In vitro macronutrient digestibility and mineral bioaccessibility of lentil-based pasta: the influence of cellular intactness

Duijsens, D.[a*], Alfie Castillo A. I.[a], Verkempinck, S.H.E.[a] Pälchen K[a], Hendrickx, M.E.[a], Grauwet, T.[a]**

[a] Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LFoRCe), Department of Microbial and Molecular Systems (M2S), KU Leuven, Kasteelpark Arenberg 22, PB 2457, 3001, Leuven, Belgium

Author's email addresses:

Duijsens, D.: dorine.duijsens@kuleuven.be

Alfie Castillo A.I.: aialfie1@gmail.com

Verkempinck, S.H.E.: sarah.verkempinck@kuleuven.be

Pälchen, K.: katharinapaelchen@outlook.de

Hendrickx, M.E.: marceg.hendrickx@kuleuven.be

Grauwet, T.: tara.grauwet@kuleuven.be

Journal: Food Chemistry

* author to whom correspondence should be addressed during submission process:

dorine.duijsens@kuleuven.be

+32 32 16 33 03 20

** author to whom correspondence should be addressed post-publication:

tara.grauwet@kuleuven.be

+32 16 32 19 47

Abstract

Recently, pulse ingredients with (partial) cellular intactness are put forward as promising innovative food ingredients with slowed macronutrient digestibility. This study compared cooking quality and nutrient (starch, protein, and mineral) digestibility/bioaccessibility of lentil-based pasta prepared from 100% raw-milled flour, and by substituting 30% of the formulation by isolated cotyledon cell powder or whole precooked powder. Formulation had little effect on cooking properties. Both amylolysis and proteolysis were significantly slowed by incorporating cellular ingredients: towards the end of simulated digestion, amylolysis was lowered by 16-25%, while differences in proteolysis became small. Cellular ingredient incorporation slightly decreased Zn and Mg but did not affect Ca and Fe bioaccessibility, overall yielding a low mineral bioaccessibility comparable to cooked whole pulses. To conclude, lentil-based pasta substituted with cellular ingredients showed improved nutritional properties (*i.e.*, high in digestible protein and slowed amylolysis), with perspectives for the development of different innovative foods with targeted nutritional properties.

Key words

Lentil noodles; nutrient bioencapsulation; INFOGEST; amylolysis; proteolysis; microstructure

List of abbreviations

DM	Dry matter
WM	Wet matter
RM	Raw-milled lentil flour
ICC	Isolated cotyledon cell powder

46	WL	Whole precooked lentil flour
47	100%RM	Pasta prepared from 100% raw-milled flour
48	30%ICC	Pasta prepared from 70% raw-milled flour and 30% ICC powder
49	30%WL	Pasta prepared from 70% raw-milled flour and 30% WL powder
50	PSD	Particle size distribution
51	DSC	Differential scanning calorimetry
52	SSF	Simulated salivary fluid
53	SGF	Simulated gastric fluid
54	SIF	Simulated intestinal fluid
55	ADR	Average daily requirement

1 Introduction

A shift towards more plant-based diets seems inevitable for feeding the rising human population healthily and sustainably. Pulses are increasingly put forward as part of these diets as they are rich in (slowly) digestible starch, protein, fiber, minerals, and bioactive compounds (*e.g.*, phytates and polyphenols). While pulses are generally rich in minerals, the bioaccessibility and absorption of minerals are mostly low due to the presence of antinutrient chelators *e.g.*, phytic acid, pectin, and polyphenols (Duijsens et al., 2021). As pulses could be an important protein source in healthy and sustainable diets, their contribution to human mineral supply is important. Minerals play a crucial role in sustaining human health, with 1.5 to 2.0 billion people in all regions of the world suffering from one or multiple chronic mineral deficiencies (Galani, Orfila, & Gong, 2022). Poor bioaccessibility and bioavailability (often the case in plant-based foods) is considered an important cause of mineral deficiency, next to insufficient dietary intake and physiological condition of the consumer (e.g., illness) (Platel & Srinivasan, 2016).

While pulses are a staple in several parts of the world, they have become underused in most of the western world due to sensory issues, their inconvenient long cooking times, and a lack of knowledge on how to prepare them (Henn et al., 2021). Incorporating pulse-based ingredients into staples may be a useful strategy to increase pulse consumption without drastically altering consumer dietary habits (Duijsens et al., 2021). In this regard, pasta and noodles are easy-to-prepare staples, highly consumed in several regions of the world. Alternative flour sources for pasta preparation (*e.g.*, pulses) are being explored with the aim of improving nutritional properties, such as more complete amino acid profiles and reduced starch digestibility (Garcia-Valle, Bello-Pérez, Agama-Acevedo, & Alvarez-Ramirez, 2021). Purely pulse-based pasta showed important nutritional potential compared to wheat-based pasta, *e.g.*, due to higher protein (up to around 30%

versus 13-14%, respectively) and fiber (around 7% *versus* 2%, respectively) contents (Laleg, Barron, Santé-Lhoutellier, Walrand, & Micard, 2016; Rosa-Sibakov et al., 2016), while exhibiting similar starch digestibilities (Petitot, Barron, Morel, & Micard, 2010; Rosa-Sibakov et al., 2016). Moreover, pulse-based pasta showed improved cooking quality (cooking loss and texture) and nutritional properties (high protein, fiber, and resistant starch) compared to gluten-free cereal pasta (Laleg, Cassan, Barron, Prabhasankar, & Micard, 2016). Interestingly, in 100% pulse-based pasta, starch is enveloped in a thick but weak protein network, formed by electrostatic forces and hydrogen bounds which mainly strengthen upon cooking (Laleg, Barron, et al., 2016).

In pulses, macronutrients are entrapped or bioencapsulated by a cell wall and intracellular (protein) matrix. Upon cooking, the gradual solubilization of pectin in the middle lamella causes the prominent tissue failure mode to shift from cell breakage to separation. As a consequence of cellular nutrient encapsulation, during digestion of cooked pulses, enzyme diffusion towards the substrate is hindered and the hydrolysis of entrapped starch and protein retarded (Duijsens et al., 2021; Gwala et al., 2019; Pälchen, Van Den Wouwer, et al., 2022; Zahir, Fogliano, & Capuano, 2018). In contrast, pulse flours used in commercial applications such as pasta are manufactured by raw milling, mostly disrupting the microstructural complexity, rendering nutrients readily accessible to digestive enzymes. To exploit the inherent potential of the characteristic pulse microstructure, powders rich in intact cotyledon cells were produced from cooked pulses and incorporated into several foods with reduced *in vitro* and *in vivo* rates of amylolysis (Bajka et al., 2021). In this context, chickpea purees made up of isolated cotyledon cell powder showed a significantly increased satiety *in vivo*, compared to isocaloric chickpea puree without nutrient bioencapsulation (Pälchen, Bredie, et al., 2022).

Recently, the authors of this work studied the effect of manufacturing conditions on the digestion kinetics of lentil powders with (partial) cellular intactness, *i.e.*, isolated cotyledon cell powder (ICC) and whole precooked lentil powder (WL) (Duijsens, Pälchen, De Coster, et al., 2022). However, knowledge on the technofunctional and digestion properties of these innovative powders upon incorporation into highly structured foods such as pasta was still missing. Therefore, in this follow-up work, the effect of partially (30%) incorporating powders with cellular intactness into a purely lentil-based pasta formulation was studied on the pasta cooking properties. Moreover, the effect on *in vitro* amylolysis and proteolysis kinetics of these pastas was evaluated. To assess the potential of pulse-based pasta as a mineral supplier, magnesium, calcium, iron, and zinc bioaccessibility values were studied as examples of major and trace minerals.

2 Materials and Methods

2.1 Materials

Green (Dupuy) lentils (*L. culinaris*) were grown and harvested in Canada (August 2019) and donated by Casibeans (Melsele, Belgium). The material (<10% moisture) was sorted and cleaned from foreign material, followed by storage below the glass transition temperature to ensure stability (-40 °C). Chemical reagents and enzymes were purchased from Merck KGaA (Germany), except for the Total Starch Kit (Megazyme, Bray, Ireland), KCl, $MgCl_2(H_2O)_6$, NaOH, sodium potassium tartrate (Acros Organics, Geel, Belgium), $NaHCO_3$, NaCl, KH_2PO_4 (VWR, Leuven, Belgium).

2.2 Lentil ingredient preparation

Lentil flours with different microstructures were prepared as previously reported (Duijsens, Pälchen, De Coster, et al., 2022). Briefly, raw lentils were milled to pass through a 500 µm sieve mesh (Cyclotec1093 sample mill, FOSS, Sweden) (*raw-milled flour*). Additionally, lentils were

soaked (1:10 w/v, 25 °C, 16 h), cooked (1:10 w/v, 95 °C, 15 min), mixed with demineralized water (1:1 w/v), and homogenized (2 min, 3000 rpm) (IKA® T25 Ultra-Turrax, Janke and Kunkel, Germany). From these cooked lentils, two distinct powders containing structurally intact cells were prepared. Firstly, *isolated cotyledon cell flour (ICC)* was prepared by isolating cotyledon cells from the slurry by wet-sieving (amplitude 2.5 mm, 4 min) (AS200, Retsch, Germany), and lyophilizing the individual cotyledon cell fraction (40-125 µm) (Alpha 1-4 LSCplus, Martin Christ, Germany). Secondly, *whole precooked lentil flour (WL) was* prepared by lyophilizing the cooked lentil slurry as such. The freeze-dried powder was sieved (500 µm mesh), after which the (seed coat) fraction >500 µm was blended (IKA Labortechnik-A10, Germany), re-sieved, and mixed again with the other fraction (<500 µm) to obtain a homogenous powder.

