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Functional, thermal, and pasting properties of cooked carioca bean (*Phaseolus vulgaris* L.) flours

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1 **Highlights**

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- Carioca beans flour presented high content of protein, total starch, resistant starch, and dietary fiber
 - Cooked and presoaked bean presented low values of peak viscosity, final viscosity, breakdown, and setback
 - Pre-gelatinized bean flour may be useful for food development

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8 Functional, thermal, and pasting properties of cooked carioca bean (*Phaseolus vulgaris* L.) flours

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24 **Abstract**

25 This study verified if cooking presoaked beans in the steam of autoclave improves the pasting properties,
26 texture profile, water-solubility (WSI), emulsifying capacities of aged carioca bean' flours. The carioca beans
27 flour presented high content of protein (20.7 – 22.3 g·100g⁻¹), resistant starch (RS) (8.3 – 31.1 g·100g⁻¹), and
28 dietary fiber (TDF) (18.9 – 23.7 g·100g⁻¹), and the cultivar Notavel presented the highest content of total
29 dietary fiber and resistant starch for both cooked and raw flour. The pretreatment promoted an increase in
30 TDF (8.8 %, cultivar Dama) and a decrease in RS (19.5 %, 33.4 %, and 47.0 % for cultivars Imperador, Gol,
31 and Bola Cheia, respectively). Regarding the pasting properties, the heating process promoted a reduction
32 in the values of peak viscosity, final viscosity, breakdown, and setback for all carioca bean cultivars. The
33 other parameters, i.e., gel hardness, WSI, emulsifying capacity, and stability also presented a significant
34 decrease in the cooked flours. So, the pretreatment promoted a total or/partially starch pre-gelatinization
35 and the denaturation of the proteins of the flours which might increase their acceptability for food
36 development.

37 **Keywords**

38 Resistant starch; paste viscosity; hardness; emulsifying capacity; water solubility.

39 **1 Introduction**

40 Dry bean (*Phaseolus vulgaris* L.) is an important crop worldwide due to their nutritional value and because
41 they contribute to the food security of low-income people in underdeveloped countries. In 2019, Brazil was
42 the third of the world's largest producers of dry beans, with an estimated production of 2.9 million tons, in
43 an area of around 2,600,000 ha (FAO, 2019). Dry beans are a product of great economic and social
44 importance in Brazil because it is an economic alternative for agricultural exploitation in small farms in a
45 large amount of Brazilian rural regions. That is, family farming is responsible for the production of 70 % of
46 national beans (considering all types of beans) (Silva, 2017). The carioca bean - grains with a light cream
47 color and with the presence of light brown streaks - is the most cultivated in Brazil. They look slightly like
48 pinto beans, except for the stripes instead of spots in the tegument and the smaller size of the grains.

49 Due to the seasonality of bean production in Brazil and other countries, the storage of carioca beans is
50 necessary to maintain bean supply throughout the year. Nevertheless, improper storage conditions cause
51 undesirable changes of carioca beans, e.g., the browning of the integument (Bento, Ferreira, Bassinello, &
52 Oomah, 2021c; Bento et al., 2020). So, after harvesting, grains of the carioca type quickly lose their
53 commercial value because some genotypes darken very quickly due to chemical changes, related to the
54 oxidation of proanthocyanidin (Bento et al., 2021d; Coelho et al., 2020). This depreciation is because the
55 consumers reject dark grains since it is associated with old grains and with a long cooking time (Bento et al.,
56 2021c; Bento et al., 2020). The aged carioca beans that quickly darkens and consequently lose their
57 commercial value can be used as ingredients in food formulation. So, making flour from aged carioca beans
58 can contribute to the sustainability of the bean production chain since the use of the bean flour could add
59 value to the aged bean. Similarly, flour from broken beans (i.e., the bean byproduct) is useful for food
60 development since they present a similar nutritional value compared to the whole grain (Bento et al.,
61 2021b; Gomes et al., 2015).

62 The heat treatment of pulses to produce flours can change the technological properties of the
63 flours, for example, it reduces the value of the breaking and final viscosities and the tendency to retrograde
64 (Sun et al., 2018). In our previous studies we showed that making flour from cooked beans allows the
65 development of food staff with high sensorial acceptance and depending on the type of bean (e.g., colorful

66 beans or black and carioca byproduct) the flour may be adequate for different food development (Bento et
67 al., 2021a; Bento et al., 2021b). This happens because different bean genotypes present distinct amounts of
68 protein, dietary fibers, resistant starch, and chemical profile which influence their technological properties,
69 i.e., water and oil absorption, emulsification, water solubility, and gelation properties (Gupta, Chhabra, Liu,
70 Bakshi, & Sathe, 2018; Lin & Fernández-Fraguas, 2020; Ramírez-Jiménez, Reynoso-Camacho, Mendoza-Díaz,
71 & Loarca-Piña, 2014). The flour made from colorful bean presoaked in water (6 h) and cooked in the steam
72 of autoclave (5 min) presented a higher amount of resistant starch and lower viscosities values (Bento et
73 al., 2021a). Thus, these flours from pretreated beans were advantageous for food systems application
74 when high levels of supplementation with pulse components are desired without causing a major texture
75 discrepancy (Bento et al., 2021a; Felker, Kenar, Byars, Singh, & Liu, 2018). Unfortunately, there is limited
76 research on the impact of their major elements on the functional characteristics of flours and the
77 physicochemical properties of carioca bean flours, which are primordial for the further development of
78 food products made with bean flours (Romero & Zhang, 2019).

79 Considering the importance of the technological properties of flour for the further development of food
80 products made with carioca bean flours, studies that propose the development of aged carioca bean flours
81 from cooked grains and their physicochemical evaluation are justified. They would provide information
82 about technological properties changes and help with the use of bean flours by industries, improving the
83 nutritional quality of processed foods, and still would attend to specific consumer's demands. Therefore,
84 the present study aimed to verify if cooking presoaked beans in the steam of autoclave improve the pasting
85 properties, texture profile, water-solubility, emulsifying capacities of aged carioca bean flours.

86 **2 MATERIAL AND METHODS**

87 **2.1 Plant material**

88 Dry bean from the commercial carioca group were selected from the Active Germplasm Bank of Embrapa
89 Arroz e Feijão, in Santo Antônio de Goiás, Goiás State, Brazil: BRSMG Madrepérola (Ma), TAA Dama (Da),
90 BRS Notável (No), IAC Imperador (Im), TAA Gol (Gol) and TAA Bola Cheia (BC). The grains were cultivated in
91 the experimental fields of Embrapa Arroz e Feijão, on the Capivara farm, in the same municipality. After
92 harvesting and drying of beans in an oven with forced air circulation (40 °C) (final moisture \pm 12.0 %) (the
93 moisture was determined using a dielectric moisture analyzer (Grainer II PM-300, Kett Electric Laboratory)),
94 the beans underwent cleaning operations, purge, and manual selection of grains. After quartering for
95 sample homogenization, they were stored in low-density polyethylene bags, in portions of 1 kg (triplicate,
96 three biological samples for each bean cultivars) for 3 months, in a place with ambient lighting at 25°C.

