioaccessible compounds. The same was not observed in the case of the AC by ABTS that had negative values. Conversely, there was synergism on the AC by FRAP in the oral and gastric phases during the co-digestion of cowpea and orange juice. Apart from the antioxidant method itself, these contradictory results may be explained by the specific antioxidant power elicited by one or another phenolic compound depending on their chemical structure, compound-compound interactions and matrix-related interference. For this reason, the calculated differences observed for the concentrations of individual polyphenolic compounds present in samples is effectively important. As shown in Table 3, we found positive values indicating synergisms for the majority of the quantified phenolics throughout the phases of gastrointestinal simulation. The augmented concentrations of individual bioaccessible phenolics contributed to the increased value of TPC, as described above.

Table 2 Phenolic composition of orange juice, cooked cowpea and combination cooked cowpea + orange juice before and after different phases of *in vitro* digestion

	Orange juice				Cooked cowpea					Cooked cowpea + orange juice				
Compounds (mg/100 g)	Before	Gastric	Intestinal	BI (%)	Before (aqueous extract)	Oral	Gastric	Intestinal	BI (%)	Before (aqueous extract)	Oral	Gastric	Intestinal	BI (%)
Phenolic acids														
4-Hydroxybenzoic	$0.15^{aA} \pm 0.03$	nd	$0.05^{bA} \pm 0.00$	33.3^{A}	$0.41^{aB} \pm 0.00$	$0.65^{aA} \pm 0.05$	$0.85^{aA} \pm 0.05$	$1.61^{bB} \pm 0.57$	392.7^{B}	$1.36^{aC} \pm 0.02$	$0.66^{bA} \pm 0.20$	$1.71^{cB} \pm 0.24$	$1.65^{cB} \pm 0.0$	121.3^{C}
Gallic acid	$0.51^{aA} \pm 0.01$	$0.31^{bA} \pm 0.01$	$2.06^{cA} \pm 0.03$	403.3^{A}	$4.28^{aB} \pm 0.02$	$1.59^{bA} \pm 0.05$	$2.37^{cB} \pm 0.31$	$15.64^{dB} \pm 4.0$	365.4^{B}	$6.54^{aC} \pm 0.08$	$2.20^{bB} \pm 0.13$	$6.08^{aC} \pm 0.58$	$14.1^{cB} \pm 3.36$	215.6^{C}
Syringic acid	nd	$0.18^{aA} \pm 0.01$	$0.21^{bA} \pm 0.00$	–	nd	$0.92^{aA} \pm 0.05$	$0.36^{bB} \pm 0.01$	$2.12^{cB} \pm 0.01$	–	$1.96^{aA} \pm 0.00$	$0.77^{bB} \pm 0.05$	$0.77^{bC} \pm 0.02$	$2.59^{cC} \pm 0.0$	132.1^{A}
Vanillic acid	$0.16^{A} \pm 0.03$	nd	nd	–	$0.13^{aA} \pm 0.00$	$0.30^{bA} \pm 0.02$	$0.27^{bA} \pm 0.10$	$0.59^{c} \pm 0.00$	453.8	$0.30^{aB} \pm 0.02$	$0.27^{aB} \pm 0.02$	$0.32^{aA} \pm 0.06$	nd	–
Caffeic acid	$1.03^{aA} \pm 0.00$	$0.28^{bA} \pm 0.00$	$0.52^{cA} \pm 0.00$	50.5^{A}	$2.82^{aB} \pm 0.03$	$0.78^{bA} \pm 0.04$	$1.34^{cB} \pm 0.08$	$2.64^{dB} \pm 0.00$	93.6^{B}	$6.08^{aC} \pm 0.06$	$1.07^{bB} \pm 0.05$	$1.57^{cC} \pm 0.01$	$4.19^{dC} \pm 0.05$	68.9^{C}
