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Proximate composition, microstructure, and protein and starch digestibility of seven collections of Jack bean (*Canavalia ensiformis*) with different optimal cooking times

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ABSTRACT

Because of its high protein content, Jack bean (*Canavalia ensiformis*) is a promising alternative protein source. However, the utilization of Jack bean is limited due to the long cooking time to achieve palatable softness. We hypothesize that the cooking time may influence protein and starch digestibility. In this study, we characterized seven Jack bean collections with different optimal cooking times in terms of their proximate composition, microstructure and protein and starch digestibility. Kidney bean was included as a reference for microstructure and protein and starch digestibility. Proximate composition showed that Jack bean collections have a protein content ranging from 28.8 to 39.3%, a starch content ranging from 31 to 41%, a fiber content from 15.4 to 24.6%, and a concanavalin A content in the range 35–51 mg/g dry cotyledon. Particle sizes ranging between 125 and 250 μm were chosen as a representative sample of the whole bean to characterize microstructure and digestibility of the seven collections. Confocal laser microscopy (CLSM) revealed that Jack bean cells have an oval shape and contain starch granules embedded in a protein matrix similar to kidney bean cells. The diameter of Jack bean cells was measured by image analysis of CLSM micrographs and ranged from 103 to 123 μm, while the diameter of starch granules was 31–38 μm, comparatively larger than that of the kidney bean starch granules. Isolated intact cells were used to determine the starch and protein digestibility in the Jack beans collections. The digestion kinetics of starch followed a logistic model, whereas the digestion kinetics of protein followed a fractional conversion model. We found no correlation between optimal cooking time and kinetic parameters of protein and starch digestibility, implying that optimal cooking time is not predictive of protein and starch digestibility. In addition, we tested the effect of reduced cooking times on protein and starch digestibility on one Jack bean collection. The result showed that reducing cooking time significantly reduces starch digestibility, but not protein digestibility. The present study contributes to our understanding of the effect of food processing on protein and starch digestibility in legumes.

1. Introduction

Legumes are considered sustainable staple foods since they generate less greenhouse gases than food from animal sources (Poore & Nemecek, 2018). Underutilized legumes, i.e. legumes that have not been used as a feeding crop on a global scale, are one of the primary strategies for improving global food security. This is because the legumes can be used not only as a support for food security challenges in developing countries but also as a sustainable protein source in the protein transition towards plant-based proteins (Padulosi et al., 2013). One such underutilized legume is Jack bean (*Canavalia ensiformis*). Jack bean is grown in tropical and subtropical regions in South America, West Africa, Asia and the South Pacific (Sharasia et al., 2017). The legume has a protein content of 23–35%, a carbohydrate content of 46–65%, and a fiber content of 5–11% (Sridhar & Seena, 2006). The main concern related to Jack bean human consumption is its content of a lectin named concanavalin A. Lectins have several physiological effects, such as agglutination of erythrocyte, binding of glycoprotein in the small intestine, and

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inhibition of digestive enzymes (Lajolo & Genovese, 2002). Concanavalin A can be denatured by heat treatment, and soaking before heating has been reported to improve its inactivation (Carlini & Udedibie, 1997).

During cooking of legumes, solubilization of pectin in the middle lamella occurs, to an extent depending on the cooking intensity, together with starch gelatinization and protein denaturation. This solubilization causes cell separation upon mechanical disintegration and is most likely to be the rate limiting factor for textural alteration rather than the gelatinization and protein denaturation (Chigwedere et al., 2018). The solubilization of pectin can be delayed due to the complex linkages between pectin molecules and other cell wall components in the middle lamella.

Furthermore, changes in the cell wall may have nutritional significance because the cell wall is made up of indigestible carbohydrates. In intact cells, the cell wall can reduce nutrient digestibility by acting as a barrier to enzyme diffusion. It has been reported that the cell wall delays the access of the enzyme to the substrate, thus influencing protein and starch digestibility (Dhital et al., 2016; Rovalino-Córdova et al., 2018). Different intensities of cooking influence cell wall permeability. In case of Canadian wonder beans and Bambara groundnuts, different levels of cell wall permeability alter the rate of enzyme diffusion and influence starch digestibility (Gwala et al., 2020; Pallares Pallares et al., 2018). In soybean, cooking process intensities significantly affect protein digestibility, but the magnitude of the effect is modest (Zahir et al., 2021). It is also important to mention that, in legume cotyledon cells, starch granules are embedded in the protein matrix in the densely packed cytoplasm, which may further limit α-amylase diffusion to starch (Rovalino-Córdova et al., 2019). This additional barrier effect exerted by the protein matrix can be modulated by cooking time through the extent of protein denaturation and protein conformation (Carbonaro et al., 2012; Rovalino-Córdova et al., 2019).

To the best of our knowledge, whether differences in cooking times may affect the digestibility of starch and protein has not been studied for Jack beans. In this study, we, therefore, aimed to investigate the influence of optimal cooking time on *in vitro* protein and starch digestibility using seven Jack bean collections. We collected Jack bean samples with a wide range of cooking times from different collections. We characterized the proximate composition, starch, fiber, and concanavalin A of the raw material and observed the corresponding microstructure at optimal cooking time. Furthermore, different cooking intensities were applied to one of the collections to investigate further the effect of heat treatment on protein and starch digestibility.

2. Materials and methods

2.1. Materials

Jack bean (*Canavalia ensiformis*) collections were harvested from seven locations in Indonesia: Banyuwangi, Kebumen, Wonogiri, Malang, Yogyakarta, Lampung, and Temanggung. Kidney bean was purchased from a local supermarket (Jumbo, the Netherlands). On arrival at the laboratory, Jack beans were sorted to remove any damaged seeds. Some of the beans were immediately analyzed for their cooking time (section 2.4), and the rest were stored at −80 °C until further use. Pepsin from porcine gastric mucosa (4205 U/mg), trypsin from porcine pancreas (309 U/mg), pancreatin from porcine pancreas (2.5 U/mg trypsin activity; 41 U/mg amylase activity) were purchased from Sigma-Aldrich (Missouri, USA) and amyloglucosidase (3300 U/mL) was supplied by Megazyme Inc. (Bray, Ireland). All other chemicals were analytical grade unless stated otherwise.