97 **2.2 Flour preparation**

98 To obtain the flour from raw grains, the beans were washed into running water and then dried, in an oven
99 (Nova Ética, 400/5, Brazil) at 60 °C with air circulation, for 1 - 2 hours (until final moisture of 10-12%).

100 Afterward, the beans were ground in a hammer mill with a sieve of 20 mesh. For cooked flour, the washed
101 grains were soaked for 6 hours in water (1:2 w/v), and then the washed beans were placed in 1 L beakers
102 without the addition of water and then cooked with the steam from the autoclave (121 °C at 1.1 kg·cm²)
103 (Primatec, CS, Brazil) for 5 min (Bento et al., 2021a). After that, the cooked grains were dried in an oven
104 (60 °C) with air circulation for 8 - 12 hours (until final moisture of 10-12%), and then it was ground in a
105 hammer mill. Three repetitions of each carioca bean flour were obtained (raw and cooked) and showed
106 similar granulometry, with particle sizes between 106 µm and 425 µm. A flowchart describing the flour
107 preparation is presented in Supplementary figure 1.

108 **2.3 Protein content, total dietary fiber (TDF), and resistant starch (RS)**

109 The nitrogen content by the micro-Kjeldahl method and then multiplied by a factor of 6.25 to obtain the
110 crude protein content, according to AOAC (2016), method number 979.09. The TDF was determined using a
111 standardized enzymatic-gravimetric method (K-TDFR Kit, obtained from Megazyme International Ireland,
112 Bray, Ireland), according to method number 985.29 (AOAC, 2016). RS content of bean flour was determined
113 using an RS assay kit (cat. no. K-RSTAR, obtained from Megazyme International Ireland, Bray, Ireland), with
114 some modifications. Briefly, pancreatic α-amylase and amyloglucosidase were added directly to 100 mg of
115 bean flour in 50 mL test tubes, and tubes were incubated at 37 °C for 16 h with shaking (100 rpm). After the
116 addition of ethanol and centrifugation, the supernatant (nonresistant starch - NRS) was removed and the
117 precipitate was homogenized using a magnetic stirrer. To solubilize RS, 2 M KOH was added to the
118 homogenized precipitate on the ice bath. Sodium acetate buffer (1.2 M, pH 3.8) was added and incubated
119 with amyloglucosidase to convert the solubilized RS to glucose. The glucose content of the RS fraction was
120 determined by the glucose oxidase/peroxidase reagent (GOPOD) method.

121 **2.4 Pasting properties and gel hardness**

122 The sample (3.5 g with adjusted moisture of 14 g·100g⁻¹) with 25.0 mL of distilled water were analyzed in a
123 Rapid Visco Analyser (Perten Instruments, RVA 4500, Macquarie Park, Australia), using the flour method
124 (RVA Method 5, Version 4, March 2010). The suspension was kept at 25 °C for 2 min, heated (14 °C·min⁻¹) at
125 95 °C and kept at this temperature for 3 min, and cooled (14 °C·min⁻¹) at 25 °C. The pasting properties
126 evaluated were paste temperature, peak viscosity, final viscosity, breakdown, and setback, expressed in
127 centipoise (cP). After being subjected to the Rapid Visco Analyzer, the samples were kept refrigerated (7 °C)
128 overnight, and later they were analyzed with a texturometer (TA HD Plus Stable Micro Systems, Surrey,
129 England). The gel hardness was measured with a 20 mm cylindrical probe at a temperature of 25 °C
130 according to Wani, Sogi, Wani, Gill, and Shivhare (2010), with a test speed of 0.5 mm·s⁻¹, a pre-test speed of
131 1.0 mm·s⁻¹, a post-test speed of 10.0 mm·s⁻¹, with force contact depth of 10 gf, and with probe penetration
132 distance/depth of 6 mm.

133 **2.5 Thermal properties**

The thermal properties were determined using a differential scanning calorimeter (TA Instruments, Q20, New Castle, UK). The samples (2 mg, dry weight) were weighed in aluminum containers, suitable for the equipment. Distilled water (6 μ L) was added, and the sample holders were sealed in a specific press. These were kept for 12 h at room temperature and heated in the range between 35 and 120 $^{\circ}$ C, at a heating rate of 10 $^{\circ}$ C \cdot min $^{-1}$. From the obtained curve, the temperature of peak gelatinization and vitrea transition was calculated using the TA Universal Analysis application (TA Instruments, New Castle, UK).

2.6 Water solubility index and the water and oil absorption index

The water solubility index (WSI) and the water absorption index (WAI) were determined according to the method described by Anderson, Conway, Pfeifer, and Griffin Junior (1969). The samples (2.5 g) were weighed into previously tared centrifuge tubes and 30 mL of distilled water was added. The tubes were shaken in a water bath for 30 min at 25 $^{\circ}$ C and then centrifuged at 3000 g for 15 min. The supernatants were carefully removed into 10 mL volumetric flasks. The WAI was calculated using equation 1, and the result was expressed in g of precipitate per g of dry matter. To determine the oil absorption index (OAI), the same methodology with adaptations was also used, since the water was replaced by soybean oil. The WSI was calculated from the ratio between the mass of the dry residue of the supernatant (evaporation residue) and the sample weight multiplied by 3 (indicating the correction for the total supernatant volume since only 10 mL of the 30 mL was used) and the result expressed in g \cdot 100 g^{-1} (Equation 2).

$$WAI = \frac{\text{Precipitate weight}}{\text{Sample weight (d.w.)}} \quad (1)$$

$$WSI = \left\{ \left[\frac{\text{Evaporation residue}}{\text{Sample weight (d.w.)}} \right] 3 \right\} 100 \quad (2)$$

2.7 Emulsifying capacity and stability

The sample (0.35 g) was weighed into a graduated centrifuge tube (10 mL) and 2.5 mL of distilled water was added. The tubes were shaken in a vortex for 30 seconds and then 2.5 mL of corn oil was added. After, the tubes were vortex for 90 s and then centrifuged at 500 g for 5 min (Kaur & Singh, 2005). The emulsifying activity was calculated by dividing the volume of the emulsified layer by the total volume before centrifugation. The stability of the emulsion was determined, following the same procedure to determine the emulsifying activity. However, before centrifuging the samples, they were subjected to heat treatment at 85 $^{\circ}$ C for 15 min and centrifuged after cooling. Emulsion stability was expressed as the percentage of the remaining emulsifying activity after heating.