2.3 Lentil pasta preparation

To produce pasta, free starch and protein are necessary in the flour to form a continuous phase (Laleg, Cassan, et al., 2016). Therefore, a ratio of substitution with the (partially) cellular ingredients was chosen at which it was hypothesized that *(i)* there would be a limited effect on the possibility to form a continuous phase, a dough with an acceptable consistency, and subsequently a pasta with acceptable cooking properties (meaning sufficient free starch and protein were present), and *(ii)* enough intact cellular material would be present to significantly affect starch digestion kinetics. Based on these requirements and previous results for partial substitution of wheat-based food formulations by cellular ingredients (Bajka et al., 2021), a ratio of 70:30 (w/w, wet basis) raw-milled flour with ICC and WL powders was selected for this work. It was hypothesized that, applying this ratio, the formed network, mainly consisting of free proteins, would entrap free starch granules, as well as cotyledon cells (provided by ICC and WL), finally delivering a pasta with acceptable cooking properties but significantly slowed starch digestion.

From here on, pasta samples made from pure raw-milled flour, 30% ICC powder, and 30% WL powder will be referred to as 100%RM, 30%ICC, and 30%WL, respectively.

2.3.1 Dough preparation

The moisture required for dough preparation was determined for each flour mixture using a farinograph-E (Brabender, Germany) with a 50 g mixing bowl (Sigma mixer S 50, Brabender, Germany). Briefly, 43 g of flour (dry matter) was weighed into a temperature-controlled mixer with continuously rotating blades (63 rpm). Upon water addition, the resistance of the formed dough to mixing was measured. Different moisture additions were tested iteratively, targeting a standard dough consistency of 500 B.U. (Patel & Chakrabarti-Bell, 2013). For each pasta, 300 g of flour (mixture) was kneaded (10 min, 100 rpm) in the mixing bowl of a Thermomix TM6 mixer (Vorwerk, Germany). The predetermined amount of water (reported in **Table 1**) was gradually added during the first seconds of kneading. The obtained dough was rested in a plastic bag at 4 °C for 30 min.

2.3.2 Pasta preparation

Pasta preparation was based on the procedures of Gallegos-Infante et al. (2010) and Giuberti, Gallo, Cerioli, Fortunati, & Masoero (2015). The kneaded dough was divided into small balls (approximately 30 g). Each dough ball was sheeted through the rollers of a manual pasta machine (Atlas 150, Marcato, Italy), twice per setting (0, 1, and 2 of 4.8, 3.8, and 3.3 mm, respectively). The laminated dough was cut into strips using a spaghetti chitarra attachment (Atlas 50, Marcato, Italy) (diameter 2 mm, length approximately 10 cm). The pasta was dried on trays using natural air convection (16 h, 25 °C) and then stored in glass jars in a dry and dark environment until utilization.

2.3.3 Determination of cooking properties and cooking quality

Optimal cooking time (OCT). For each pasta type, the optimal cooking time was determined following the approved 66-50 method (AACC 2000) (Laleg, Cassan, et al., 2016; Petitot & Micard, 2010). Briefly, the minimum cooking time was determined as the cooking time at which the starchy core disappeared (qualitatively) upon squeezing between two glass plates. Then, the OCT was determined as the minimal cooking time plus one minute (**Table 1**). In view of the evaluation of starch digestibility, DSC analysis confirmed complete starch gelatinization upon cooking for the OCT (*data not shown).*

Cooking loss. For each pasta type, a duplicate analysis of cooking loss was carried out by cooking 15 g pasta in 250 mL demineralized water. Cooking losses were calculated using Eq.1 (Laleg, Barron, et al., 2016). Additionally, starch and protein losses upon cooking were determined, considering the respective content in the cooked and uncooked pasta samples.

$$Cooking\ loss\ (\%, dm) = \frac{cooked\ pasta(g,\ DM) - uncooked\ pasta\ (g,\ DM)}{uncooked\ pasta\ (g,\ DM)} \times 100\% \qquad \text{Eq.1}$$

Water uptake. The water uptake was determined gravimetrically, upon cooking pasta in duplicate as stated above, according to Eq.2.

$$Water\ uptake\ (\%) = \frac{cooked\ pasta\ (g,\ WM) - uncooked\ pasta(g,\ WM)}{uncooked\ pasta\ (g,\ WM)} \qquad \text{Eq.2}$$

2.4 Characterization of produced spaghetti

2.4.1 Microscopy

Cooked pasta samples were analyzed using an Olympus BX-51 light microscope (Olympus, Optical Co. Ltd, Tokyo, Japan) with 10x objectives and cellSense Standard® software. A thin slice

(about 1 mm) of pasta was squeezed between a microscope slide and a cover glass to visualize the cooked samples.

2.4.2 Proximate analysis

The composition of each produced (uncooked and cooked) pasta was determined. Starch content was determined in duplicate (total starch kit, AA/AMG, Megazyme Inc. Bray, Ireland). Nitrogen content was analyzed in duplicate by automated Dumas analysis (CE instrument, Thermofischer Scientific, Walthom, USA), applying a 5.4 conversion factor to calculate the crude protein content (De Almeida Costa, Da Silva Queiroz-Monici, Pissini Machado Reis, & De Oliveira, 2006). The moisture content was determined in duplicate as the mass difference upon oven drying (Jouan, France) (100 °C, 24 h). The lipid content was determined according to Janssen et al. (2018), in triplicate. The ash content was determined in triplicate as the weight loss upon complete incineration (muffle furnace, 550 °C, 20 h). Hereafter, the Mg, Ca, Fe, and Zn contents were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Gwala et al., 2020). To do so, the obtained ashes were dissolved in 10 mL MilliQ-water containing 1% nitric acid solution and stored overnight (24 h, 25 °C). Subsequently, the solutions were filtered using a 0.45 µm membrane filter (Chromaphil® A-45/25, Macherey-Nagel, Germany). Mineral analysis was carried out by ICP-OES (ICAP 7000 series, Thermo Fisher Scientific), radially at 285 and 318 nm for Mg and Ca, and axially at 238 and 206 nm for Fe and Zn. Before each run and after 20 samples, a calibration using SPS-SW2 was performed. Certified reference materials (BCR-129 and BCR-679, Joint Research Centre, European Commission) were analyzed to ensure accuracy of the quantification (recoveries in *Supplementary material 1*). Finally, the fiber-rich residue (containing high amounts of fiber and sugars among which undigestible galacto-oligosaccharides) was calculated by difference from the starch, protein, moisture, lipid, and ash content.

2.5 *In vitro* digestion of pasta samples

In vitro digestion of lentil pastas with different microstructural properties was executed following the static INFOGEST protocol (Brodkorb et al., 2019). To study the digestion process as a function of time, digestion was performed in independent, individual tubes (5 gastric and 13 small intestinal time points) for each considered sample. Enzyme blanks (*i.e.*, no sample but all reagents) were prepared for the gastric and small intestinal phase.

Immediately before digestion, lentil pasta was cooked (OCT), rinsed with demineralized water to stop the cooking process, and drained for 2 min. To mimic the particle size distribution upon mastication of spaghetti (ranging 5 – 30 mm with an average of 10 mm according to Hoebler et al. (1998)), cooked samples were cut into pieces of about 10 mm with a knife, as previously applied by Freitas & Le Feunteun (2019). 1.25 g of sample were weighed into each reaction tube and the oral, gastric, and small intestinal phases were simulated exactly as reported earlier (Duijsens, Pälchen, De Coster, et al., 2022). During simulation of the different digestion phases, conditions (pH, temperature, and enzyme activity) were kept constant, ensuring that time-dependent enzyme-substrate interactions (and metabolite release) can be studied under physiologically relevant conditions. Samples were taken at predetermined time points followed by thermal enzyme inactivation (5 min, 98 °C). Digests were centrifuged (2000 g, 5 min, 25 °C) and separated into a soluble supernatant containing bioaccessible nutrients, and an insoluble pellet. Both fractions were snap-frozen using liquid nitrogen and stored until analysis (-40 °C).