2.2. Production of Jack bean cotyledon powder

Raw Jack beans were manually dehulled, freeze-dried, and ground using a ball mill (MM 400, Retsch) for 60 s at a frequency 1/30 s. The powder was sieved using 425 μm to remove big particles. The big particles were milled again at the same condition as previously described. The powder that passed through the sieve was collected and stored at room temperature until further analysis.

2.3. Proximate composition, starch, fiber, and concanavalin A

Proximate composition was analyzed on cotyledon powder. Nitrogen was quantified with the Dumas combustion method (FlashEA 1112NC, Thermo Scientific). The protein content was calculated by multiplying the nitrogen value by the conversion factor 6.25. We selected this conversion factor to be able to compare with other studies. Different conversion factors have been reported for legumes (Mariotti et al., 2008). Our data is likely an overestimation of the protein content, which would require a specific factor for Jack bean to calculate an accurate value. Lipid content was determined with the Soxhlet method by using petroleum ether as a solvent. Ash content was analyzed by incinerating the sample at 550 °C for 16 h following an AOAC 923.03 method. Carbohydrate content was calculated by difference, i.e. the residual weight after subtracting the amount of protein, fat, and ash. Moisture content was determined after drying a known amount of samples overnight in an oven at 105 °C to a constant weight. Starch content was determined on cotyledon powder according to the protocol of the Total Starch Assay (amyloglucosidase/α-amylase method) (Megazyme Inc., Bray, Ireland). Fiber content was calculated by subtracting starch from carbohydrate content. All measurements were performed in duplicate and presented on dry weight.

Concanavalin A content was analyzed on the protein extract of the cotyledon powder by using an ELISA kit from CloudClone (SEC179Ge, Texas, USA). The protein was extracted by using the manufacturer's standard protocol with modification. Cotyledon powder was mixed with 0.02 M phosphate buffer saline (pH 7.3) (1:8 w/v) under shaking condition (1000 rpm at 25 °C) for 1.5 h. The mixture was centrifuged at 10500g for 10 min, and the supernatant was collected and stored at −20 °C. Additionally, cooked Jack bean powder from the Malang collection was extracted for protein using the same method previously described. The bean was cooked following the method in section 2.5. After dehulling, the bean was freeze-dried, milled, and sieved as described in section 2.2. The assay was performed according to the protocol in triplicate.

2.4. Determination of the optimal cooking time

Finger pressing was used to measure cooking time where the cooking degree is presented as % cooked bean seeds, following the protocol described in a previous study (Kinyanjui et al., 2015; Wahome et al., 2023). One hundred Jack bean seeds were soaked in demineralized water (1:3 w/v) for 16 h at 25 °C and subjected to a thermal treatment at 97 °C. During this process, 10 beans were taken out every 30 min and were categorized as "cooked" once they easily disintegrated when pinched between a thumb and forefinger. The cooked degree was calculated as the ratio of the number of easily pressed beans with the number of beans taken in each sampling. The cooking profile was expressed as the percentage of cooked beans at different cooking times and was modelled by using a logistic model with three kinetic parameters (Eq. (1)).

$$y = \frac{a}{1 + e^{[b-kt]}} \quad (1)$$

y is the value of cooked beans (%), t is the cooking time (min), and a, b and k are the kinetic parameters. Minimization of residual sum of squares (RSS) was used for the estimation of the parameters. The model was used to extrapolate the optimal cooking time, defined as the time at which 90% beans were cooked (CT_{90}). The determination of the optimal cooking time was performed in duplicate for each of the collections.

2.5. Isolation of cotyledon cells

Isolation of intact cotyledon cells was carried out as described in a previous study (Rovalino-Córdova et al., 2018) with modifications. In brief, 30 g beans were soaked and cooked under the same condition as previously described in section 2.4. The shortest cooking time that gave 100% of cooked beans (CT_{100}) was used instead of CT_{90} to ensure cell separation and prevent the collection of residual cell clusters or the rupture of the cells during mechanical grinding. Banyuwangi, Kebumen, and Wonogiri collections were cooked for 180 min, Malang, Yogyakarta, and Lampung for 210 min, and Temanggung for 270 min. We included kidney bean as a reference bean for microstructure and digestibility analysis. Kidney beans were boiled for 45 min; this cooking time was previously evaluated using the same cooking time determination method as Jack bean (data not shown). All cooked beans were dehulled, and the seed coats were discarded. The cotyledons were mashed with mortar and pestle and then wet-sieved and the particles with size ranging between 125 and 250 μm were selected for further characterization. This range was selected because it gave the highest yield while mostly containing individual cells, therefore representing a homogeneous sample of the whole beans. Particles that remained at 125 μm were flushed with demineralized water three times to remove free starch granules. The cotyledon cells were collected and stored at 5 °C after adding sodium azide with a concentration of 0.02% in the final mixture.

We randomly selected one Jack bean collection to eliminate the compositional differences caused by different collections. Additional cotyledon cells were isolated from the Malang collection after processing the collection at two different cooking times (90 and 135 min). Therefore, isolated cells from the Malang collection were generated from three different cooking times. The selection of cooking times represents different % cooked beans (see Fig. 1). The higher the % cooked beans, the lower the hardness of the cooked beans (Wainaina et al., 2021). This means that samples at different cooking times and % cooked beans would have different hardness levels. As a result, we could compare, after *in vitro* digestion, the digestion kinetics of the same collection at three different hardness levels.

2.6. In vitro digestion

In vitro digestion was performed in duplicate according to the harmonized INFOGEST protocol with modifications (Brodkorb et al., 2019; Zahir et al., 2018). In the oral phase, 5 g cotyledon cells of Jack beans and kidney bean were added with simulated salivary fluid (SSF) without salivary α-amylase. In the gastric phase, simulated gastric fluid (SGF) was added to the previous mixture and adjusted to pH 3 with HCl. $CaCl_2$ was added to the mixture, and finally porcine pepsin (2000 U/mL enzyme concentration in the final mixture). The tubes were incubated at 37 °C for 2 h. Aliquots were taken at digestion times (t) 0 and 120 min during the gastric phase. Absolute ethanol and Pefabloc (0.1 M) were added immediately into the aliquots to stop the activity of amylase and proteases, respectively. The ratio to sample was 4:1 and 1:20 for absolute ethanol and Pefabloc. At the end of the gastric phase, the pH was adjusted to pH 7 with NaOH to stop the enzymatic reaction. For the intestinal phase, gastric chyme was combined with simulated intestinal fluids (SIF), bile solution, $CaCl_2$, porcine trypsin, and pancreatic enzyme (100 U/mL of trypsin activity and 200 U/mL of amylase activity in the final mixture). The tubes were incubated at 37 °C for 2 h. Aliquots were taken at 15, 30, 60, 120 min during intestinal phase. The t = 120 min for the gastric phase was used as t = 0 for the intestinal phase. Absolute ethanol and Pefabloc were added, as above mentioned. Incubation in all of the phases were performed under mixing conditions using rotatory equipment at 20 rpm. A blank digestion was prepared by using Milli-Q water instead of cotyledon cells and was run under the same digestion condition. All aliquots were centrifuged at 20000g for 10 min, and the ethanolic and pefabloc-added supernatants were collected and stored at −20 °C until use for the digestible starch and digestible protein analysis.