2.8 Statistical analyses

All results were obtained in triplicate, are presented as means \pm standard deviation. Levene test was applied to verify the variance homogeneity (normality test), and the data were evaluated by the ANOVA (analysis of variance), followed by the Tukey test ($p < 0.05$). A general description of the data was obtained by Principal Component Analysis (PCA) of the normalized data based on Pearson's correlation matrix provided by XLSTAT software (Addinsoft, 2021).

3 Results and discussion

3.1 Protein, dietary fiber, total starch, and resistant starch content

The protein content presented a slight variation between the cultivars. The flours of the cultivar Ma showed the highest value of protein ($22.31 \text{ g}\cdot 100\text{g}^{-1}$), while the cultivar No was the lowest one ($20.90 \text{ g}\cdot 100\text{g}^{-1}$) (Table 1). Bean's protein content is comparable to pea ($18.7\text{--}22.3 \text{ g}\cdot 100\text{g}^{-1}$), lentil ($25.1 \text{ g}\cdot 100\text{g}^{-1}$), and faba bean ($26.5 \text{ g}\cdot 100\text{g}^{-1}$) (Abdel-Aal, Ragaee, Rabalski, Warkentin, & Vandenberg, 2018; Byanju, Hojilla-Evangelista, & Lamsal, 2021; Young et al., 2020). Besides, bean protein provides health benefits due to the presence of bioactive peptides that acts in anti-inflammatory responses, metabolism of protein and carbohydrate, antioxidant, and immune system modulation (Alves et al., 2021; Luna-Vital, Mojica, González de Mejía, Mendoza, & Loarca-Piña, 2015).

Carioca beans also presented high content of dietary fiber, between 18.89 to $23.7 \text{ g}\cdot 100\text{g}^{-1}$. The flour of beans is a source of fiber, and it had more fiber than lentil ($4.11 \text{ g}\cdot 100\text{g}^{-1}$), green pea ($6.51 \text{ g}\cdot 100\text{g}^{-1}$) (Byanju et al., 2021), and yellow pea ($16.5 \text{ g}\cdot 100\text{g}^{-1}$) (Setia et al., 2019). The thermal pretreatment did not influence the TDF, with exception of the flours of cultivar Da that presented an increase in TDF in the cooked flour (Table 1). This increase in dietary fiber is due to protein-fiber complexes formed by chemical modification caused by the cooking process (Wang, Hatcher, Toews, & Gawalko, 2009). Many of the nutritional benefits from consuming beans have been largely accredited to their dietary fiber content (Vergara-Castañeda et al., 2010).

The flours of the cultivar Da showed the highest content of resistant starch ($29 \text{ g}\cdot 100\text{g}^{-1}$), whereas the cultivar Ma had the lowest one ($9 \text{ g}\cdot 100\text{g}^{-1}$) (Table 1). These results are low than those found for Pinto bean and black bean (around $35 \text{ g}\cdot 100\text{g}^{-1}$) (Escobedo, Mora, & Mojica, 2019). However, most of the flour of raw beans presented more resistant starch than the yellow pea ($2 \text{ g}\cdot 100\text{g}^{-1}$) (Vatansever, Rao, & Hall, 2020), and cooked lentils ($3.0 \text{ g}\cdot 100\text{g}^{-1}$) (Johnson et al., 2015). The content of RS starch may be influenced by several factors, such as the composition of the bean flour (e.g., the fibers content) and the starch characteristics (i.e., the crystallinity of starch) since starch with high content of amylose tends to make the major amount of RS related to their chain-length. Additionally, the enzymatic content of the bean flour influences the content of RS since the natural RS present in plant material is due to the enzymatic de-branching of the amylose and amylopectin branch (Hung, Vien, & Lan Phi, 2016). RS present low digestibility and are composed of soluble and insoluble fibers and non-digestible sugars which is fermented by gut microbiota in the colon. The fermentation of RS by these microorganisms produces short-chain fatty acids, such as butyric acid. These acids are known to improve several biological mechanisms such as modulate postprandial lipemia and blood pressure control (Barber, Kabisch, Pfeiffer, & Weickert, 2020; Mullins & Arjmandi, 2021; Reverri et al., 2015; Vergara-Castañeda et al., 2010).

The pretreatments decreased the RS content of the flours of cultivars Im, Gol, and BC. Other research also observed a reduction in the RS when the grains were soaked before cooking (Santiago-Ramos, Figueroa-Cárdenas, Véles-Medina, & Salazar, 2018). The cooking process of beans causes starch gelatinization which increases the starch digestibility and upon cooling they form retrograded starch (less digestible) (Liu, Ragaee, Marcone, & Abdel-Aal, 2020). However, the formation of less digestible starch is dependent on the starch composition (i.e., amylose and amylopectin ratio) of the bean cultivar. Moreover, the formation of RS due the recrystallisation of the starch fractions can be improved by an additional heating/cooling treatment (Ramírez-Jiménez et al., 2014; Ramírez-Jiménez, Reynoso-Camacho, Tejero, León-Galván, & Loarca-Piña, 2015).

3.2 Pasting properties and gel hardness

211 The pasting temperature of the raw bean flours was between 80.7 – 84.1 °C and the flour of cultivar BC
212 presented the highest one (Table 2). Other flours of pulses also presented high pasting temperatures, e.g.,
213 yellow pea (79.3 °C) (Waduge et al., 2017), and kidney bean (89.4 – 94.9 °C) (Wani, Andrabi, Sogi, & Hassan,
214 2020). The high pasting temperatures are due to the presence of non-starch components (i.e., proteins,
215 oligosaccharides, cellulose, etc.). These compounds compete with the starch for water, which reduces the
216 water availability increasing the pasting temperature. Besides, the RS contributes to a higher resistance to
217 swelling and rupturing (Lin & Fernández-Fraguas, 2020; Romero & Zhang, 2019). Additionally, flours of
218 pulses contain a high amount of amylose (around 30 %) compared to cereal (around 10 %). The high
219 content of amylose might result in a high gelatinization temperature due to the orientation of amylose
220 chains relative to one another, or strong interactions between starch chains, which increases the stability of
221 the granules to rupture under mechanical agitation (Frohlich et al., 2021; Li, Prakash, Nicholson, Fitzgerald,
222 & Gilbert, 2016; Lin & Fernández-Fraguas, 2020). These aforementioned factors raise the minimum
223 temperature to cook the bean flours as well as the temperature at which the viscosity begins to increase
224 during the heating process.