2.5.1 Determination of digested starch

Amylolysis was quantified in the small intestinal phase since amylase was only incorporated at this stage of the simulation. The extent of starch digestion (%) was evaluated using the dinitrosalicylic (DNS) method, exactly as described earlier (Duijsens, Pälchen, De Coster, et al.,

2022). The concentration of reducing sugars in each sample was calculated using a maltose calibration curve, and the maltose concentration was converted to starch using a conversion factor of 0.95 (Eq.3).

$$Digested\ starch\ (\%) = \frac{maltose\ equivalents \times 0.95}{total\ starch\ content} \times 100 \qquad \text{Eq.3.}$$

The DNS method quantifies reducing groups, using maltose as a reference. Starch hydrolysis by (pancreatic) amylase mostly yields maltose and maltotriose, but other products such as glucose can be formed too, possibly causing an overestimation in the obtained amylolysis extent (Warren, Zhang, Waltzer, Gidley, & Dhital, 2015). The chromatographic quantification of different starch hydrolysis products can be a strategy to avoid such overestimations. Comparison of DNS with HPAEC-PAD revealed an overestimation of final amylolysis extents determined by DNS of around 10% (Gwala et al., 2019), while amylolysis kinetics generally followed similar patterns. In this regard, *in vitro* digestion assays are suited to deliver insight into digestion patterns and rates and allow the comparison of samples under fixed conditions (*e.g.*, enzymatic activity) (Warren et al., 2015). However, these simulations are less suited for the predictions of exact amylolysis extents *in vivo* (Duijsens, Pälchen, Guevara-Zambrano, et al., 2022; Warren et al., 2015). Therefore, the DNS method is a suitable, widely applied, and rapid method for the analysis and comparison of amylolysis kinetics and patterns, even though it does not distinguish between different formed metabolites.

2.5.2 Determination of digested protein

Protein digestion was evaluated using the spectrophotometric o-phthaldialdehyde (OPA) assay, quantifying α-amino groups released upon proteolysis, according to Zahir et al. (2018), as previously described in detail (Duijsens, Pälchen, De Coster, et al., 2022).

To quantify the *total α-amino groups present in the undigested sample (NH_{2total})*, 5 mg of sample was hydrolyzed in 1 mL 6 N HCl (24 h, 110 °C) in duplicate. Afterwards, the acid was removed by rotary evaporation (Hei-Vap Core, Heidolph, Germany), samples were diluted in 5 mL MilliQ, and filtered (pore size 0.25 µm). Proteolysis was then quantified in two ways. The *readily bioaccessible fraction (NH_{2TCA})* was quantified by precipitating large peptides from the digestive supernatant (through addition of 3.2% TCA) (Zahir et al., 2018). After centrifugation (10 000 g, 25 °C, 30 min) (Microfuge 22R, Beckman Coulter, US), the resulting supernatant was filtered. The small peptides in the TCA-treated supernatant were considered to be absorbable at intestinal brush border. In parallel, the *hydrolyzed readily bioaccessible fraction ($NH_{2TCA,hydro}$)* was evaluated. $NH_{2TCA,hydro}$ represents the same fraction as NH_{2TCA}, but expressed in terms of amino acid constituents and thus more easily relatable to the total protein amount (*i.e.*, α-amino groups) in the undigested sample (NH_{2total}). For this, the TCA-soluble peptides were hydrolyzed in 6 N HCl (16 h, 110 °C), the acid was evaporated, the samples were redissolved, and filtered.

For each set of analyses, OPA reagent was (freshly) prepared according to Zahir et al. (2018). Detected amino groups were converted into serine equivalents using an L-serine standard curve (12.5-100 mg/mL). The analysis was carried out as stated earlier (Duijsens, Pälchen, De Coster, et al., 2022), and the extent of proteolysis was calculated as indicated in equations 4 and 5.

$$readily\ bioaccessible\ protein = \frac{NH_{2TCA} - NH_{2initial}}{NH_{2total}} * 100\% \quad \text{Eq.4}$$

$$hydrolyzed\ readily\ bioaccessible\ protein = \frac{NH_{2TCA,hydro} - NH_{2initial}}{NH_{2total}} * 100\% \quad \text{Eq.5}$$

2.5.3 Determination of mineral bioaccessibility

The mineral bioaccessibility was determined (Eq.6) according to Gwala, Kyomugasho et al. (2020), from the digestive supernatants (see *Section 2.4.2*). Mineral bioaccessibility was evaluated

at 5 small intestinal time points (0,10, 90, 120, and 150 minutes). Since the data revealed no effect of small intestinal digestion time on mineral bioaccessibility (in accordance with literature (Gwala et al., 2020; Rousseau, Pallares Pallares, Vancoillie, Hendrickx, & Grauwet, 2020)), an average mineral bioaccessibility was calculated over the considered digestion times.

$$Mineral\ bioaccessibility = \frac{mineral\ conc.\ in\ digest - mineral\ conc.\ in\ blank}{mineral\ conc.\ in\ the\ sample} \times 100\% \quad \text{Eq.6}$$

2.6 Kinetic modelling and statistical analysis

As elaborated earlier, macronutrient digestion kinetics were studied by determining digestion products in each of the produced digests. Each of these digests represents an independent, consecutive measurement of the same digestive behavior as recently reviewed (Duijsens, Pälchen, Guevara-Zambrano, et al., 2022). Macronutrient digestibility of different samples was evaluated by integrating the data of all kinetic points by mathematical modelling, and subsequently comparing the obtained curves and estimated parameters.

To integrate the kinetic data of macronutrient digestion during the small intestinal phase, a first order fractional conversion model was applied (Gwala et al., 2019), shown in equation 7, with C the extent (%) of macronutrient hydrolysis at a certain digestion time. C_0 was defined as the hydrolysis extent at the beginning of the small intestinal phase (t=0) (and thus the end of the gastric phase). The final hydrolysis extent C_f (%) and the rate constant k (min^{-1}) were simultaneously estimated by nonlinear regression (SAS version 9.4, SAS Institute, Inc., Cary, NC, USA). The fit of the modelled curves was assessed by calculating R^2_{adj} and drafting residual and parity plots. Significant differences between the parameters were evaluated based on 95% confidence intervals.

For the case of starch digestion, since no salivary amylase was incorporated into the simulated oral phase, it was assumed no orogastric amylolysis took place (C_0=0). Moreover, the initial reaction

rate ($v_{initial}$) was calculated from the slope of the tangent line to the modelled amylolysis curve at time 0.

$$C(t) = C_f + (C_0 - C_f) * e^{-k*t} \quad \text{Eq.7}$$

To assess significant differences between mean compositions and mineral bioaccessibilities, one-way ANOVA and Tukey's HSD tests ($p<0.05$) were performed (JMP16, SAS Institute Inc., Cary, NC, USA), while 95% confidence intervals were used for fiber-rich residues.

3 Results and discussion

3.1 Cooking properties and quality of pasta

The cooking properties of the pasta samples were summarized in **Table 1.**

The **moisture addition** required to form the 100%RM dough (49%) was similar to the amount reported by for raw-milled fava bean dough (42%) (Rosa-Sibakov et al., 2016). A higher moisture addition (70-74%) was observed for the 30%ICC and 30%WL doughs. Plausibly, this could be caused by the *(i)* higher water holding capacity of flours with cellular intactness (Bajka et al., 2021) and *(ii)* lower water absorption of native semi-crystalline starch (present in raw-milled flour) as compared to pre-gelatinized starch (present in precooked ICC and WL powders) (Noordraven, Bernaerts, Mommens, Hendrickx, & Van Loey, 2021).

A slightly longer **OCT** was observed for the 100%RM spaghetti (15 min) compared to the other samples (12 min). In literature, OCT has been related to the (ungelatinized) starch content of the pasta. Indeed, 30%ICC and 30%WL were partially composed of a thermally pretreated ingredient in which starch was pregelatinized and protein denatured, possibly explaining their shorter OCT.

Dry matter losses (10-16%) observed for the three pasta samples are similar to values reported earlier for purely pulse-based pasta (e.g. 13% for lentil (Laleg, Cassan, et al., 2016) and 11% for

fava bean pasta (Rosa-Sibakov et al., 2016)). In contrast, cooking losses of 6 to 8% dry matter were reported for wheat semolina pasta, while a value of 9% was reported for wheat-based pasta partially substituted with fava bean flour (Laleg, Barron, et al., 2016; Rosa-Sibakov et al., 2016). A higher cooking loss in pulse-based pasta compared to wheat-based formulations (partially substituted with pulse flour or not) was attributed to a weaker, more water-soluble protein network formed upon cooking of pulse-based pasta compared to the covalently bound wheat gluten network 100. In the context of wheat-based pasta, fiber was previously reported to physically interfere with the formation of a continuous gluten network in pasta (Dodi et al., 2021). Plausibly, fiber (present in higher amounts in RM and WL powders due to the seed coat present) could disrupt the continuity of the non covalently bound pulse protein network in the pasta samples studied here as well, hereby increasing cooking loss.

The 30%ICC pasta presented lower DM losses upon cooking than the two other samples. Due to the composition of the ICC powder (100% isolated cotyledon cells), it is hypothesized that the higher degree of nutrient bioencapsulation may have contributed to the limited leaching in the 30%ICC pasta compared to the other samples. Moreover, the 30%ICC flour mixture contained relatively fewer large seed coat particles (such as seed coat material) compared to the other two pasta samples. Next to differences in microstructure (*i.e.*, degree of nutrient bioencapsulation) and contribution of seed coat particles, the higher dry matter loss of the 100%RM compared to the other two samples may be due to the longer OCT applied for this sample as well. However, this research aimed to compare pasta quality and digestion attributes of pasta cooked to an equivalent palatable point (requiring different OCTs) at which starch gelatinization was complete.