2.6.1. Determination of in vitro protein digestibility

Free amino groups released after *in vitro* digestion were measured using a spectrophotometric assay as described in a previous study (Zahir et al., 2018). The degree of protein hydrolysis (DH%) was calculated using Eq. (2) as follows:

$$DH\% = \frac{NH_{2final} - NH_{2initial}}{NH_{2acid} - NH_{2initial}} \times 100 \quad (2)$$

Where NH_{2final} is the free amino groups content in the hydrolysate of the digested sample, $NH_{2initial}$ is the free amino groups content in the hydrolysate of the undigested sample (at time 0 of gastric phase), NH_{2acid} is the free amino groups content after hydrolysis of sample with 6 N HCl at 110 °C for 24 h.

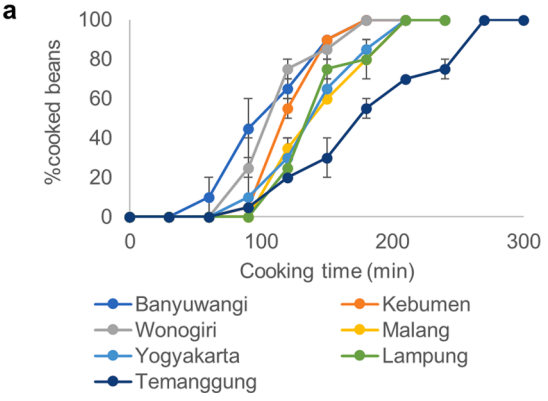
Data from the protein hydrolysis were modelled by using a fractional conversion model (Gwala et al., 2020) from duplicate data (Eq. (3)).

$$Protein_t = Protein_f + \left(Protein_i - Protein_f\right) \times e^{-kt} \quad (3)$$

where $Protein_t$ (%) is the DH% at time t of digestion, $Protein_f$ (%) is the estimated plateau at extended digestion times, and $Protein_i$ (%) is the value of DH% at the beginning of the small intestinal phase. k represents the reaction rate constant (min^{-1}). Residuals and RSS were used to assess the goodness-of-fit of the model.

2.6.2. Determination of in vitro starch digestibility

Ethanolic supernatants were subjected to amyloglucosidase treatment to convert products of amylase hydrolysis into glucose (Rovalino-Córdova et al., 2019). D-glucose assay procedure was performed to quantify the amount of glucose at digestion time (0 and 120 min during



b

Collections	CT_{90} (min)
Banyuwangi	147 ± 5
Kebumen	142 ± 7
Wonogiri	137 ± 5
Malang	189 ± 8
Yogyakarta	182 ± 4
Lampung	173 ± 8
Temanggung	254 ± 6

Fig. 1. Cooking profile (panel a) and CT_{90} (panel b) of the seven Jack bean collections. Data expressed as mean ± standard deviation from two replicates. CT_{90}: estimated time to achieve 90% cooked beans.

gastric phase, 15, 30, 60, and 120 min during intestinal phase) using a protocol of GOPOD FORMAT K-GLUC 09.14 (Megazyme Inc., Bray, Ireland). A conversion factor of 0.9 was used to convert glucose to starch. The starch hydrolysis was presented as grams of hydrolyzed starch/100 g of dry starch.

Data from the starch hydrolysis were modelled by using a logistic model (Eq. (4)) as reported in several studies (Gwala et al., 2020; Pallares Pallares et al., 2018) from duplicate data.

$$Starch_t = \frac{Starch_f}{1 + e^{\left[\frac{4 \times k_{max}}{Starch_f}(\lambda - t) + 2\right]}} \quad (4)$$

where $Starch_t$ (%) is the digested starch at time t, $Starch_f$(%) is the plateau of the digested starch, k_{max} (%starch/min) is the rate constant, and λ (min) is lag phase time. Residuals and RSS were used to assess the goodness-of-fit of the model.

2.7. Microscopy analysis

Intact cells of seven Jack bean collections and kidney bean, as a reference, were visualized using confocal laser scanning microscope (LSM-510, Zeiss). Cell walls were stained with 0.1% calcofluor white, and protein bodies were visualized with 0.0001% rhodamine B. Staining was performed overnight to ensure the complete penetration of dyes on the cell wall and protein, respectively. Microscope setting with 405 nm blue/violet laser for calcofluor white and 543 nm HeNe laser for rhodamine B were applied. All images were taken at objective 20x EC Plan-Neofluar (0.50) and processed with Zen blue software v.2.3 edition (Carl Zeiss, Oberkochen, Germany).

Images were processed using ImageJ (Fiji) v.1.52 Fuji software. All images were converted into 8-bit binary before adjusting the threshold. The "invert" and "fill hole" tools were applied to the images to fill the area of starch granules and enabled the selection of cells along the cell's perimeter. Additionally, the "watershed" tool was used if the cells were clustered. The cell area was selected using the wand tool and was measured. Cell diameter (D_{cell}) was calculated from the cell area, assuming the cells were round. For starch area measurement, we eliminated the cell wall by inactivating the calcofluor white panel and used only the rhodamine B panel, which contained starch granules. The images were converted into 8-bit binary, and the threshold was adjusted. The watershed tool was then used to separate the overlap area of the starch granules. The area of the starch granule was selected and measured. Similar to cell diameter, starch granule diameter (D_{starch}) was calculated assuming the granule was round. When selecting the area of starch granule, the processed image was compared with the raw image to ensure that the selected area conforms to a single starch granule. For each Jack bean collection, 100 measurements of D_{cell} and D_{starch} were collected.