Chlorogenic acid	$0.27^{aA} \pm 0.03$	$0.13^{bA} \pm 0.00$	$0.35^{cA} \pm 0.03$	129.6^{A}	$2.13^{aB} \pm 0.03$	$1.56^{bA} \pm 0.10$	$1.94^{cB} \pm 0.10$	$3.56^{cB} \pm 0.07$	167.1^{B}	$6.98^{aC} \pm 0.20$	$1.82^{bA} \pm 0.34$	$3.92^{cC} \pm 0.01$	$5.34^{dC} \pm 0.08$	76.5^{C}
Ferulic acid	$0.28^{aA} \pm 0.09$	$0.07^{bA} \pm 0.00$	nd	–	$0.69^{aB} \pm 0.03$	$0.23^{bA} \pm 0.02$	$0.21^{bB} \pm 0.00$	$0.17^{cA} \pm 0.00$	24.6^{A}	$0.13^{aC} \pm 0.01$	$0.23^{bA} \pm 0.03$	$0.36^{cC} \pm 0.03$	$0.41^{cB} \pm 0.05$	315.4^{B}
p-coumaric acid	$0.34^{aA} \pm 0.00$	$0.46^{bA} \pm 0.00$	nd	–	nd	$0.43^{aA} \pm 0.02$	$0.75^{aB} \pm 0.08$	$1.73^{bA} \pm 0.00$	–	$3.88^{aB} \pm 0.00$	$0.57^{bB} \pm 0.02$	$1.24^{cC} \pm 0.15$	$2.96^{dB} \pm 0.02$	76.3^{B}
Flavonoids														
(−)-epicatechin	$0.19^{aA} \pm 0.04$	nd	$0.40^{bA} \pm 0.06$	210.5^{A}	$4.65^{aB} \pm 0.15$	$1.22^{bA} \pm 0.08$	$1.58^{cA} \pm 0.10$	$2.64^{dB} \pm 0.34$	56.8^{B}	$7.28^{aC} \pm 0.21$	$1.01^{bA} \pm 0.17$	$2.40^{cB} \pm 0.40$	$4.20^{dC} \pm 0.18$	57.7^{B}
(+)-catechin	$0.71^{aA} \pm 0.03$	$0.24^{bA} \pm 0.01$	nd	–	nd	$0.66^{aA} \pm 0.11$	$1.12^{bB} \pm 0.15$	$0.91^{b} \pm 0.19$	–	nd	$0.77^{aA} \pm 0.09$	$0.86^{aC} \pm 0.07$	nd	–
Apigenin	0.44 ± 0.00	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	–
Hesperidin	$11.5^{aA} \pm 0.47$	$6.31^{bA} \pm 0.17$	$4.24^{cA} \pm 0.23$	36.9^{A}	nd	nd	nd	nd	–	$13.2^{aB} \pm 0.49$	$3.89^{b} \pm 0.19$	$8.57^{cB} \pm 0.26$	$7.45^{dB} \pm 0.82$	56.4^{B}
Kaempferol	1.64 ± 0.01	nd	nd	–	nd	nd	nd	nd	–	nd	nd	nd	nd	–
Myricetin	$2.43^{aA} \pm 0.03$	$0.80^{bA} \pm 0.00$	nd	–	$7.60^{aB} \pm 0.03$	$0.70^{bA} \pm 0.01$	$2.75^{cB} \pm 0.03$	$4.43^{dA} \pm 0.02$	58.3^{A}	nd	$1.35^{aB} \pm 0.10$	$3.72^{bC} \pm 0.06$	$8.79^{cB} \pm 0.04$	–
Naringenin	$0.46^{A} \pm 0.07$	nd	nd	–	$0.72^{aB} \pm 0.15$	$0.12^{bA} \pm 0.00$	nd	nd	–	$1.59^{aC} \pm 0.06$	$0.15^{bB} \pm 0.01$	$0.28^{c} \pm 0.00$	nd	–
Naringin	0.30 ± 0.01	nd	nd	–	nd	$0.78^{A} \pm 0.03$	nd	nd	–	$3.28^{a} \pm 0.27$	$0.90^{bB} \pm 0.03$	$1.99^{c} \pm 0.03$	$2.13^{c} \pm 0.13$	64.9
Quercetin	$0.75^{aA} \pm 0.00$	$1.26^{bA} \pm 0.01$	$1.31^{cA} \pm 0.06$	174.7^{A}	nd	$0.62^{A} \pm 0.00$	nd	nd	–	$12.7^{aB} \pm 0.02$	$1.37^{bB} \pm 0.01$	$4.01^{cB} \pm 0.00$	$8.89^{dB} \pm 0.04$	70.0^{B}
Rutin	nd	nd	0.11 ± 0.03	–	$1.44^{aA} \pm 0.03$	$0.74^{bA} \pm 0.05$	nd	nd	–	$1.02^{aB} \pm 0.09$	$0.54^{bB} \pm 0.1$	$0.50^{b} \pm 0.01$	nd	–
Taxifolin	nd	$0.10^{aA} \pm 0.01$	$0.27^{bA} \pm 0.00$	–	$0.92^{aA} \pm 0.03$	$0.32^{bA} \pm 0.00$	$2.29^{cB} \pm 0.13$	$0.75^{dB} \pm 0.00$	81.5^{B}	$3.75^{aB} \pm 0.16$	$0.21^{bB} \pm 0.01$	$0.64^{cC} \pm 0.04$	$1.01^{dC} \pm 0.03$	26.9^{C}
Tyrosol	$0.14^{A} \pm 0.01$	nd	nd	–	$0.63^{B} \pm 0.02$	nd	nd	nd	–	$0.56^{C} \pm 0.04$	nd	nd	nd	—