In terms of **water uptake** (approximately 290%), the studied pastas triplicated in weight upon cooking. Similar water uptake was reported for a wheat-based pasta enriched with raw-milled

common bean flour (Gallegos-Infante et al., 2010), produced manually similarly to the pasta in this work. In contrast, water absorption of 165% was reported for a 100% raw-milled lentil flour pasta (Laleg, Cassan, et al., 2016), and similar values were found for other wheat-based pastas partially enriched with pulse flour (Laleg, Barron, et al., 2016; Petitot & Micard, 2010), both prepared by extrusion. Therefore, it is expected that variations in water uptake of pasta samples strongly depend on the manufacturing method and final dimensions of the pasta, rather than the cellular integrity of the employed flour type.

3.2 Microstructure of cooked pasta

Figure 1 shows representative micrographs of the microstructure of the (cooked) pasta before and after 90 min of small intestinal digestion. These images confirm the (at least partial) persistence of intact cotyledon cells, during dough handling, pasta manufacturing, drying, cooking, as well as simulated digestion.

Each of the produced pastas was composed of a continuous phase of free protein and starch provided by the raw-milled flour. While the raw-milled flour consisted of small fractionated material (among which free starch granules and protein bodies) and tissue fragments (such as cell clusters and seed coat particles), the pure ICC powder was composed of 100% individual cells physically entrapping macronutrients, and the pure WL powder consisted of approximately 63% of individual cells (40-300 µm) next to cell clusters, seed coat particles etc. (Duijsens, Pälchen, De Coster, et al., 2022). From the previously reported particle size distributions (PSDs) of the respective powders, the fraction of individual cells in the flour mixtures could be determined, indicating the possible incidence of cells in the final pasta to be around 30% in the 30%ICC mixture, and around 20% in the 30%WL flour mix. This was confirmed via laser scattering and microscopy (*data in Supplementary material 2*).

3.3 Composition of uncooked and cooked pasta

The chemical composition of the (uncooked and cooked) pasta samples is summarized in **Table 2.** As can be seen from **Table 2**, the partial (30%) substitution of raw-milled whole lentil flour with a (partly) cellular ingredient slightly increases the protein and starch content. This is explained by the slightly higher starch and protein content in the ICC and WL flours, compared to the raw-milled one (Duijsens, Pälchen, De Coster, et al., 2022).The protein content and fiber-rich fraction of all pasta types produced here was remarkably higher as compared to wheat-based pasta (13-14% and 2.4%, respectively) (Laleg, Barron, et al., 2016; Rosa-Sibakov et al., 2016).

The fiber-rich residue and ash (mineral) content of 100%RM was slightly higher as compared to the 30%WL and 30%ICC pasta, due to partial replacement of the whole, raw-milled lentil ingredient by a precooked ingredient. Upon cooking, the ash content decreased due to mineral leaching. In more detail, **Figure 2A** shows the Fe, Zn, Mg, and Ca contents of the cooked and uncooked pasta samples. The mineral contents determined for the uncooked raw-milled pasta are in accordance with previously reported values in raw green lentils (Suliburska & Krejpcio, 2014).

For each of the considered pasta types, the Fe content did not significantly decrease upon cooking, similarly as during cooking of Bambara groundnuts and common beans (Gwala et al., 2020; Rousseau, Celus, et al., 2020). In pulses, non-ferritin Fe is expected to preferentially complex with phytates, minimizing leaching during cooking (Gwala et al., 2020; Moore et al., 2018). In contrast, the Mg and Zn content of the 100%RM significantly decreased upon cooking. Due to limited interactions with chelators, Mg ions leach more easily compared to other minerals as reviewed earlier (Duijsens et al., 2021). No significant decrease in Ca content was observed upon pasta cooking, in contrast with the small but significant decrease in Ca content upon cooking of Bambara groundnuts and common beans (Gwala et al., 2020; Rousseau, Celus, et al., 2020). Seed coat

particles present in the pulse flour are rich in fiber (see **Table 2**), oxalic acid, and polyphenols among others, which may have a protective effect against Ca leaching (Lombardi-Boccia et al., 1998).

3.4 Starch hydrolysis upon *in vitro* digestion of pasta

Starch hydrolysis kinetics upon *in vitro* small intestinal digestion of lentil pasta are shown in **Figure 3A**. All studied pastas first showed a period of increasing amylolysis, reaching a plateau upon long digestion times. The data were therefore modelled using the fractional conversion model (Eq. 7), with the estimated kinetic parameters summarized in **Table 3.** The fraction of starch which remains undigested upon 120 min of small intestinal digestion has been defined as resistant starch. Moreover, the extent of starch hydrolysis at 90 minutes of small intestinal digestion was reported to correlate to *in vivo* GI and can be a useful measure of starch digestibility (Edwards, Cochetel, Setterfield, Perez-Moral, & Warren, 2019). Therefore, next to the estimated final level of digestion (C_f), C_{120} and C_{90} were compared among the studied samples.

Amylolysis in 100%RM occurred faster and, after 120 min of *in vitro* small intestinal digestion, to a higher extent (103%) compared to 30%WL (83%) and 30%ICC (72%) pasta, quantitatively supported by the significant differences in the kinetic parameters k, $v_{initial,}$ and C_{120} in **Table 3**. These results indicate that incorporating ingredients with cellular intactness (ICC and WL) into a pulse-based pasta could significantly reduce the rate and extent of starch hydrolysis, highlighting their potential to delay starch hydrolysis in such applications.

Amylolysis was slower in the 30%ICC compared to the 30%WL, which could be attributed to the differences in PSD and relative amounts of individual cells incorporated (see *Section 3.2*). The flour mixture for 30%ICC contained ~30% individual cells, compared to ~20% for the 30%WL pasta (*section 3.3*). The difference between ICC and WL powder composition was not limited to

the cellular fraction, as WL also contained seed coat particles (*i.e.*, fiber) (Duijsens, Pälchen, De Coster, et al., 2022). In general, a higher pulse fiber content has been linked to retarded amylolysis explained by non-specific interactions with amylases (Dhital, Gidley, & Warren, 2015). However, the addition of fiber in wheat-based pasta has been shown to disrupt the continuity of the protein matrix, rendering starch granules more susceptible to enzymatic degradation, increasing starch digestion kinetics by 15% (Dodi et al., 2021). While the higher fiber content of 30%WL and 100%RM may have caused additional disruption of the continuous protein phase, its effect on amylolysis kinetics could (partially) be counteracted by the presence of bioencapsulated starch.

Clearly, starch hydrolysis was fastest for the 100%RM, reaching about 53% of amylolysis within the first 20 minutes of small intestinal digestion. Oppositely, the RM flour (evaluated in a heat-treated suspension) reached about 80% of starch digestion during the first 20 min of the small intestinal simulation (Duijsens, Pälchen, De Coster, et al., 2022). In contrast to the suspended system, pasta consists of a densely packed protein network surrounding starch, decreasing its accessibility for digestive enzymes. These findings indicate that the pasta structure was the major rate-determining physical barrier hindering amylolysis. Subsequently, by incorporating bioencapsulated starch (*i.e.*, 30%ICC and 30%WL), amylolysis can be slowed down further. These results confirmed once more to what extent nutrient digestibility can be influenced by both micro- and macro- structural properties of the food in which they are incorporated, highlighting the need for digestion studies for specific food applications.

In the available literature, a similar extent of 99% amylolysis upon 120 min of small intestinal digestion was reported for an extruded, dehulled raw-milled lentil pasta (Laleg, Cassan, et al., 2016), while around 75% was reported for a raw-milled chickpea macaroni (Garcia-Valle et al., 2021). Logically, next to nutrient bioencapsulation, differences in exact digestion kinetics among

samples can be caused by differences in pasta size, shape, thickness, and preparation technique (extrusion, drying temperature) (Petitot et al., 2009), determining the accessibility of the starch to amylases. However, these studies applied *in vitro* digestion protocols different from INFOGEST, rendering comparison difficult. Therefore, a commercial extruded (non-whole meal) wheat-based reference spaghetti was evaluated using the INFOGEST protocol (*data in Supplementary material 3*). The wheat-based pasta exhibited a significantly slower starch hydrolysis ($v_{initial}$) compared to the 100%RM pasta (approximately 80% after 90 minutes) while reaching a similar final level (approximately 100%), explained by both the presence of a covalently bound gluten network and the absence of a hull fraction. Generally, amylolysis of the commercial wheat-based pasta followed a similar course to the 30%WL, while amylolysis of 30%ICC was significantly (s)lower (in terms of $v_{initial}$ and extent for each digestion time).

3.5 Protein hydrolysis upon in vitro digestion of pasta

The formation of bioaccessible protein upon digestion of cooked pasta made of lentil flour mixes with different degrees of cellular intactness is shown in **Figure 3B.**

At the end of the gastric phase, the amount of bioaccessible protein (NH_{2TCA}) varied between 8 and 12% compared to around 5% for a commercial wheat-based spaghetti (*data in Supplementary material 3*). Petitot et al. (2009) reported 3-8% gastric proteolysis for wheat semolina pasta, while Laleg, Barron et al. (2016) found that during 30 min of *in vitro* (pepsin) proteolysis, only 4% of protein digestion was reached, compared to 6% for a wheat-based pasta enriched with 35% fava bean flour. While proteolysis is expressed in different terms throughout literature, and values are thus hard to compare, these results indicate that lentil proteins in a 100% (raw-milled) lentil-based pasta might be slightly more accessible to hydrolytic enzymes as compared to covalently linked gluten in wheat-based pasta.