2.8. Particle size distribution of the isolated intact cells samples

Standard percentile (Dv_{10}, Dv_{50}, Dv_{90}) and mean diameter of cells ($D_{3,2}$, $D_{4,3}$) were determined using a Mastersizer 3000 laser diffraction particle size analyzer with a hydro HV dispersion system (Malvern Panalytical Ltd., Malvern, UK). Intact cells were suspended in deionized water (1 g/ml). The particle refractive index was set at 1.46 for legume cells (Edwards et al., 2020). Dispersant refractive index and particle absorption index were set at 1.33, and 0.01, respectively. All measurements were performed within 12–14% obscuration range.

2.9. Statistical analysis

Kinetics parameters were estimated using the Solver add-in in Microsoft Excel by minimizing the residual sum of squares. Analysis of variance was used to compare the nutritional properties and kinetic parameters of protein and starch digestion across collections. Pearson correlations were used to examine the correlations between kinetic parameters of protein and starch digestion and cooking time (CT_{90}), geometrical properties ($D_{4,3}$, D_{cell}, and D_{starch}) and proximate composition as well as between protein and starch digestion. Both the variance and correlation analysis were run using GraphPad Prism 9. The level of significance was set at $p < 0.05$.

3. Results

3.1. Cooking profile, proximate composition, starch, fiber, and concanavalin A of seven Jack bean collections with different cooking times

Fig. 1a shows the cooking profiles of the seven Jack bean collections. It can be seen that the kinetics of the cooking profile follow the logistic model (Supplementary Fig. 1). The Jack bean collections exhibited different optimal cooking times ranging from 137 to 254 min (Fig. 1b).

Table 1 shows the proximate composition, starch, and concanavalin A of the seven Jack bean collections. Jack bean protein content ranged from 28.8 to 39.3% of the dry weight. The highest protein content was found in the Kebumen collection. Carbohydrates ranged from 53.8 to 63.1% of the dry weight, with a starch content ranging from 31 to 41% of the dry weight. The collections contain a significant amount of dietary fiber, with an average of 21.5%. The content of concanavalin A varied between 35 and 51 mg/g dry cotyledon.

3.2. Microstructure characteristics of seven Jack beans collections

An overview of Jack bean and kidney bean cotyledon cells is shown in Fig. 2. Jack bean intact cotyledon cells have an oval shape similar to kidney bean cells. Jack bean has big starch granules embedded in the protein matrix and encapsulated by the cell walls. Mostly single cells and a limited amount of small cluster cells with two or three cells (yellow arrow in Fig. 2) were observed on samples obtained by mashing and sieving with 125–250 μm filter.

Table 2 shows the diameters of cell and starch granules of the particles of size ranging between 125 and 250 μm of the seven Jack bean collections measured by particle analyzer and image analysis. Jack bean collections show different cell diameters as measured by either a particle analyzer ($D_{4,3}$) or calculated from the images (D_{cell}). We observed a lower diameter value based on the microscopy (103–123 μm) compared to the particle analyzer (123–165 μm). For starch granules, similar starch granule diameters (D_{starch}) were observed in the seven Jack bean collections (31–38 μm). It is clear that Jack beans have a bigger starch granule diameter than kidney bean (29 μm).

3.3. In vitro protein and starch digestion kinetics of Jack bean collections with different optimal cooking time

The kinetic parameters of starch and protein digestion are presented in Table 3. A logistic model was used to model the starch digestion in intact cotyledon cells as described in previous reports (Pallares Pallares et al., 2018; Rovalino-Córdova et al., 2019). The model has three kinetic parameters: a lag phase at the initial phase of digestion (λ), rate constant (k_{max}) and digested starch at the final intestinal phase ($Starch_f$). Jack bean starch was digested slowly at the initial phase of digestion, as shown by the λ values, ranging from 14.0 to 34.9 min. The lag phase was followed by a rapid increase in starch digestion and reached final values ranging from 38.1 to 63.9%. Compared to kidney bean, Jack bean collections showed higher starch digestion rate, which resulted in a higher final starch digestion ($Starch_f$). For protein digestibility, we observed differences in initial protein digestion ($Protein_i$), rate constant (k), and final digested protein ($Protein_f$) during the intestinal phase among the seven jack bean collections. We observed a low % digested protein at the beginning of the intestinal phase of digestion, showing a limited ability of pepsin to digest and generate free α-amino groups during the gastric

Table 1
Protein, fat, ash, carbohydrate, starch, fiber, and concanavalin A content of seven Jack bean collections.

Collections	Protein (%db)	Fat (%db)	Ash (%db)	Carbohydrate (%db)	Starch (%db)	Fiber (%db)	Concanavalin A (mg/g dry cotyledon)
Banyuwangi	36.8 ± 0.0^{b}	4.4 ± 0.6^{a}	2.8 ± 0.1^{d}	56.1 ± 0.5^{bc}	32.0 ± 0.7^{c}	24.0 ± 1.3^{a}	51.5 ± 1.3^{a}
Kebumen	39.3 ± 0.1^{a}	3.5 ± 0.0^{a}	3.4 ± 0.1^{b}	53.8 ± 0.2^{c}	31.0 ± 1.4^{c}	22.7 ± 0.4^{ab}	43.0 ± 4.6^{ab}
Wonogiri	28.8 ± 0.3^{e}	4.9 ± 1.1^{a}	3.3 ± 0.1^{bc}	63.1 ± 1.2^{a}	38.5 ± 1.0^{ab}	24.6 ± 2.2^{a}	45.1 ± 4.9^{ab}
Malang	36.5 ± 0.2^{bc}	4.4 ± 0.6^{a}	3.0 ± 0.1^{cd}	56.1 ± 0.9^{bc}	39.3 ± 1.9^{a}	16.8 ± 2.9^{bc}	43.5 ± 3.5^{ab}
Yogyakarta	35.6 ± 0.1^{d}	3.3 ± 0.1^{a}	3.8 ± 0.1^{a}	57.2 ± 0.1^{b}	33.0 ± 0.9^{c}	24.2 ± 0.7^{a}	48.9 ± 4.5^{a}
Lampung	35.8 ± 0.1^{cd}	4.0 ± 0.0^{a}	2.7 ± 0.1^{d}	57.5 ± 0.2^{b}	34.7 ± 0.2^{bc}	22.8 ± 0.1^{ab}	44.9 ± 0.9^{ab}
Temanggung	35.6 ± 0.4^{d}	4.4 ± 0.3^{a}	3.8 ± 0.1^{a}	56.3 ± 0.6^{bc}	40.9 ± 0.8^{a}	15.4 ± 1.4^{c}	35.8 ± 2.9^{b}

Different superscript letters within the same column indicate significant differences ($p < 0.05$). dry basis (db). Data expressed as mean ± standard deviation from two replicates for protein, fat, ash, carbohydrate, starch, and fiber and three replicates for concanavalin A.