225 The pretreatment of the grains promoted a starch pre-gelatinization, and this phenomenon was
226 more evident on the flours of Da_C, Ma_C, Gol_C, and BC_C because it did not present a paste temperature
227 (Table 2). The flours of cultivar No_C and Im_C presented a paste temperature, which indicates that these
228 grains were not completely cooked during the heat pretreatment. Therefore, its flour retains part of the
229 native starch granules since it was not completely pregelatinized.

230 The beans flours presented a range for peak viscosity between 48.3 - 1376.7 cP, and the raw flour
231 presented the highest one compared to the flours of cooked beans (Table 2). This result is comparable to
232 the peak viscosity of fava bean (1152 cP), yellow pea (1544 cP), and pea (1542 cP) flours (Frohlich et al.,
233 2021; Vatansever et al., 2020; Young et al., 2020). The breakdown, the difference between the maximum
234 and minimum viscosities at constant temperature (95 °C), is associated with gel stability. Therefore, lower
235 breakdown suggested that the paste is more stable during cooking (Zhang et al., 2019). The content of
236 amylose and the extent of amylose leaching also influence the breakdown of pulses flours. Thus, the raw
237 flour of cultivars Ma, Im, and Gol might show a high amount of amylose since these presented the highest
238 viscosity values (Table 2). The final viscosity presented a range between 1204 – 1987 cP for raw bean flour,
239 and between 74 – 672 cP for cooked bean flours. The setback oscillated between 28 and 695 cP, where the
240 raw flour presented the highest values (e.g., Ma, Im, and Gol). Low values of setback represent a small
241 tendency to retrogradation, i.e., the starch molecules have low mobility which retains the water into the
242 gel matrix (Demiate et al., 2016; Li et al., 2016). The differences observed in pasting properties between the
243 bean cultivars are a result of their starch composition (ratio of amylose and amylopectin), starch
244 crystallinity, and the content of non-starch components (Frohlich et al., 2021; Lin & Fernández-Fraguas,
245 2020; Romero & Zhang, 2019).

246 In general, the flours of cooked beans presented low values of viscosity compared with the raw ones (Table
247 2, Supplementary figure 2). This is because the starch granules of these samples were previously
248 gelatinized, at least part of them (Simons & Hall iii, 2018). Consequently, the partial depolymerization of
249 amylose and amylopectin promoted by the heating process produces short linear and branched chains. This
250 happens in both crystalline and amorphous regions of the granule starch reducing the gel-forming power,
251 swelling capacity, and viscosity values (Hung et al., 2016).

252 The gel hardness, which represents the compressive strength, presented a variation between 0.01 –
253 3.04 N, where the highest values were observed in the raw flour (Table 2). This property is related to the
254 retrogradation of the flour. Thus, flours with high content of amylose present a high setback and gel
255 hardness due to the recrystallization of amylose molecules (Weber, Collares-Queiroz, & Chang, 2009). So,

the high gel hardness of cultivars Ma, Im, and Gol also suggest that these cultivars present a high amount of amylose. The heat pretreatment reduced the gel hardness due to starch pre-gelatinization.

3.3 Thermal properties

The flours from raw beans exhibited two endotherm peaks (Table 3, Supplementary Figure 3), the first one corresponding to starch gelatinization and the second one to protein denaturation and the melting of amylose-lipid complexes (Santiago-Ramos et al., 2018). The peak temperatures (around 80 °C) of raw flours were higher than those reported for other pulses, i.e., faba bean (73 – 75 °C), lentil (70 – 71 °C), and pea (72 – 73 °C) (Abdel-Aal et al., 2018). The temperature of gelatinization is influenced by the starch composition and the presence of non-starch compounds. Which explains the difference between the beans with the other pulses as well as between different bean cultivars. The presence of the non-starch compound in the bean flour is responsible for the low enthalpy energy (Table 3) compared to the isolate bean starch (13 - 15 J·g⁻¹) (Demiate et al., 2016). Some cooked flours did not present the first endotherm peak, which is following the paste properties results, showing that the starch of the flours from cultivars Da_C, Ma_C, Gol_C, and BC_C were pregelatinized during the cooking process (Table 2 and 3, Supplementary Figure 4). For the cooked flours Im_C and Da_C the first endothermic peak presented an increase of temperature compared to their raw flours. This occurred due to the presence of retrograded starch which usually needs more temperature to start forming a gel.

Both sorts of flours (raw and cooked) from all studied bean cultivars presented the second endothermic peak (between 90 and 108 °C) (Table 3), which is due to the melting of amylose-lipid complexes due to the existence of them in flour from beans (Santiago-Ramos et al., 2018; Wani et al., 2020). At this range of temperature also occurs the denaturation of proteins since the peak denaturation temperatures of albumins and vicilin are 87 – 98 °C and 98.8 °C, respectively (Santiago-Ramos et al., 2018). Therefore, the differences between the bean cultivars in the temperature and the enthalpy energy may be due to the protein composition of the cultivars as well as the contents of amylose and lipids.

3.4 Water and oil absorption and water solubility

The water solubility index (WSI) varied between 27 – 30 g·100g⁻¹ and the flours from cultivars Ma presented the maximum value and the cultivar No had the lowest one (Figure 1). The bean flour solubility is mainly influenced by their protein composition and is related to the hydrophobicity of their amino acids (Boye, Zare, & Pletch, 2010; Los, Demiate, Prestes Dornelles, & Lamsal, 2020). The heat treatment promoted a reduction in the WSI for all bean cultivars (Figure 1). These reductions might be due to the protein denaturation which changes the protein solubility, as well as to the starch gelatinization that modifies the cellular medium which might entrap the protein fraction (Alfaro-Diaz et al., 2021). Solubility is a critical factor to consider when developing products based on beans since some bean proteins have low solubility in their original state. Proteins with low solubility promote the formation of very thick suspensions after hydration, which are not suitable for making soups nor for producing low viscosity drinks such as milk substitutes (Vogelsang-O'Dwyer, Zannini, & Arendt, 2021). On the other hand, flours with low values of WSI are appropriate for pasta and baked products development (Bento et al., 2021b).

The water absorption index (WAI) is the ability of the flour to entrap water into its molecular structure. The WAI varied between 3.9 – 5.1 g·g⁻¹ and the flours from cultivar No presented the maximum value and the cultivars Ma, Da, and BC the lowest ones (Figure 1). The results were higher than those found for raw kidney bean flour (1.21–1.53 g·g⁻¹) (Wani et al., 2020). The WAI is affected by the ratio of hydrophilic proteins and carbohydrates amounts in the flours since their strong bonds of the hydrogens of the polar or charged side chains are responsible for contributing to the increased capacity to absorb water

299 (Prasad, Singh, & Anil, 2012). The pretreatment increases the WAI, except for the flour from No_C which
300 presented a reduction in WAI (Figure 1). The increase in WAI observed in the flour of cooked beans is a
301 consequence of the protein denaturation, which exposes some earlier hidden peptide bonds and polar side
302 chains resulting in an increased ability to trap and keep hold water molecules. Additionally, the heat
303 process promotes starch gelatinization, which also could increase WAI due to the greater loss of molecular
304 order and crystalline structure (Lin & Fernández-Fraguas, 2020).