During the small intestinal phase, the formation of bioaccessible protein (NH_{2TCA} and $NH_{2TCA,hydro}$) followed a fractional conversion behavior, with the rate of proteolysis decreasing with proceeding digestion. While no plateau was reached within 180 min of simulation, the data were anyhow modelled using the fractional conversion model to allow comparison of the proteolytic behavior of the different considered samples, with estimated parameters shown in **Table 3.** While the modelled curves fit the data well, the estimated final values require cautious interpretation.

At each considered digestion time during the gastric and small intestinal phases, the extent of protein hydrolysis was higher for 100%RM compared to the 30%ICC and 30%WL pasta. In literature, bioencapsulation of protein in intact pulse cotyledon cells has indeed been linked to decreased protein digestibility (Pälchen, Van Den Wouwer, et al., 2022; Zahir et al., 2018). 30%ICC and 30%WL pasta showed a quite similar behavior in terms of bioaccessible protein (NH_{2TCA}) release, while a higher plateau was observed for 30%WL compared to 30%ICC pasta in terms of hydrolyzed bioaccessible protein ($NH_{2TCA,hydro}$). This difference could be attributed to the lower fraction of individual cells with entrapped macronutrients, and the relatively higher fraction of seed coat material possibly disrupting the protein matrix, in the WL powder (Dodi et al., 2021; Laleg, Barron, et al., 2016). However, upon long digestion times, the observed difference in digestion extent (C_f) between 100%RM, 30%ICC, and 30%WL became statistically insignificant in terms of amino acid constituents ($NH_{2TCA,hydro}$). While the partial substitution of raw-milled flour by ICC or WL powder in lentil pasta thus significantly decreased starch digestion kinetics (*section 3.4*), the difference in formation of bioaccessible protein is rather limited, especially in the case of 30%WL pasta.

Generally, the evolution of hydrolyzed bioaccessible protein ($NH_{2TCA,hydro}$) in the 100%RM pasta followed a similar trend compared to a heat-treated suspension of raw-milled lentil flour (Duijsens,

Pälchen, De Coster, et al., 2022), reaching similar final values but with a significantly lower rate constant. It can therefore be expected that the pasta structure would have a (limited but) rate-determining effect on the conversion of protein into absorbable units in absolute terms.

Protein digestibility of 100%RM was higher than for a reference wheat-based pasta (approximately 20% upon 120 min small intestinal digestion, *data in Supplementary material 3*). An increased protein digestibility of 100% pulse-based pasta compared to a commercial wheat-based pasta could be expected due to the formation of a weaker (non covalently bound soluble) protein network and its disruption by seed coat particles and fiber. In literature, protein digestibility values of about 42% were found for wheat semolina pasta, and 46% for wheat pasta substituted with 35% fava bean flour (Laleg, Barron, et al., 2016). However, comparison with the current data is difficult due to different applied methods for digestion simulation and analysis. Moreover, absolute levels of protein digestibility may be affected by differences in pasta diameter and compactness of the pasta, in turn affected by the manufacturing steps as well (*e.g.*, manually made *versus* extrusion, drying temperature) (Petitot et al., 2009). More insight into the protein network formed in these pasta samples as well as its exact hydrolysis patterns could be obtained through e.g., HPLC-SEC (Duijsens et al., 2023; Petitot et al., 2009).

3.6 Mineral bioaccessibility of pasta

The Ca, Mg, Fe, and Zn bioaccessibilities of cooked 100%RM, 30%ICC, and 30%WL pasta are shown in **Figure 2B**. In accordance with data reported on different pulse types (Gwala et al., 2020; Rousseau, Celus, et al., 2020), mineral bioaccessibility did not reach 100% in the pasta samples and significant mineral chelation thus occurred.

Ca bioaccessibility reached values between 60 and 72% and did not significantly differ between different pasta samples. Correspondingly, a Ca bioaccessibility of 59% was found for green lentils

(Suliburska & Krejpcio, 2014), and similar values were reported for cooked Bambara groundnuts and common beans (Gwala et al., 2020; Rousseau, Celus, et al., 2020), indicating no significant effect of lentil flour incorporation into pasta on Ca bioaccessibility (in %).

Mg was found the most bioaccessible mineral in lentil-based pasta in this study (65 to 90%), in similarity to several other cooked pulses and attributed to its inherent lower potential to interact with antinutrients such as pectin (Gwala et al., 2020; Rousseau, Celus, et al., 2020; Suliburska & Krejpcio, 2014) (see *section 3.3*).

Fe bioaccessibility was low in all samples (10 to 14%). Varying values have been reported for Fe bioaccessibility in literature (Gwala et al., 2020; Hemalatha, Platel, & Srinivasan, 2007; Rousseau, Celus, et al., 2020; Suliburska & Krejpcio, 2014). Fe bioaccessibility was shown to be significantly reduced by complexation by phytic acid in pulses and cereals (Hemalatha et al., 2007; Moore et al., 2018).

The determined **Zn** bioaccessibility is in line with values determined for other pulses (Gwala et al., 2020; Rousseau, Celus, et al., 2020). Importantly, it should be taken into account that competition effects could arise, as Zn chelation by pectin was found to decrease upon increasing Ca concentration (Rousseau et al., 2019). In the lentil-based pastas of this study, the Ca content is over 20 times higher as compared to Zn, and competition for chelation is thus a plausible mechanism.

The decrease in Mg and Zn bioaccessibility upon incorporation of ingredients with cellular intactness (100%RM>30%WL>30%ICC) is remarkable. In this context, Rousseau, Pallares Pallares et al. (2020) identified that, rather than cellular intactness, the presence of mineral antinutrients in foods (*i.e.,* phytic acid and pectin) caused mineral chelation reducing

bioaccessibility in cooked common beans. Therefore, a possible explanation could be that thermal pretreatment of ingredients (ICC and WL) influenced the composition and ratio of antinutrients and minerals, ultimately affecting bioaccessibility. During thermal pretreatment of ICC and WL, more bioaccessible (soluble and unbound) minerals could have preferably leached out compared to chelated minerals. Moreover, mineral bioaccessibility has been described to be affected by the formation of Zn-protein complexes (Hemalatha et al., 2007). Consequentially, a higher extent of (gastric) protein solubilization in an open system (i.e. 100%RM) could explain a higher bioaccessibility as compared to systems with cellular intactness (30%ICC and 30%WL), as postulated by Gwala, Kyomugasho, et al. (2020). However, while the effect of gastric proteolysis was not studied, no effect of (small intestinal) digestion time was observed on mineral bioaccessibility (*data not shown*).

To determine if significant amounts of minerals could be accessible for absorption in the small intestine upon the consumption of one portion (100g) of cooked pasta (mineral content shown in **Figure 2A**), bioaccessibility was expressed in absolute amounts and in terms of the average daily requirements (ADR) (*data in Supplementary material 4*). One portion could offer around 5, 10-13, 12-15, and 5-9 % of the ADR of Ca, Mg, Zn, and Fe, respectively. Especially for Fe, 100 g pasta would deliver an appreciable bioaccessible amount (up to 15% of ADR). These values are in accordance with previously reported values for cooked whole common beans (Duijsens et al., 2021; Rousseau, Celus, et al., 2020).

Generally, it can be concluded that while lentils contain significant amounts of minerals (**Figure 2A**), the mineral quality of the produced pastas is limited. Based on literature, a possible strategy for increasing mineral bioaccessibility in lentil-based pasta could be to produce ingredients from

dehulled lentils, decreasing the total antinutrient, but also mineral and fiber content (Rousseau, Celus, et al., 2020).

4 Conclusion

The effect of incorporating ingredients with cellular intactness into raw-milled lentil pasta flour formulations (100%RM, 30%ICC, and 30%WL) was investigated on cooking properties, composition, macronutrient digestibility, and mineral bioaccessibility.

While the effects on cooking properties were limited, 30%ICC and 30%WL had slightly higher protein and starch, but lower fiber and ash contents, compared 100%RM pasta. 30%ICC and 30%WL pasta showed a slowed amylolysis compared to 100%RM pasta. The pasta structure could be identified as the major rate-determining physical barrier for amylolysis, enhanced by partial nutrient bioencapsulation. The attenuation of proteolysis was attributed to bioencapsulation as well, while the effect on final protein digestibility was limited. The incorporation of a (partially) cellular ingredient into the pasta formulation moderately decreased Zn and Mg bioaccessibility, while this was not the case for Ca and Fe. To conclude, the lentil-based pastas prepared here have a large potential as a food with improved nutritional properties (high in digestible protein and slow(er) starch digestion). However, similarly to cooked pulses, their potential as sources of bioaccessible minerals is limited.

Further investigation should lead to pastas with improved techno-functional properties (*e.g.*, textural properties) and insights into (sensorial) consumer acceptability of these products. The use of different flour blends (*e.g.*, wheat semolina combined with ICC or WL powder) could deliver pastas with improved cooking quality and interesting nutritional properties. In the context of an industrial application, it should be confirmed if nutrient bioencapsulation prevails upon more harsh

processing as well, *e.g.*, extrusion and high temperature drying. Besides, *in vivo* studies should be carried out, testing the current findings in terms of physiologically relevant parameters such as glycemic index and satiety/satiation.