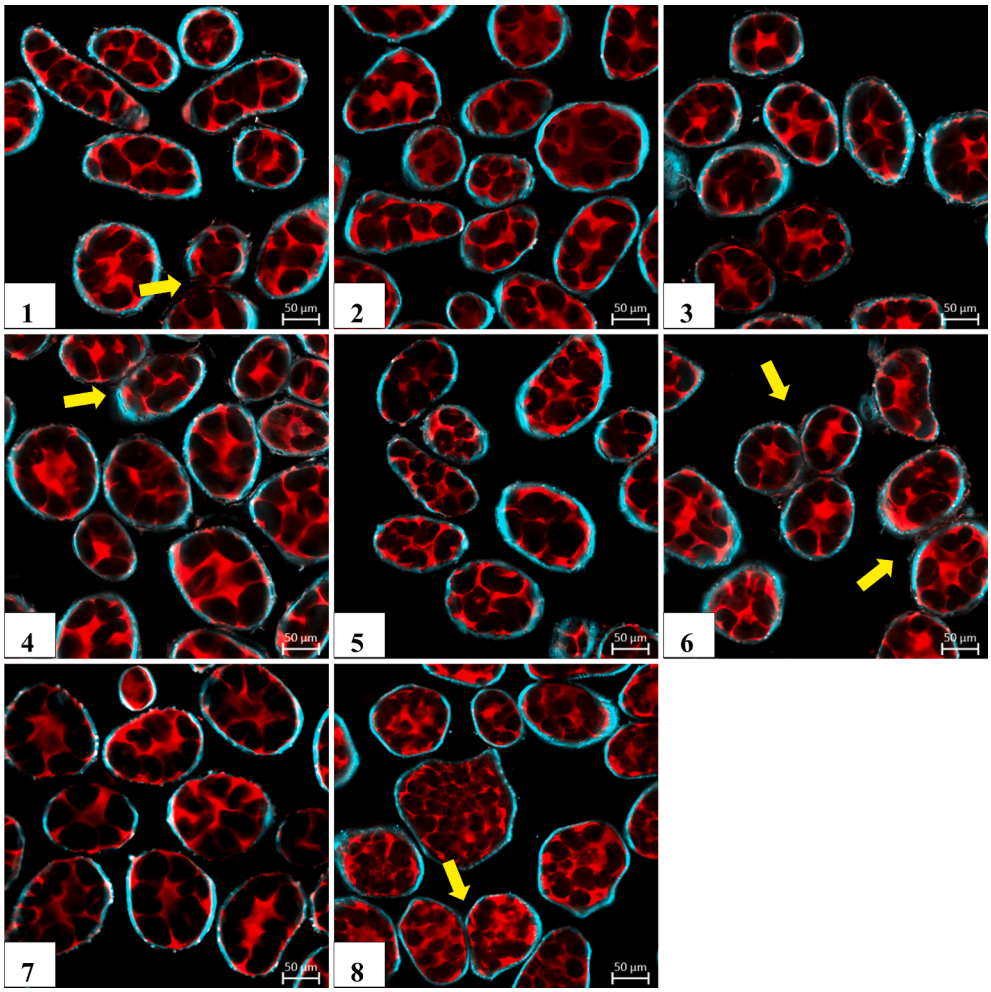


Fig. 2. Representative confocal laser microscopy images of cooked Jack bean (1–7) and Kidney bean cells (8). Proteins are displayed in red, cell walls are displayed in light blue. Banyuwangi (1), Kebumen (2), Wonogiri (3), Malang (4), Yogyakarta (5), Lampung (6), Temanggung (7), Kidney bean (8). Yellow arrows indicated cluster of cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

phase. During intestinal digestion, the amount of digested protein increased, and Jack bean showed a % digested protein ranging from 21.6 to 30.8% at the end of the intestinal phase. In general, the final % digested protein in Jack beans was, on average (25.2%), slightly lower than kidney bean (31%).

Pearson correlation coefficients between kinetic parameters of starch and protein digestibility and CT_{90} are summarized in Table 4. No significant correlation was found between CT_{90} and any of the kinetic parameters of starch and protein digestibility, meaning that the effect of optimal cooking times on starch and protein digestion is limited. Moreover, we examined the correlation between kinetic parameters of protein and starch digestion and diameter of cells, diameter of starch granules, and proximate composition (Table 4). The results showed a limited effect of the cells and starch granules size on starch and protein digestibility, perhaps due to the narrow variability of the geometrical properties of the seven Jack bean collections used in this study. We did find a significant positive correlation between carbohydrate content and starch digestion rate constant. A negative correlation at $p < 0.1$ was observed between protein content and starch digestion rate constant (k_{max}) ($p = 0.057$) as well as final digested protein ($Protein_f$) ($p = 0.062$).

Table 2
Standard percentile (Dv_{10}, Dv_{50}, Dv_{90}) and mean diameter of cell ($D_{3,2}$, $D_{4,3}$, D_{Cell}) and starch granule (D_{starch}) of seven Jack bean collections and one kidney bean collection.