305 The oil absorption index (OAI) varied between 2.1 – 1.9 g·g⁻¹ and the flours from cultivars BC presented the
306 maximum value and the cultivar Gol had the lowest one (Figure 2). These results were higher than those
307 found for chickpea flour (0.62 g·g⁻¹) (Gupta et al., 2018). The OAI of the flour is related to the ability of the
308 protein to link with the fat molecules, which is a very important characteristic, since the fat acts as a flavor
309 retainer and increases the food palatability. Electrostatic and hydrogen bonds are the forces involved in the
310 lipid-protein interaction. The water and oil binding capacity of food proteins depends on intrinsic factors
311 such as amino acid composition, protein conformation, and surface polarity or hydrophobicity (Vaidya,
312 Solanke, & Gaware, 2016). The heat treatment promoted a slight reduction in OAI on the flour from BC and
313 Ma cultivars, a slight increase in OAI on the flours of Gol and Im, and it was not able to affect the OAI of the
314 flours from No and Da cultivars (Figure 2). An enhanced OAI would be due to a higher amount of non-polar
315 groups at the protein surface in contact with the neighboring oil (Lin & Fernández-Fraguas, 2020). The
316 reduction of OAI observed in the cooked flours from BC and Ma can be interesting in the point of health
317 since they may be used in fried products to provide reduced fat content and calories (Gupta et al., 2018).

318 3.5 Emulsifying properties

319 The emulsifying capacity (EC) ranged between 51 to 64 %, with the raw flour from cultivar No presenting
320 the highest value and the cooked flour Da_C the lowest EC (Figure 3). The formation of emulsions is mainly
321 due to the reduction in the interfacial tension of oil droplets in aqueous systems and electrostatic repulsion
322 among them (Wani et al., 2020). The dissimilarity for EC among the cultivars is due to the
323 hydrophilic/hydrophobic proportions of amino acids in the major storage proteins present in these seeds
324 (Foschia, Horstmann, Arendt, & Zannini, 2017; Gupta et al., 2018). The polysaccharides help to stabilize it
325 by increasing viscosity. So usually, pulses proteins with high solubility have high foaming capacity,
326 emulsification, and gelatinization (Boye et al., 2010; Los et al., 2020). Therefore, the reduction of EC
327 observed in most of the flours is related to the reduction of viscosity of these flours due to starch
328 gelatinization.

329 The flours from cultivars No and Im presented the highest emulsion stability (ES) (60 %) (Figure 3).
330 Pulses with excellent emulsifying properties allow the development of emulsion-based drinks and milk
331 alternative drinks without emulsifiers (Alavi, Chen, & Emam-Djomeh, 2021; Vogelsang-O'Dwyer et al.,
332 2021). The heat pretreatment reduced their ES with exception of the flours from cultivar Ma_C (Figure 3).
333 This reduction may be related to the protein denaturation which reduces the degree of the folded structure
334 resulting in an unstable interfacial layer due to the low molecular interactions into the sub-surface. Another
335 point that explains the reduction or even the increase in the ES is the protein profile of the flour. For
336 example, globular proteins, like globulin (~ 70 % of bean proteins), present more conformational
337 limitations, therefore it adsorbs slowly and only partially unfold at the interface, hence exhibiting poor
338 emulsification power (Lin & Fernández-Fraguas, 2020).

339 The cooked flours compared with the native material (raw flours) presented a reduction in the values of
340 WSI, pasting properties (peak viscosity, final viscosity, breakdown, and setback), hardness, emulsifying
341 capacity, and emulsifying stability (Figure 4). This happens because of starch pre-gelatinization and the

denaturation of the proteins present in the flours during the cooking step. For instance, precooked flours may be suitable for biscuits development since the heat process contributes to the formation of complexes between amylose and lipids improving the functional properties of the flours, such as lowering the stickiness of biscuits and modifying viscosity profiles (reducing it). Moreover, the flours of pretreated beans may present more fragmentation of their components as a resulting of denaturation and gelatinization which has been related to a desirable smooth texture and also may be responsible for the stable suspensions in thin porridge (Kamau, Nkhata, & Ayua, 2020).

4 Conclusion

The carioca beans flour presented high content of protein, resistant starch, and dietary fiber, and the flour from cultivar Notavel presented the highest content of total dietary fiber and resistant starch for both cooked and raw flours. Additionally, the protein, resistant starch, and dietary fiber content of bean flour are higher than other pulses flours such as pea and lentil, which efforts the high nutritional value of bean flours. The bean flours made from cooked beans presented a reduction in the values of pasting properties (peak viscosity, final viscosity, breakdown, and setback), hardness, water-solubility, emulsifying capacity, and emulsification stability in all carioca bean flours. Hence, the proposed method (i.e., soaking the beans in water for 6 h followed by cooking it under the steam of autoclave for 5 min) was able to promote starch pre-gelatinization and the denaturation of the proteins of the flours (at least part of it). Pre-gelatinized bean flour processed from presoaked beans in the steam of autoclave may be useful for food development (such as snacks, soups, cakes, pasta, etc.) increasing their acceptability as a base ingredient since it might present appropriate functional properties. Therefore, the application of cooked flour in the preparation of new food products still needs more study. Also, there is a gap in information about the nutritional value of cooked carioca bean flour (e.g., the bioavailability of minerals and protein digestibility). So, both application and nutritional studies are needed for better use of this pulse flour.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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540 **Table 1.** Protein, total dietary fiber, resistant starch, and total starch content in dry weight ($\text{g} \cdot 100\text{g}^{-1}$) of
 541 different raw and cooked carioca bean flour.