Acknowledgements

D. Duijsens is a PhD researcher funded by the Research Foundation Flanders (FWO - Grant no. 1S23321N). S.H.E. Verkempinck is a Postdoctoral Researcher funded by the Research Foundation Flanders (FWO - Grant no. 1222420N). K. Pälchen is a PhD fellow funded by European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie (Grant no. 765415). The authors acknowledge the financial support of the KU Leuven Research Fund.

Conflict of interest

None

References

Bajka, B. H., Pinto, A. M., Ahn-Jarvis, J., Ryden, P., Perez-Moral, N., van der Schoot, A., Stocchi, C., Bland, C., Berry, S. E., Ellis, P. R., & Edwards, C. H. (2021). The impact of replacing wheat flour with cellular legume powder on starch bioaccessibility, glycaemic response and bread roll quality: A double-blind randomised controlled trial in healthy participants. *Food Hydrocolloids*, *114*(December 2020), 106565. https://doi.org/10.1016/j.foodhyd.2020.106565

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, *14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

De Almeida Costa, G. E., Da Silva Queiroz-Monici, K., Pissini Machado Reis, S. M., & De Oliveira, A. C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry*, *94*(3), 327–330. https://doi.org/10.1016/j.foodchem.2004.11.020

Dhital, S., Gidley, M. J., & Warren, F. J. (2015). Inhibition of α-amylase activity by cellulose: Kinetic analysis and nutritional implications. *Carbohydrate Polymers*, *123*, 305–312. https://doi.org/10.1016/j.carbpol.2015.01.039

Dodi, R., Bresciani, L., Biasini, B., Cossu, M., Scazzina, F., Taddei, F., D'egidio, M. G., Dall'asta, M., & Martini, D. (2021). Traditional and non-conventional pasta-making processes: Effect on in vitro starch digestibility. *Foods*, *10*(5), 1–10. https://doi.org/10.3390/foods10050921

607 Duijsens, D., Pälchen, K., De Coster, A., Verkempinck, S. H. E., Hendrickx, M. E., & Grauwet,
608 T. (2022). Effect of manufacturing conditions on in vitro starch and protein digestibility of
609 (cellular) lentil-based ingredients. *Food Research International*, *158*(August 2022).
610 https://doi.org/10.1016/j.foodres.2022.111546

611 Duijsens, D., Pälchen, K., Guevara-Zambrano, J. ., Verkempinck, S. H. ., Infantes-Garcia, M. .,
612 Hendrickx, M. ., Van Loey, A., & Grauwet, T. (2022). Strategic choices for in vitro food
613 digestion methodologies enabling food digestion design. *Trends in Food Science &*
614 *Technology*, *126*, 61–72. https://doi.org/10.1016/j.tifs.2022.06.017

615 Duijsens, D., Gwala, S., Pallares, A. P., Pälchen, K., Hendrickx, M., & Grauwet, T. (2021). How
616 postharvest variables in the pulse value chain affect nutrient digestibility and bioaccessibility.
617 *Comprehensive Reviews in Food Science and Food Safety*, *June*, 1–30.
618 https://doi.org/10.1111/1541-4337.12826

619 Duijsens, D., Pälchen, K., Verkempinck, S., Guevara Zambrano, J., Hendrickx, M., Van Loey, A.,
620 & Grauwet, T. (2023). Size exclusion chromatography to evaluate in vitro proteolysis: a case
621 study on the impact of microstructure in pulse powders. *Food Chemistry*, *418*, 135709.
622 https://doi.org/10.1016/j.foodchem.2023.135709

623 Edwards, C. H., Cochetel, N., Setterfield, L., Perez-Moral, N., & Warren, F. J. (2019). A single-
624 enzyme system for starch digestibility screening and its relevance to understanding and
625 predicting the glycaemic index of food products. *Food and Function*, *10*(8), 4751–4760.
626 https://doi.org/10.1039/c9fo00603f

627 Freitas, D., & Le Feunteun, S. (2019). Oro-gastro-intestinal digestion of starch in white bread,
628 wheat-based and gluten-free pasta: Unveiling the contribution of human salivary α-amylase.

Food Chemistry, *274*(August 2018), 566–573. https://doi.org/10.1016/j.foodchem.2018.09.025

Galani, Y. J. H., Orfila, C., & Gong, Y. Y. (2022). A review of micronutrient deficiencies and analysis of maize contribution to nutrient requirements of women and children in Eastern and Southern Africa. *Critical Reviews in Food Science and Nutrition*, *62*(6), 1568–1591. https://doi.org/10.1080/10408398.2020.1844636

Gallegos-Infante, J. A., Rocha-Guzman, N. E., Gonzalez-Laredo, R. F., Ochoa-Martínez, L. A., Corzo, N., Bello-Perez, L. A., Medina-Torres, L., & Peralta-Alvarez, L. E. (2010). Quality of spaghetti pasta containing Mexican common bean flour (Phaseolus vulgaris L.). *Food Chemistry*, *119*(4), 1544–1549. https://doi.org/10.1016/j.foodchem.2009.09.040

Garcia-Valle, D. E., Bello-Pérez, L. A., Agama-Acevedo, E., & Alvarez-Ramirez, J. (2021). Structural characteristics and in vitro starch digestibility of pasta made with durum wheat semolina and chickpea flour. *Lwt*, *145*(January). https://doi.org/10.1016/j.lwt.2021.111347

Giuberti, G., Gallo, A., Cerioli, C., Fortunati, P., & Masoero, F. (2015). Cooking quality and starch digestibility of gluten free pasta using new bean flour. *Food Chemistry*, *175*, 43–49. https://doi.org/10.1016/j.foodchem.2014.11.127

Gwala, S., Kyomugasho, C., Wainaina, I., Rousseau, S., Hendrickx, M., & Grauwet, T. (2020). Ageing, dehulling and cooking of Bambara groundnuts: Consequences for mineral retention and: In vitro bioaccessibility. *Food and Function*, *11*(3), 2509–2521. https://doi.org/10.1039/c9fo01731c

Gwala, S., Wainana, I., Pallares Pallares, A., Kyomugasho, C., Hendrickx, M., & Grauwet, T. (2019). Texture and interlinked post-process microstructures determine the in vitro starch

digestibility of Bambara groundnuts with distinct hard-to-cook levels. *Food Research International*, *120*(February), 1–11. https://doi.org/10.1016/j.foodres.2019.02.022

Hemalatha, S., Platel, K., & Srinivasan, K. (2007). Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *Journal of Trace Elements in Medicine and Biology*, *21*(1), 1–7. https://doi.org/10.1016/j.jtemb.2006.10.002

Henn, K., Goddyn, H., Bøye Olsen, S., Bredie, W. L. P., Olsen, S. B., & Bredie, W. L. P. (2021). Identifying behavioral and attitudinal barriers and drivers to promote consumption of pulses: A quantitative survey across five European countries. *Food Quality and Preference*, *98*, 104455. https://doi.org/10.1016/j.foodqual.2021.104455

Hoebler, C., Karinthi, A., Devaux, M.-F. F., Guillon, F., Gallant, D. J. G. G., Bouchet, B., Melegari, C., & Barry, J.-L. L. (1998). Physical and chemical transformations of cereal food during oral digestion in human subjects. *British Journal of Nutrition*, *80*(5), 429–436. https://doi.org/10.1017/s0007114598001494

Janssen, F., Wouters, A. G. B., Pareyt, B., Gerits, L. R., Delcour, J. A., Waelkens, E., & Derua, R. (2018). Wheat (Triticum aestivum L.) lipid species distribution in the different stages of straight dough bread making. *Food Research International*, *112*(May), 299–311. https://doi.org/10.1016/j.foodres.2018.06.038

Laleg, K., Barron, C., Santé-Lhoutellier, V., Walrand, S., & Micard, V. (2016). Protein enriched pasta: Structure and digestibility of its protein network. *Food and Function*, *7*(2), 1196–1207. https://doi.org/10.1039/c5fo01231g

Laleg, K., Cassan, D., Barron, C., Prabhasankar, P., & Micard, V. (2016). Structural, culinary, nutritional and anti-nutritional properties of high protein, gluten free, 100% legume pasta.