Collections	Particle size analysis					Image analysis	
	Dv_{10} (μm)	Dv_{50} (μm)	Dv_{90} (μm)	$D_{3,2}$ (μm)	$D_{4,3}$ (μm)	D_{cell} (μm)	D_{starch} (μm)
Banyuwangi	92^{d}	138^{d}	209^{d}	132^{d}	146^{d}	112 ± 18^{b}	33 ± 7^{bc}
Kebumen	79^{f}	119^{f}	177^{f}	94^{g}	124^{f}	113 ± 20^{b}	32 ± 8^{cd}
Wonogiri	90^{e}	138^{d}	211^{d}	131^{e}	145^{d}	103 ± 19^{c}	32 ± 7^{cd}
Malang	101^{b}	156^{a}	241^{a}	148^{a}	165^{a}	109 ± 19^{bc}	38 ± 7^{a}
Yogyakarta	80^{f}	124^{e}	190^{e}	103^{f}	130^{e}	107 ± 17^{bc}	31 ± 7^{cd}
Lampung	102^{ab}	154^{ab}	234^{b}	147^{ab}	162^{b}	110 ± 20^{bc}	33 ± 6^{bc}
Temanggung	103^{a}	153^{b}	231^{b}	147^{b}	161^{b}	123 ± 21^{a}	36 ± 8^{ab}
Kidney bean	97^{c}	147^{c}	226^{c}	140^{c}	155^{c}	112 ± 22^{b}	29 ± 6^{d}

Values with different superscript letters in the same column are significantly different ($p < 0.05$). Dv_{10}: the size of particle below which 10% of the particles are found, Dv_{50}: median of the volume distribution, Dv_{90}: the size of particle below which 90% of the particles are found, $D_{3,2}$: the surface weighted mean, $D_{4,3}$: the volume weighted mean, D_{cell}: diameter of cell measured by image analysis, D_{starch}: diameter of starch granule measured by image analysis.

Table 3
Kinetic parameters of *in vitro* protein and starch digestion of cells isolated from seven Jack bean collections and one kidney bean collection.

Collections	Protein digestibility				Starch digestibility			
	$Protein_i$(%)	k(protein%/min)	$Protein_f$(%)	RSS	λ(min)	k_{max}(starch%/min)	$Starch_f$(%)	RSS
Banyuwangi	2.1 ± 0.5^{b}	0.036 ± 0.002^{ab}	24.4 ± 0.5^{b}	3.1	17.1 ± 2.1^{cd}	1.4 ± 0.2^{a}	40.2 ± 1.0^{d}	22.4
Kebumen	1.8 ± 1.5^{b}	0.044 ± 0.009^{a}	24.6 ± 1.4^{b}	32.9	16.9 ± 2.7^{cd}	0.7 ± 0.1^{b}	38.1 ± 1.4^{d}	25.3
Wonogiri	2.3 ± 0.9^{ab}	0.022 ± 0.004^{bc}	26.8 ± 1.6^{ab}	13.5	14.0 ± 1.9^{d}	1.5 ± 0.2^{a}	57.2 ± 1.5^{b}	36.5
Malang	0.8 ± 0.6^{a}	0.017 ± 0.002^{c}	30.8 ± 1.7^{a}	6.4	22.5 ± 2.2^{bcd}	1.1 ± 0.1^{ab}	57.6 ± 1.5^{b}	26.3
Yogyakarta	1.2 ± 0.4^{b}	0.020 ± 0.002^{bc}	21.6 ± 0.8^{b}	2.4	19.4 ± 1.5^{bcd}	1.2 ± 0.1^{ab}	63.9 ± 1.2^{a}	17.8
Lampung	1.1 ± 0.6^{b}	0.015 ± 0.003^{c}	22.4 ± 1.9^{b}	5.9	34.9 ± 5.8^{a}	1.2 ± 0.3^{ab}	50.3 ± 2.6^{c}	92.4
Temanggung	2.5 ± 0.8^{ab}	0.035 ± 0.004^{ab}	25.9 ± 0.9^{ab}	9.3	29.1 ± 2.7^{ab}	1.0 ± 0.1^{ab}	56.6 ± 1.5^{b}	26.7
Kidney bean	1.0 ± 0.4^{a}	0.015 ± 0.001^{c}	31.0 ± 1.3^{a}	2.6	26.2 ± 1.3^{abc}	0.7 ± 0.0^{b}	39.2 ± 0.5^{d}	3.3

Protein and starch digestibility from two replicates data are fitted to fractional conversion model and logistic model, respectively. $Protein_i$: digested protein at the beginning of the intestinal phase; k: protein digestion rate constant; $Protein_f$: digested protein at end of the intestinal phase; λ: digested starch at beginning of the intestinal phase; k_{max}: starch digestion rate constant; $Starch_f$: digested starch at the end of the intestinal phase. Values with different superscript letters in the same column indicate significant differences ($p < 0.05$).

Table 4
Correlation matrix of starch/protein digestibility and cooking time (CT_{90}), geometrical properties ($D_{4,3}$, D_{Cell}, D_{starch}) protein, fat, ash, carbohydrate, starch, fiber, and concanavalin A.

Kinetic parameters	CT_{90}	$D_{4,3}$	D_{cell}	D_{starch}	Protein	Fat	Ash	Carbohydrate			Concanavalin A
									Starch	Fiber	
λ	0.61	0.67	0.44	0.37	0.22	−0.08	−0.16	−0.21	0.30	−0.47	−0.45
k_{max}	−0.28	0.25	−0.58	−0.21	−0.74	0.61	−0.35	0.78*	0.17	0.42	0.54
$Starch_f$	0.45	0.30	−0.24	0.11	−0.52	0.07	0.51	0.49	0.61	−0.25	−0.18
$Protein_i$	0.05	−0.15	0.39	−0.26	−0.34	0.42	0.27	0.24	0.17	0.01	−0.28
k	−0.04	−0.49	0.59	−0.14	0.44	−0.15	0.21	−0.51	−0.35	−0.04	−0.18
$Protein_f$	−0.26	0.23	−0.38	0.21	−0.73	0.76*	−0.04	0.68	0.59	−0.09	−0.17

Asterisk symbol indicates a significant correlation $p < 0.05$ (2-tailed).

3.4. Effect of different cooking time on protein and starch digestion in one Jack bean collection

Starch and protein digestibility of the Malang collection cooked at different times are presented in Fig. 3. The processing intensity clearly showed an influence on starch digestion. We found a significant increase in starch digestibility by prolonging the cooking time (Supplementary Table 1). On the contrary, a limited effect of cooking time was observed on protein digestibility. We can observe that increasing processing intensity reduced the initial protein digestion from 1.35 to 0.78%, with no significant effect on the rate constant and the final digested protein (Supplementary Table 1).