Values are the mean of two independent determinations ± standard deviation. Different lowercase letters represent significant difference among the digestion phases for each sample. Different uppercase letters represent significant differences among samples for each phase of digestion or BI (Tukey's test. $P \leq 0.05$). BI, bioaccessibility index; nd, not detected.

Table 3 Mean differences of antioxidant activity, total phenolic content (TPC) and individual phenolic compounds for evaluating synergisms of the co-digestion of cooked cowpea and orange juice at different phases of *in vitro* digestion

	Oral	Gastric	Intestinal
ABTS	−0.09	−2.83	−6.65
FRAP	3.01	1.79	−2.16
TPC	21.82	18.79	34.29
Phenolic acids			
4-Hydroxybenzoic acid	0.01	0.86	−0.01
Gallic acid	0.61	3.40	−3.63
Syringic acid	−0.15	0.23	0.26
Vanillic acid	−0.03	0.05	−0.59
Caffeic acid	0.29	−0.05	1.03
Chlorogenic acid	0.26	1.85	1.43
Ferulic acid	0.00	0.08	0.24
p-coumaric acid	0.14	0.03	1.23
Flavonoids			
(−)-epicatechin	−0.21	0.82	1.17
(+)-catechin	0.11	−0.50	−0.91
Apigenin	0.00	0.00	0.00
Hesperidin	3.89	2.26	3.21
Kaempferol	0.00	0.00	0.00
Myricetin	0.65	0.17	4.36
Naringenin	0.03	0.28	0.00
Naringin	0.12	1.99	2.13
Quercetin	0.75	2.75	7.58
Rutin	−0.20	0.50	−0.11
Taxifolin	−0.11	−1.75	−0.01
Tyrosol	0.00	0.00	0.00

Calculation of the difference was performed according to Reber *et al.* (2011).