673 *PLoS ONE*, *11*(9), 1–19. https://doi.org/10.1371/journal.pone.0160721

674 Lombardi-Boccia, G., Lucarini, M., Di Lullo, G., Del Puppo, E., Ferrari, A., & Carnovale, E.
675 (1998). Dialysable, soluble and fermentable calcium from beans (Phaseolus vulgaris L.) as
676 model for in vitro assessment of the potential calcium availability. *Food Chemistry*, *61*(1–2),
677 167–171. https://doi.org/10.1016/S0308-8146(97)00040-X

678 Moore, K. L., Rodríguez-Ramiro, I., Jones, E. R., Jones, E. J., Rodríguez-Celma, J., Halsey, K.,
679 Domoney, C., Shewry, P. R., Fairweather-Tait, S., & Balk, J. (2018). The stage of seed
680 development influences iron bioavailability in pea (Pisum sativum L.). *Scientific Reports*,
681 *8*(1), 1–11. https://doi.org/10.1038/s41598-018-25130-3

682 Noordraven, L. E. C., Bernaerts, T., Mommens, L., Hendrickx, M. E., & Van Loey, A. M. (2021).
683 Impact of cell intactness and starch state on the thickening potential of chickpea flours in
684 water-flour systems. *LWT*, *146*(March), 111409. https://doi.org/10.1016/j.lwt.2021.111409

685 Pälchen, K., Bredie, W. L. P., Duijsens, D., Alfie, A. I., Hendrickx, M., Loey, A. Van, Raben, A.,
686 & Grauwet, T. (2022). Effect of processing and microstructural properties of chickpea-flours
687 on in vitro digestion and appetite sensations. *Food Research International*, 111245.
688 https://doi.org/10.1016/j.foodres.2022.111245

689 Pälchen, K., Van Den Wouwer, B., Duijsens, D., Hendrickx, M. E., Van Loey, A., & Grauwet, T.
690 (2022). Utilizing Hydrothermal Processing to Align Structure and In Vitro Digestion Kinetics
691 between Three Different Pulse Types. *Foods*, *11*(2), 206.
692 https://doi.org/10.3390/foods11020206

693 Patel, M. J., & Chakrabarti-Bell, S. (2013). Flour quality and dough elasticity: Dough sheetability.
694 *Journal of Food Engineering*, *115*(3), 371–383.

https://doi.org/10.1016/j.jfoodeng.2012.10.038

Petitot, M., Barron, C., Morel, M. H., & Micard, V. (2010). Impact of Legume Flour Addition on Pasta Structure: Consequences on Its In Vitro Starch Digestibility. *Food Biophysics*, *5*(4), 284–299. https://doi.org/10.1007/s11483-010-9170-3

Petitot, M., Brossard, C., Barron, C., Larré, C., Morel, M. H., & Micard, V. (2009). Modification of pasta structure induced by high drying temperatures. Effects on the in vitro digestibility of protein and starch fractions and the potential allergenicity of protein hydrolysates. *Food Chemistry*, *116*(2), 401–412. https://doi.org/10.1016/j.foodchem.2009.01.001

Petitot, M., & Micard, V. (2010). Legume-Fortified Pasta. Impact of Drying and Precooking Treatments on Pasta Structure and Inherent In Vitro Starch Digestibility. *Food Biophysics*, *5*(4), 309–320. https://doi.org/10.1007/s11483-010-9180-1

Platel, K., & Srinivasan, K. (2016). Bioavailability of Micronutrients from Plant Foods : An Update Bioavailability of Micronutrients from Plant Foods : An Update. *Critical Reviews in Food Science and Nutrition ISSN:*, *56*(10), 1608–1619. https://doi.org/10.1080/10408398.2013.781011

Rosa-Sibakov, N., Heiniö, R. L., Cassan, D., Holopainen-Mantila, U., Micard, V., Lantto, R., & Sozer, N. (2016). Effect of bioprocessing and fractionation on the structural, textural and sensory properties of gluten-free faba bean pasta. *LWT - Food Science and Technology*, *67*, 27–36. https://doi.org/10.1016/j.lwt.2015.11.032

Rousseau, S., Celus, M., Duijsens, D., Gwala, S., Hendrickx, M., & Grauwet, T. (2020). The impact of postharvest storage and cooking time on mineral bioaccessibility in common beans. *Food and Function*, *11*(9), 7584–7595. https://doi.org/10.1039/d0fo01302a

Rousseau, S., Kyomugasho, C., Celus, M., Yeasmen, N., Hendrickx, M. E., & Grauwet, T. (2019). Zinc bioaccessibility is affected by the presence of calcium ions and degree of methylesterification in pectin-based model systems. *Food Hydrocolloids*, *90*(December 2018), 206–215. https://doi.org/10.1016/j.foodhyd.2018.12.019

Rousseau, S., Pallares Pallares, A., Vancoillie, F., Hendrickx, M., & Grauwet, T. (2020). Pectin and phytic acid reduce mineral bioaccessibility in cooked common bean cotyledons regardless of cell wall integrity. *Food Research International*, *137*(May), 109685. https://doi.org/10.1016/j.foodres.2020.109685

Sulibursk a, J., & Krejpcio, Z. (2014). Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains and nuts. *Journal of Food Science and Technology*, *51*(3), 589–594. https://doi.org/10.1007/s13197-011-0535-5

Warren, F. J., Zhang, B., Waltzer, G., Gidley, M. J., & Dhital, S. (2015). The interplay of α-amylase and amyloglucosidase activities on the digestion of starch in in vitro enzymic systems. *Carbohydrate Polymers*, *117*, 185–191. https://doi.org/10.1016/j.carbpol.2014.09.043

Zahir, M. A., Fogliano, V., & Capuano, E. (2018). Food matrix and processing modulates in vitro protein digestibility in soybean. *Food & Function*, *9*(12), 6326–6336. https://doi.org/10.1039/C8FO01385C

Table 1: Cooking properties of 100% RM, 30%ICC, and 30%WL lentil pasta produced in this study. Dough moisture addition, optimal cooking time (OCT), dry matter loss, protein loss, and starch loss are indicated in % dry matter ± standard deviation.

Lentil pasta	Dough moisture addition (%)	OCT (min)	Water uptake (%)	Dry matter loss (%)	Protein loss (%)	Starch loss (%)
100%RM	49	15	291.4 ± 0.2	15.3 ± 0.1	9.1 ± 0.1	17.0 ± 0.1
30%ICC	74	12	297.2 ± 0.9	10.2 ± 0.2	2.3 ± 0.2	6.0 ± 0.2
30% WL	70	12	289.5± 1.7	16.0 ± 0.4	8.7 ± 0.4	13.6 ± 0.4

100%RM: lentil pasta made of 100% raw-milled lentil flour; 30%ICC: lentil pasta made of 70% raw-milled flour and 30% isolated cotyledon cell powder; 30%WL: lentil pasta made of 70% raw-milled and 30% whole precooked lentil powder.

Table 2: Chemical composition (g/100g dry matter (DM)) of uncooked and cooked lentil pasta samples. Different superscript letters within a column indicate significant differences between the mean values (Tukey's range test $p<0.05$ except for 95% confidence interval for calculated fiber-rich residue).

	Lentil pasta	Dry matter (g/100g)	Starch (g/100g DM)	Protein (g/100g DM)	Ash (g/100g DM)	Lipid (g/100g DM)	Fiber-rich residue[1] (g/100g DM)
Uncooked	100%RM	92.37 ± 0.01^{a}	44.56 ± 0.05^{d}	20.64 ± 0.18^{c}	2.80 ± 0.01^{a}	2.82 ± 0.10^{b,c}	29.17 ± 0.34^{a}
	30%ICC	91.03 ± 0.01^{b}	50.20 ± 0.61^{c}	20.93 ± 0.43^{c,b}	1.99 ± 0.01^{b}	2.57 ± 0.23^{c}	24.31 ± 0.79^{b}
	30%WL	91.11 ± 0.01^{c}	46.24 ± 0.58^{d,b}	21.25 ± 0.06^{b}	2.06 ± 0.01^{b}	2.67 ± 0.20^{c}	27.78 ± 0.63^{a}
Cooked	100%RM	19.98 ± 0.01^{d}	45.37 ± 0.33^{d}	22.18 ± 0.01^{a}	0.88 ± 0.01^{c}	3.59 ± 0.39^{a,b}	27.99 ± 0.59^{a}
	30%ICC	20.58 ± 0.00^{d}	52.56 ± 0.16^{a}	22.76 ± 0.05^{a}	0.74 ± 0.01^{c}	3.49 ± 0.44^{a,b}	20.447 ± 0.49^{c}
	30%WL	19.66 ± 0.00^{d}	47.56 ± 0.26^{b}	23.08 ± 0.02^{a}	0.58 ± 0.01^{c}	3.91 ± 0.24^{a}	24.87 ± 0.35^{b}

[1]Calculated by difference, containing both fibers and sugars (including raffinose family oligosaccharides)

100%RM: lentil pasta made of 100% raw-milled lentil flour; 30%ICC: lentil pasta made of 70% raw-milled flour and 30% isolated cotyledon cell powder; 30%WL: lentil pasta made of 70% raw-milled and 30% whole precooked lentil powder.

Table 3: Estimated kinetic parameters of the fractional conversion model for in vitro amylolysis and proteolysis of 100%RM, 30%ICC, and 30%WL pasta cooked to their optimal cooking time. Proteolysis is expressed in terms of readily bioaccessible protein (NH_{2TCA}, %) and hydrolyzed readily bioaccessible protein ($NH_{2TCA,hydro}$, %). Estimated kinetic parameters include rate constant (k, min-1) and final degree of amylolysis/proteolysis (C_f, %). Values are estimates ± standard error. For one type of analysis, different superscript letters within a column indicate significant differences between the mean values (95% confidence intervals).