4. Discussion

This study reports the variability of nutritional parameters of seven Jack bean collections having different optimal cooking times (Fig. 1). The Jack bean collections have a protein content ranging from 28.8 to 39.3% (Table 1), showing the nutritional potential of Jack bean as a source of proteins. In other studies, the protein content in whole Jack bean was reported to be 26.8% (Agbede & Aletor, 2005) and 31.9% (Vadivel & Janardhanan, 2001). The difference in the protein content is probably due to the beans analyzed here were dehulled prior to analysis. The Jack bean hull contains three times less protein than in the cotyledon (Oliveira et al., 1999). Concanavalin A content in our collections ranged from 35 to 51 mg/g dry cotyledon. This value is higher than previously reported (15–35 mg/g dry weight) (Sridhar & Seena, 2006). Once cooked, the coagulation activity can be partially lost due to denaturation (Udedibie & Carlini, 1998). We found a lower amount of concanavalin A in optimally cooked Malang collection (9.5 mg concanavalin A/g dry cotyledon) than raw bean (43.5 mg concanavalin A/g dry cotyledon), which suggests that the lectin was modified during the heat treatment process. Longer cooking times may be beneficial for removing all hemagglutination activity, and the activity of this residual

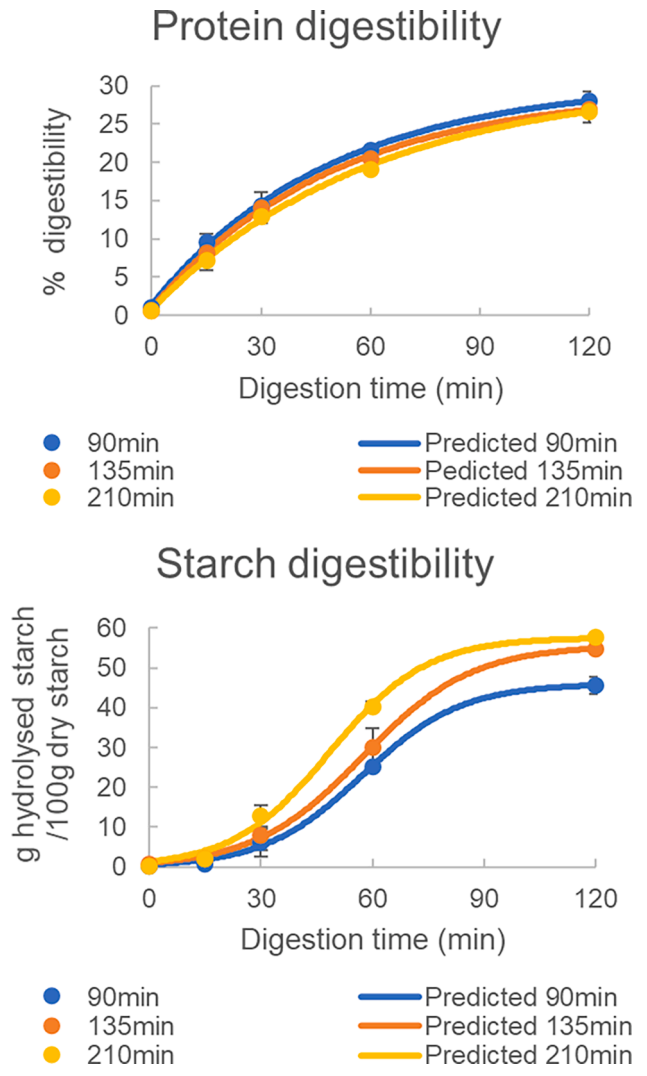


Fig. 3. Protein and starch digestibility of isolated intact cells of Jack bean cooked at different times. Malang collection was used to generate intact cells.

concanavalin A should be considered when strategies to reduce the cooking time of Jack beans are implemented.

We show that image processing can be useful as an alternative method to measure microstructure characteristics of plant material, especially when comparing samples. This is the first time that this approach is used and the first time that cell size of Jack beans is determined. However, we found that the cell size measured by image analysis is lower than the cell size measured by particle analyzer (either $D_{4,3}$ and $D_{3,2}$ values) (Table 2; Supplementary Fig. 2). This can be explained by (1) cell clusters that may have been measured by particle analyzer and not by image analysis; (2) the fact that with the 2D images, we cannot always measure the diameter of the cells in correspondence to the center of the cells. Compared to the particle analyzer, image analysis offers the flexibility to select and analyze more than one parameter of interest in one-time preparation while at the same time being able to differentiate single cells over cell clusters. This approach should be further optimized, for instance, by evaluating the sensitivity of other microstructure parameters (such as Feret's diameter or perimeter) or using 3D scanning images to locate and measure only the center of the cells.

The starch granule size of kidney beans determined by image analysis (Table 2) falls in the range of the starch granule size determined by laser diffraction in another research (Romero & Zhang, 2019). The authors reported that the size distribution and mean diameter ($D_{4,3}$) of kidney bean starch granules measured by particle analyzer are 10–55 μm and 27 μm, respectively. In our study, the mean diameter of kidney bean was 29 μm. The cell size of kidney bean in this study (112 μm) is slightly bigger than the value tentatively estimated in a previous study (100 μm) (Rovalino-Córdova et al., 2018), which can be due to the different sieve range used in our study.

To study the *in vitro* starch and protein digestibility, we used isolated intact cells for three reasons: (1) to appreciate the barrier effect of the cell wall that would have been lost if flour was used, (2) to uniform the legume particle size since it has been shown to affect macronutrients digestibility in intact plant materials (Zahir et al., 2018), and (3) intact cell is the most important microstructural fractions to represent the digestive behavior of the whole seeds cooked at optimal cooking times (Duijsens et al., 2023). Overall protein digestibility varied across collections, as seen from all kinetic parameters (Table 3). The DH% at the beginning of the intestinal phase ($Protein_i$) suggests that the pepsin can penetrate the isolated cells of Jack beans and kidney bean during the gastric phase. At the end of the intestinal phase, the digested protein ($Protein_f$) ranged from 21.6 to 30.8% showing that Jack bean collections had lower protein digestibility than kidney bean, which may affect the overall quality of Jack bean compared to kidney bean. We found no correlation between optimal cooking times and all protein digestibility kinetic parameters (Table 4). This implies that optimal cooking times from the seven Jack bean collections are not predictive of *in vitro* protein digestibility. Cooking time is mainly correlated with the progressive solubilization of pectin in the middle lamella (Chigwedere et al., 2018). When legumes are cooked at the optimal cooking time, the level of pectin solubilization is perhaps comparable, as well as the barrier effect exerted by the cell wall, and the trypsin can easily diffuse into the cells (Zahir et al., 2020). It has been reported that processing may induce protein aggregation, thus reducing protein digestibility (Miraji et al., 2021). Therefore, it is likely that different levels of aggregation may also contribute to the differences in protein digestibility across collections.