Flours	Protein	Total dietary fibers	Resistant starch
Da	20.76 ± 0.58 b	18.89 ± 0.17 e	29.00 ± 1.48 ab
Da_C	-	20.55 ± 0.06 cd	31.11 ± 1.59 a
No	20.90 ± 0.15 b	23.05 ± 0.33 a	27.60 ± 1.41 ab
No_C	-	23.72 ± 0.37 a	29.63 ± 1.51 ab
Ma	22.31 ± 0.34 a	21.49 ± 0.53 bcd	9.10 ± 0.46 f
Ma_C	-	20.88 ± 0.45 cd	8.30 ± 0.42 f
Im	21.52 ± 0.44 ab	21.20 ± 0.40 bcd	27.76 ± 1.40 ab
Im_C	-	20.80 ± 0.44 cd	22.34 ± 1.14 c
Gol	21.39 ± 0.29 ab	21.72 ± 0.61 bc	26.14 ± 1.33 b
Gol_C	-	22.50 ± 0.55 ab	17.41 ± 0.89 d
BC	21.28 ± 0.28 ab	20.37 ± 0.74 cd	26.76 ± 1.36 b
BC_C	-	20.93 ± 0.29 d	14.18 ± 0.21 e

542 *Means of three determinations ± standard deviation. Different letters on the same column represent the statistical
 543 difference ($p < 0.05$). Beans are described as **No** and **No_C** (Notavel), **Im** and **Im_C** (Imperador), **Gol** and **Gol_C** (Gol),
 544 **BC** and **BC_C** (Bola Cheia), **Da** and **Da_C** (Dama) and **Ma** and **Ma_C** (Madreperola), where the 'C' indicates cooked
 545 flours.

546 **Table 2.** Pasting properties and gel hardness of different raw and cooked carioca bean flour.

Flours	Paste temperature (°C)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Gel hardness (N)
Da	83.90 ± 0.15b	944.0 ± 30.5b	18.7 ± 7.3c	1274.0 ± 36.6de	348.7 ± 3.2c	1.76 ± 0.26bc
Da_C	-	48.3 ± 1.5g	2.7 ± 0.5d	74.3 ± 1.5j	28.7 ± 0.6g	0.03 ± 0.00e
No	83.72 ± 0.63b	952.0 ± 6.6b	22.0 ± 4.4c	1282.7 ± 10.4d	352.7 ± 10.7c	1.85 ± 0.32bc
No_C	87.37 ± 0.38a	245.0 ± 11.8de	20.0 ± 3.0c	507.3 ± 17.9g	282.3 ± 9.1d	0.72 ± 0.03d
Ma	82.32 ± 0.26c	1005.3 ± 37.2b	35.3 ± 9.9bc	1363.7 ± 29.2c	393.7 ± 23.1c	2.09 ± 0.15b
Ma_C	-	93.0 ± 3.0fg	11.3 ± 2.3d	189.3 ± 2.5i	107.7 ± 1.1f	0.01 ± 0.00e
Im	80.73 ± 0.04d	932.5 ± 0.7bc	49.5 ± 0.7b	1450.5 ± 4.9b	567.5 ± 6.4b	2.05 ± 0.12bc
Im_C	83.17 ± 0.60bc	304.3 ± 15.6d	25.3 ± 3.2c	672.0 ± 34.2f	393.0 ± 19.7c	0.84 ± 0.01d
Gol	78.77 ± 0.60e	1376.7 ± 107.5a	65.0 ± 13.5a	1987.3 ± 35.4a	695.7 ± 30.4a	3.04 ± 0.05a
Gol_C	-	190.2 ± 14.8ef	21.0 ± 7.0c	385.0 ± 62.9h	192.0 ± 55.1e	0.05 ± 0.00e
BC	84.10 ± 0.35b	832.5 ± 3.5c	36.5 ± 2.1b	1204.0 ± 1.4e	408.0 ± 1.3c	1.68 ± 0.13c
BC_C	-	67.0 ± 1.0g	5.0 ± 0.8d	118.0 ± 5.0ij	56.0 ± 4.0fg	0.02 ± 0.00e

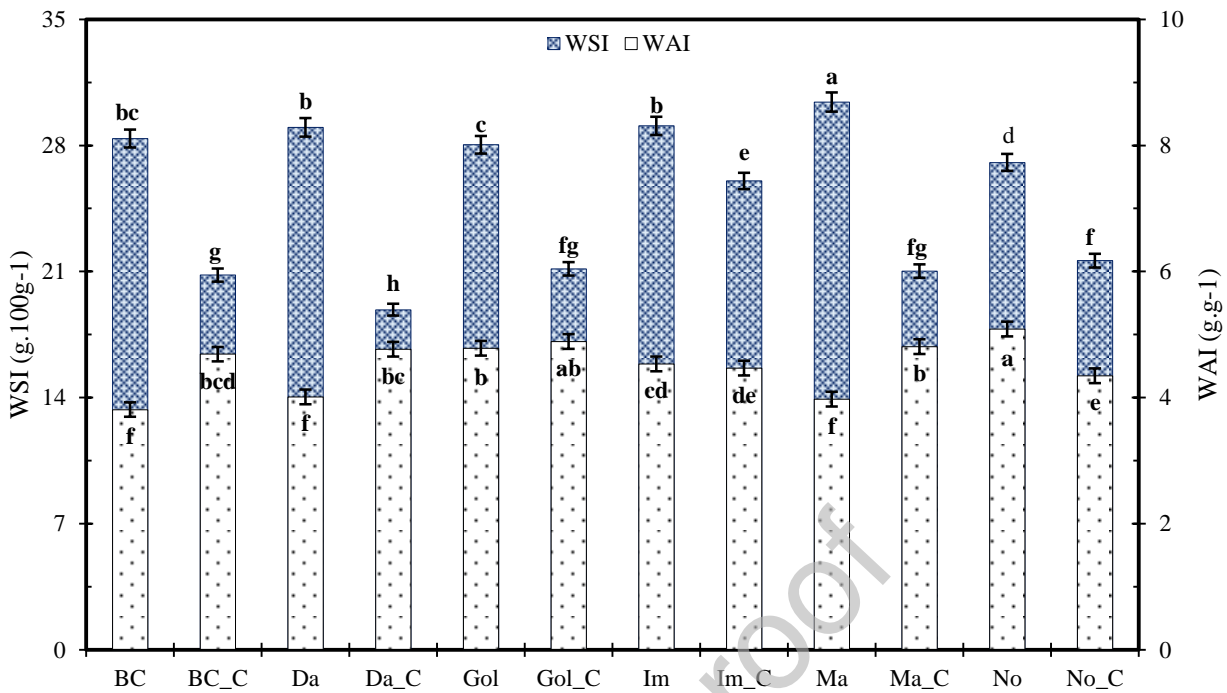
547 *Means of three determinations ± standard deviation. Different letters on the same column represent the statistical
548 difference ($p < 0.05$). Beans are described as **No** and **No_C** (Notavel), **Im** and **Im_C** (Imperador), **Gol** and **Gol_C** (Gol),
549 **BC** and **BC_C** (Bola Cheia), **Da** and **Da_C** (Dama) and **Ma** and **Ma_C** (Madreperola), where the 'C' indicates cooked
550 flours.