According to Sánchez-Rangel *et al.* (2013) the Folin–Ciocalteu protocol is not specific for phenolics, as other molecules such as reducing sugars and ascorbic acid can reduce the Folin's reagent, overestimating the results. Once again, the monitoring and quantification of individual compounds was essential to elucidate this impasse. Among 20 polyphenolics analysed, 10 showed positive synergisms when in co-digestion (higher values of antioxidant capacity and phenolics) in the intestinal phase, being highly bioaccessible (Table 3).

Nevertheless, although the AC showed positive difference on oral phase, it displayed negative differences on the intestinal phase. Considering the increase of TPC and the decrease of AC after the combination CC + OJ, it is possible that the release of phenolics showed pro-oxidant activity. This is because the phenolics can act as anti or pro-oxidant agents, and this ability depends on chemical structure, concentration, food matrix and metal ions interactions (Samra *et al.*, 2011).

Gallic acid and quercetin are the phenolics observed in greater quantity after digestion of CC + OJ (Table 2). According to Hagerman *et al.* (1998) small molecules like gallic acid and quercetin demonstrate significant pro-oxidant capacity because they are easy to oxidate. Quercetin, (−)-epicatechin and (+)-catechin present high pro-oxidant activity, as well as the mixtures of quercetin/catechin and caffeic acid/ascorbic acid (Samra *et al.*, 2011). The cowpea cultivar used in our study is biofortified with iron (60 mg kg^{-1}) and zinc (52 mg kg^{-1}) (Coelho *et al.*, 2021), which can stimulate oxidation by phenolics through their ability to reduce iron.

Cooking promoted a significant decrease of TPC, as well the AC of cowpea beans, but contributed to protein-phenolic interactions that positively affected the bioaccessibility of its phenolics. While the AC and the bioaccessibility of phenolics from orange juice decreased after the gastric and intestinal phases, in cowpea there was an increase on phenolics bioaccessibility. The co-digestion of CC + OJ led to a higher content of some phenolics; however, the antioxidant capacity decreased in most phases. The calculated differences for the concentrations of individual polyphenolic compounds suggested the occurrence of synergisms during the co-digestion of CC + OJ, and this was observed for the majority of polyphenolic compounds quantified in samples and throughout the three phases of gastrointestinal simulation. The augmented concentrations of individual bioaccessible phenolics contributed to increased TPC values. Finally, the use of a co-digestion set-up allowed to observe that the combination of orange juice with cooked cowpea led to higher content of bioaccessible polyphenols than the digestion of these samples alone, and their concentrations revealed that food synergisms are not neglectable when considering bioaccessibility behaviours during *in vitro* digestion. These findings could contribute to the research and development of functional foods and ingredients from beans and citrus fruits, besides application in food and nutrition studies and guidelines.

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Author contributions

Tuany Camila Honaiser: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); visualization (equal); writing – original draft (equal). **Stefany Grutzmann Arcari:** Formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Keli Cristina Fabiane:** Formal analysis (equal); investigation (equal); methodology (equal). **Maurisrael**

de Moura Rocha: Funding acquisition (equal); supervision (equal); writing – review and editing (equal). **Isabela Maia Toaldo Fedrigo:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); writing – review and editing (equal). **Ana Carolina Maisonnave Arisi:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); writing – review and editing (equal).

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Conflict of interest

There is no conflict of interest.

Ethical approval

Ethics approval was not required for this study.

PEER REVIEW

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Data availability statement

The data supporting this study are available on request from the corresponding author.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Representative HPLC-DAD chromatograms of non-digested orange juice. Detection was performed at 280 nm.

Figure S2. Representative HPLC-DAD chromatograms of non-digested cooked cowpea. Detection was performed at 280 nm.

Figure S3. Representative HPLC-DAD chromatograms of non-digested co-digestion sample (CC+OJ). Detection was performed at 280 nm.

Figure S4. Representative HPLC-DAD chromatograms of co-digestion sample at the end of the intestinal phase. Detection was performed at 280 nm.

Table S1. Phenolic composition of cowpea before and after cooking extracted by aqueous and methanolic acidified solvents.