Starch digestibility of Jack bean collections followed a logistic model, likely due to the accumulation of amylase in the cells as mechanistically modelled in kidney bean (Rovalino-Córdova et al., 2021). In general, all the kinetic parameters of starch digestion varied between Jack bean collections (Table 3). The slow progression of starch digestion at the initial phase (λ) reflects barriers to enzyme diffusivity due to cell wall integrity which were shown by previous reports (Pallares Pallares et al., 2018; Rovalino-Córdova et al., 2021). Jack bean collections (except Kebumen) demonstrated a higher rate constant (k_{max}) than kidney bean, resulting in a greater extent of starch digestion ($Starch_f$). We found no correlation between cooking time and any of the kinetic parameters of starch digestion (Table 4). Similar to protein digestion, optimal cooking time is not predictive of *in vitro* starch digestion in Jack beans. The differences in starch digestibility suggest that cell wall permeability is not the only factor hindering amylase diffusion. Other mechanisms may influence amylase diffusivity, such as the role of cellulose in the cell wall in binding amylase (Dhital et al., 2015) and the role of densely packed protein matrix in the cell cytoplasm (Dhital et al., 2016; Rovalino-Córdova et al., 2019).

It was reported that the protein matrix can act as an additional barrier to amylase diffusion to starch (Do et al., 2023; Rovalino-Córdova et al., 2019). We did not find any correlation between kinetic parameters of protein digestibility with starch digestibility in Jack beans (Supplementary Table 2), but we observed a negative correlation between protein content and starch digestion rate constant ($p = 0.057$) (Table 4). At the optimal cooking time, when the cell wall permeability is likely similar, the protein matrix surrounding the starch granules may have a major role in starch digestion, i.e. the higher the protein content, the slower starch digestion.

The protein/starch digestibility has no correlation with the diameter of cells and the diameter of starch granules. In this study, we isolated the cells within a narrow range (125–250 μm) that produced a small cell size variability, with a ratio between the largest and the smallest cell of

around 1.2 (Table 2). Despite this difference is magnified when the whole surface area is considered, this is still too small to produce differences in enzyme diffusion within the cells and this possibly explains the limited correlation we found in this study. An influence of particle size is reported within fractions with a larger size range (71–125 μm to 1000–2000 μm), i.e. an inverse relationship between particle size and protein digestibility (Zahir et al., 2018).

Our findings suggested that protein and starch digestibility is not correlated to optimal cooking time when different collections were used, possibly due to the variability in macronutrients that may have larger impact than cooking times on the digestibility. In order to limit the variability, we selected one collection to confirm the effect of cooking times on protein/starch digestibility in Jack bean. Using only one collection, we can expect different cell wall permeability due to a different degree of pectin solubilization. We selected Malang collection for this investigation. The results showed that increasing cooking time in one collection would increase starch digestibility but not protein digestibility (Fig. 3; Supplementary Table 1). It can be assumed that increasing cooking time would increase the permeability of cell walls to digestive enzymes as reported in previous studies (Bhattarai et al., 2018; Pallares Pallares et al., 2018; Zahir et al., 2018). The effect of the increased cell wall permeability may be more pronounced for amylase (51–54 kDa) than trypsin (23.4 kDa). The digestive enzyme with a smaller size can diffuse more quickly than the bigger ones. In soybean cells, trypsin has been shown to easily diffuse inside the cells (Zahir et al., 2020). Another study mentioned a higher affinity of amylase towards non-specific cell wall binding than proteases (Bhattarai et al., 2017). The difference in size or relative characteristics of digestive enzymes possibly explains the effect on starch digestion ($Starch_f$) and the existence of a lag phase (λ) for starch digestion that is not evident for protein digestion (Fig. 3; Supplementary Table 1). It has been reported that starch gelatinization and protein denaturation occurred during 30 min of boiling treatment in short and longer-to-cook beans (Chigwedere et al., 2018). Another study mentioned that a complete gelatinization had occurred on Canadian wonder beans (*Phaseolus vulgaris*) cooked at 95 °C for 30 min, showing no residual gelatinization enthalpy compared to a longer processing time (180 min) (Pallares Pallares et al., 2018). Therefore, in the present study, we can assume that the starch would be fully gelatinized even after the shortest cooking time (90 min). The increase in starch digestibility with cooking time is in agreement with a previous study for Bambara groundnut (Gwala et al., 2020). In contrast, prolonged cooking times have no effect on the final starch digestion for lentil (Duijsens et al., 2023) and black bean (Pälchen et al., 2022). In this study, the lack of effect on protein digestibility was found, similar to earlier report on other *Canavalia* species (Torres et al., 2016). Longer cooking times may enable more protein unfolding, making the protein more prone to enzymatic attack. On the other hand, prolonged cooking times may produce protein aggregation, which is known to decrease protein digestibility (Zahir et al., 2021). It could be possible that the heating time utilized in our study (90 to 210 min) generated more protein aggregation, therefore counteracting the effect of an increase cell wall permeability to trypsin (Gwala et al., 2020).

5. Conclusion

In this study, we have characterized the proximate composition of seven Jack bean collections. The collections can be a potential source of protein with a valuable amount of fiber. We found that optimal cooking time cannot be used as the only factor to predict starch and protein digestibility in Jack beans. However, increasing cooking time for a single collection would increase starch digestion but not protein digestibility. Furthermore, the current study demonstrated for the first time quantification of cells and starch granules size in Jack bean using image analysis. Image analysis has the advantage of excluding cell clusters, thus improving the accuracy of cell size quantification. Reliable results can be obtained for starch granules without the need for starch isolation as compared to the particle distribution analyzer. The findings of this study suggest that strategies for reducing cooking time in Jack bean will be advantageous for lowering starch digestibility without impairing protein digestibility.

CRediT authorship contribution statement

Fiametta Ayu Purwandari: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Christien Westerbos:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Keumwoo Lee:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Vincenzo Fogliano:** Conceptualization, Writing – review & editing, Visualization, Supervision, Project administration. **Edoardo Capuano:** Conceptualization, Methodology, Visualization, Supervision, Writing – review & editing, Project administration, Resources.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112956.

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