551 **Table 3.** Thermal properties of flour of raw and cooked beans from different cultivars: **No** and **No_C** (Notavel), **Im** and **Im_C** (Imperador), **Gol** and **Gol_C** (Gol), **BC**
 552 and **BC_C** (Bola Cheia), **Da** and **Da_C** (Dama), and **Ma** and **Ma_C** (Madreperola), where the 'C' indicates cooked flours.

Flours ¹	Peak 1				Peak 2			
	T _{onset} (°C)	T _{peak} (°C)	T _{end} (°C)	ΔH (J g ⁻¹)	T _{onset} (°C)	T _{peak} (°C)	T _{end} (°C)	ΔH (J g ⁻¹)
Da	75.56 ± 0.15 b	80.34 ± 0.08 bc	88.03 ± 1.74 b	2.43 ± 0.63 abc	92.79 ± 0.08 b	97.14 ± 0.07 b	102.25 ± 0.01 b	1.17 ± 0.05 b
Da_C	-	-	-	0.00 ± 0.00 f	91.46 ± 0.24 b	96.29 ± 0.48 b	103.01 ± 2.37 b	1.66 ± 0.78 ab
No	75.70 ± 0.03 b	80.47 ± 0.33 bc	85.91 ± 0.96 c	1.68 ± 0.58 cd	99.84 ± 0.23 a	102.30 ± 0.10 a	107.47 ± 0.15 a	1.87 ± 0.01 ab
No_C	78.33 ± 0.15 a	85.26 ± 0.18 a	94.60 ± 0.95 a	1.32 ± 0.01 de	94.69 ± 1.59 b	98.86 ± 1.64 b	103.79 ± 1.38 b	0.62 ± 0.12 c
Ma	74.25 ± 0.22 c	79.84 ± 0.05 c	86.46 ± 0.43 b	2.41 ± 0.43 abc	93.21 ± 0.10 b	96.23 ± 0.01	102.67 ± 0.27 b	2.14 ± 0.20 a
Ma_C	-	-	-	0.00 ± 0.00 f	92.55 ± 1.21 b	97.25 ± 1.16 b	101.83 ± 2.04 b	1.13 ± 0.24 b
Im	77.01 ± 1.14 a	80.17 ± 0.64 bc	87.77 ± 0.15 b	2.98 ± 0.55 ab	99.96 ± 0.01 a	102.52 ± 0.10 a	107.30 ± 0.05 a	1.56 ± 0.01 b
Im_C	76.76 ± 0.14 ab	81.55 ± 0.63 b	89.80 ± 2.18 b	1.96 ± 0.23 bcd	92.74 ± 0.78 b	97.19 ± 0.20 b	102.66 ± 0.61 b	1.53 ± 0.24 b
Gol	74.77 ± 0.59 bc	79.57 ± 0.98 c	87.36 ± 0.87 b	2.92 ± 0.28 a	93.33 ± 1.23 b	97.38 ± 2.70 b	102.52 ± 2.25 b	1.29 ± 0.16 b
Gol_C	-	-	-	0.00 ± 0.00 f	93.20 ± 0.87 b	97.27 ± 0.91 b	102.54 ± 1.11 b	0.72 ± 0.06 c
BC	76.34 ± 0.24 ab	80.36 ± 0.37 bc	86.21 ± 0.37 b	1.45 ± 0.19 de	99.74 ± 0.33 a	102.43 ± 0.47 a	107.27 ± 0.51 a	1.72 ± 0.24 ab
BC_C	-	-	-	0.00 ± 0.00 f	92.09 ± 1.06 b	96.16 ± 0.84	100.99 ± 1.36 b	0.77 ± 0.15 c

553 Results are presented as the mean of three replicates ± standard deviation; ¹Flours: raw and cooked (presoaked beans cooked for 5 min). T_{onset}: Onset temperature; T_{peak}: Peak
 554 temperature; T_{end}: Conclusion temperature. Different letters in the columns show statistical differences between the preparation method (p<0.05).

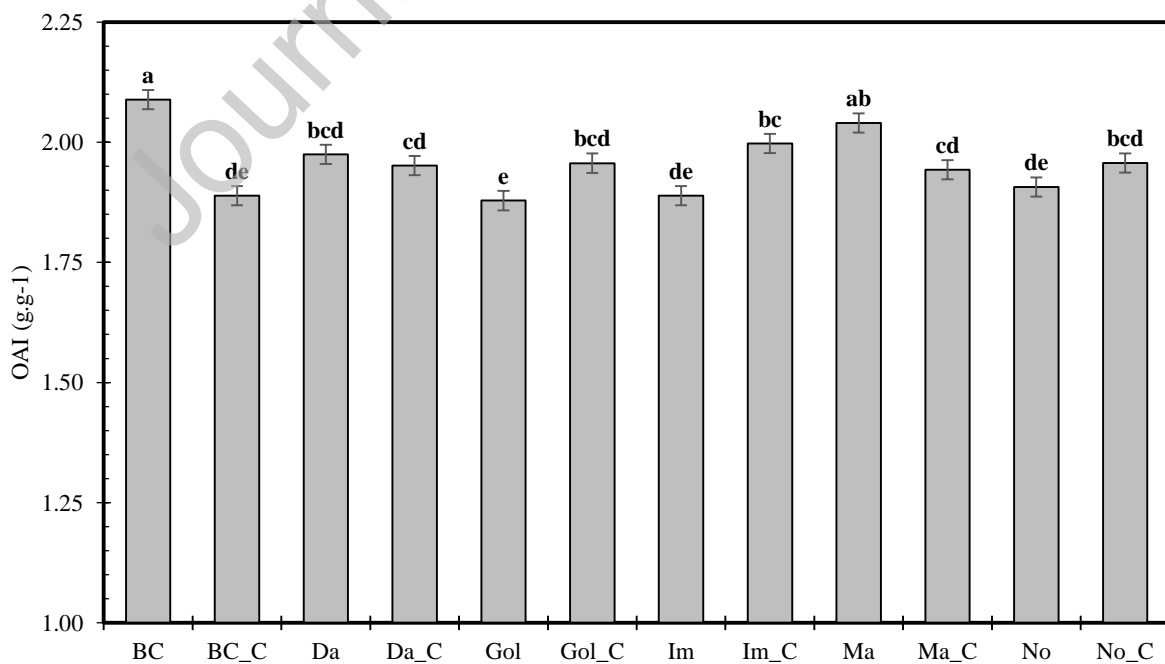
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556

557 **Figure 1.** Water solubility index (WSI) and water absorption index (WAI) of raw and cooked flours of
 558 different carioca bean cultivars. Beans are described as **No** and **No_C** (Notavel), **Im** and **Im_C** (Imperador),
 559 **Gol** and **Gol_C** (Gol), **BC** and **BC_C** (Bola Cheia), **Da** and **Da_C** (Dama), and **Ma** and **Ma_C** (Madreperola),
 560 where the 'C' indicates cooked flours.

561



562

563 **Figure 2.** Oil absorption index (OAI) of raw and cooked flours of different carioca bean cultivars. Beans are
564 described as **No** and **No_C** (Notavel), **Im** and **Im_C** (Imperador), **Gol** and **Gol_C** (Gol), **BC** and **BC_C** (Bola
565 Cheia), **Da** and **Da_C** (Dama) and **Ma** and **Ma_C** (Madreperola), where the 'C' indicates cooked flours.

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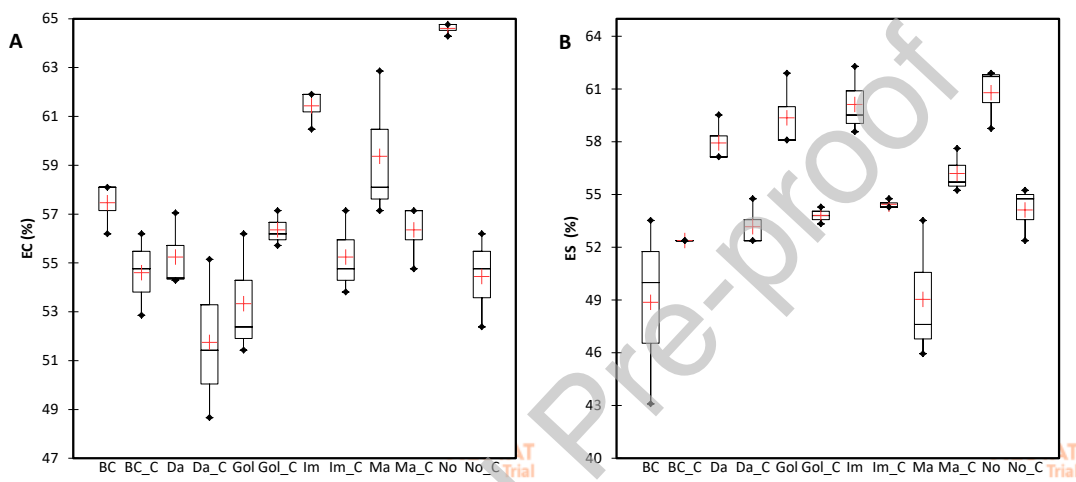
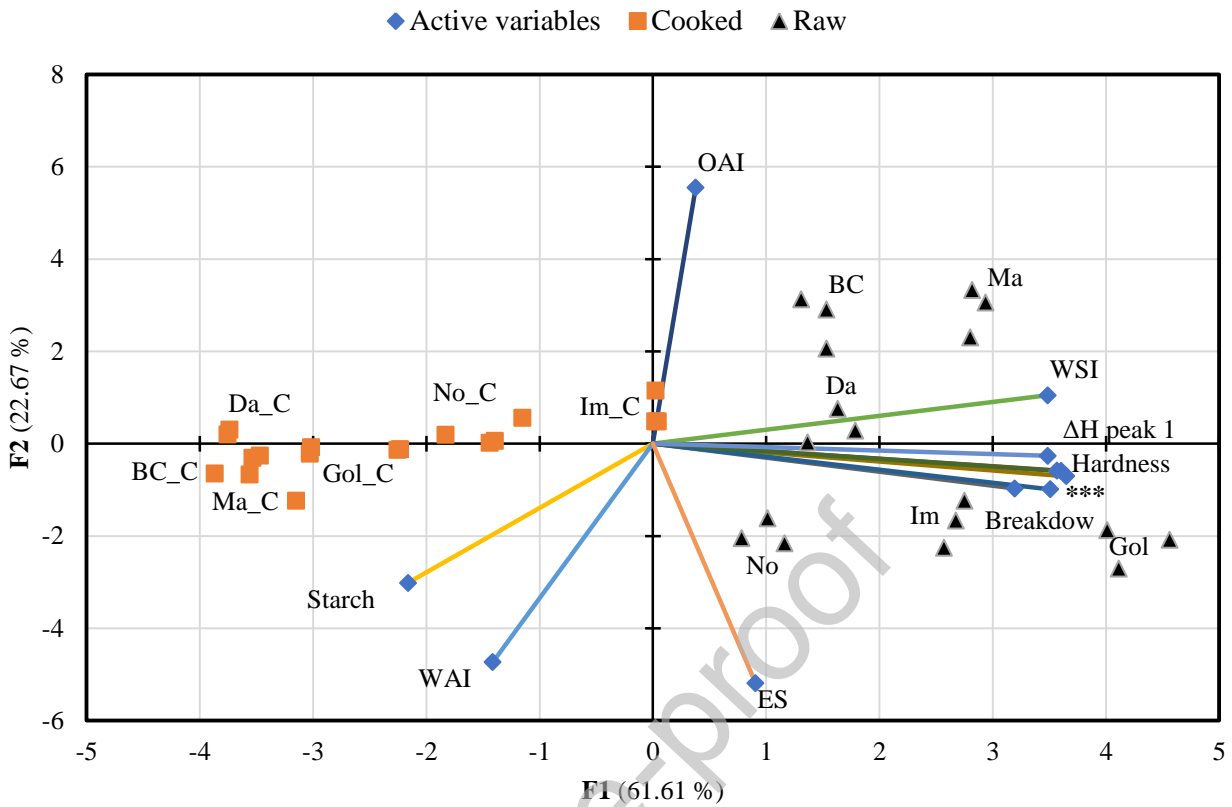


Figure 3. Emulsify capacity (EC) (A) and emulsion stability (ES) (B) of raw and cooked flours of different carioca bean cultivars. Beans are described as **No** and **No_C** (Notavel), **Im** and **Im_C** (Imperador), **Gol** and **Gol_C** (Gol), **BC** and **BC_C** (Bola Cheia), **Da** and **Da_C** (Dama), and **Ma** and **Ma_C** (Madreperola), where the 'C' indicates cooked flours.

571



572

573 **Figure 4.** Principal component analyses of the technological properties of raw and cooked flours of different
 574 carioca bean cultivars. Beans are described as **No** and **No_C** (Notavel), **Im** and **Im_C** (Imperador), **Gol** and
 575 **Gol_C** (Gol), **BC** and **BC_C** (Bola Cheia), **Da** and **Da_C** (Dama) and **Ma** and **Ma_C** (Madreperola), where the
 576 'C' indicates cooked flours. OAI: oil absorption index; WSI: water solubility index; ***: peak viscosity, final
 577 viscosity, and setback; ES: emulsion stability; WAI: water absorption index.