Amylolysis						
Lentil pasta	***k* (min^{-1})**	***C_f* (%)**	**R^2_{adj}**	**$V_{initial}$ (%min^{-1})**	**C_{90} (%)**	**C_{120} (%)**
100%RM	0.035 ± 0.005^{a}	102.3 ± 5.3^{a}	0.99	3.55 ± 0.55^{a}	97.8 ± 3.8^{a}	100.7 ± 4.5^{a}
30%ICC	0.023 ± 0.003^{a}	77.286 ± 3.5^{b}	0.99	1.75 ± 0.22^{c}	67.3 ± 1.8^{c}	72.2 ± 2.2^{c}
30%WL	0.027 ± 0.003^{a}	85.99 ± 3.3^{a,b}	0.99	2.33 ± 0.27^{b}	78.5 ±2.1^{b}	82.7 ± 2.5^{b}

Proteolysis						
	Readily bioaccessible protein (NH_{2TCA})			**Hydrolyzed readily bioaccessible protein ($NH_{2TCA,hydro}$)**		
Lentil pasta	***k* (min^{-1})**	***C_f* (%)**	**R^2adj**	***k* (min^{-1})**	***C_f* (%)**	**R^2adj**
100%RM	0.013 ± 0.001^{a}	42.7 ± 1.5^{a}	0.99	0.014 ± 0.003^{a,b}	99.6 ± 5.6^{a}	0.99
30%ICC	0.020 ± 0.003^{a}	26.8 ± 1.1^{b}	0.99	0.010 ± 0.001^{b}	91.1 ± 5.4^{a}	0.99
30%WL	0.006 ± 0.001^{b}	44.7 ± 3.8^{a}	0.99	0.023 ± 0.002^{a}	91.2 ± 2.5^{a}	0.99

100%RM: lentil pasta made of 100% raw-milled lentil flour; 30%ICC: lentil pasta made of 70% raw-milled flour and 30% isolated cotyledon cell powder; 30%WL: lentil pasta made of 70% raw-milled and 30% whole precooked lentil powder.

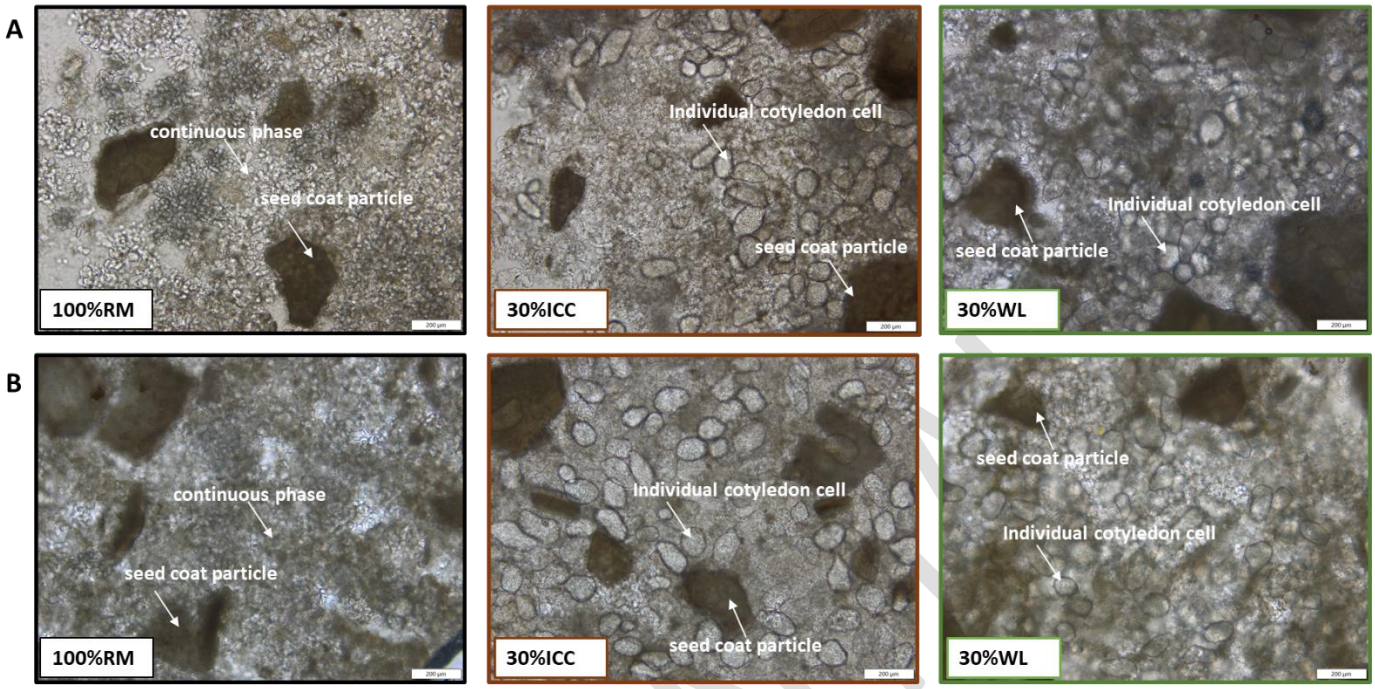


Figure 1: Representative micrographs of 100%RM (−), 30%ICC (−), 30%WL (−) pasta cooked to the optimal cooking time, A) before, and B) upon 90 minutes of in vitro small intestinal digestion (magnification 10x, scale bar 200 µm). Arrows indicate the continuous phase, seed coat particles, and individual cotyledon cells.

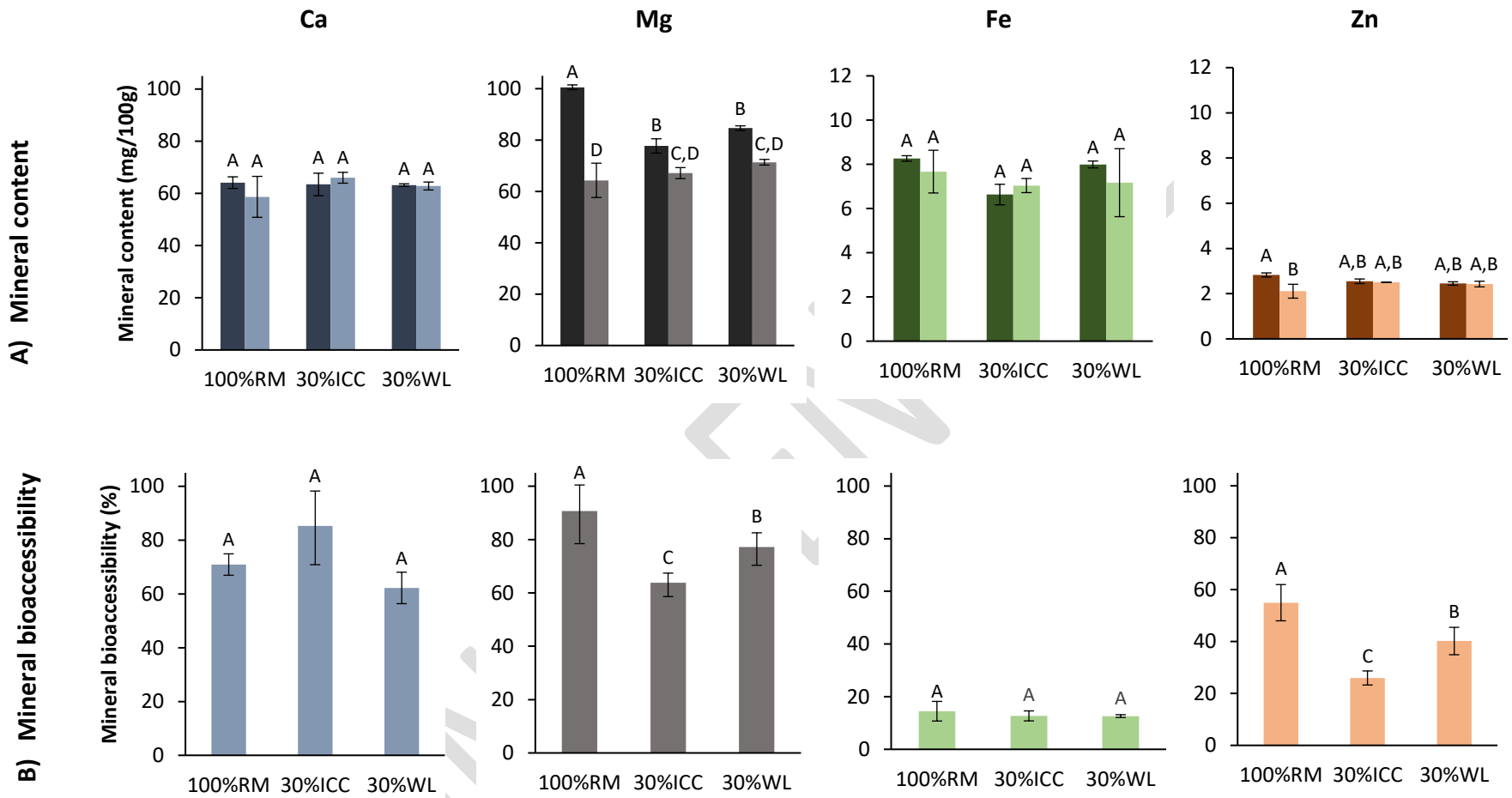


Figure 2: A) Ca (■/■) ,Mg (■/■) and B) Fe (■/■), and Zn(■/■) content (in mg/100g dry matter) of 100%RM, 30%ICC, and 30%WL lentil pasta before and upon cooking, respectively. B) Ca (■), Mg(■), Fe(■), and Zn (■) bioaccessibility in 100%RM, 30%ICC, and 30%WL lentil pasta cooked to their optimal cooking time. Dark colors indicate mineral content before cooking, while the bright colors indicate the mineral content/bioaccessibility of the corresponding pasta after cooking for the determined optimal cooking time. For mineral content (A) and bioaccessibility (B), within a mineral type, different letters indicate significant difference of the mean content (Tukey's range test, $p<0.5$).

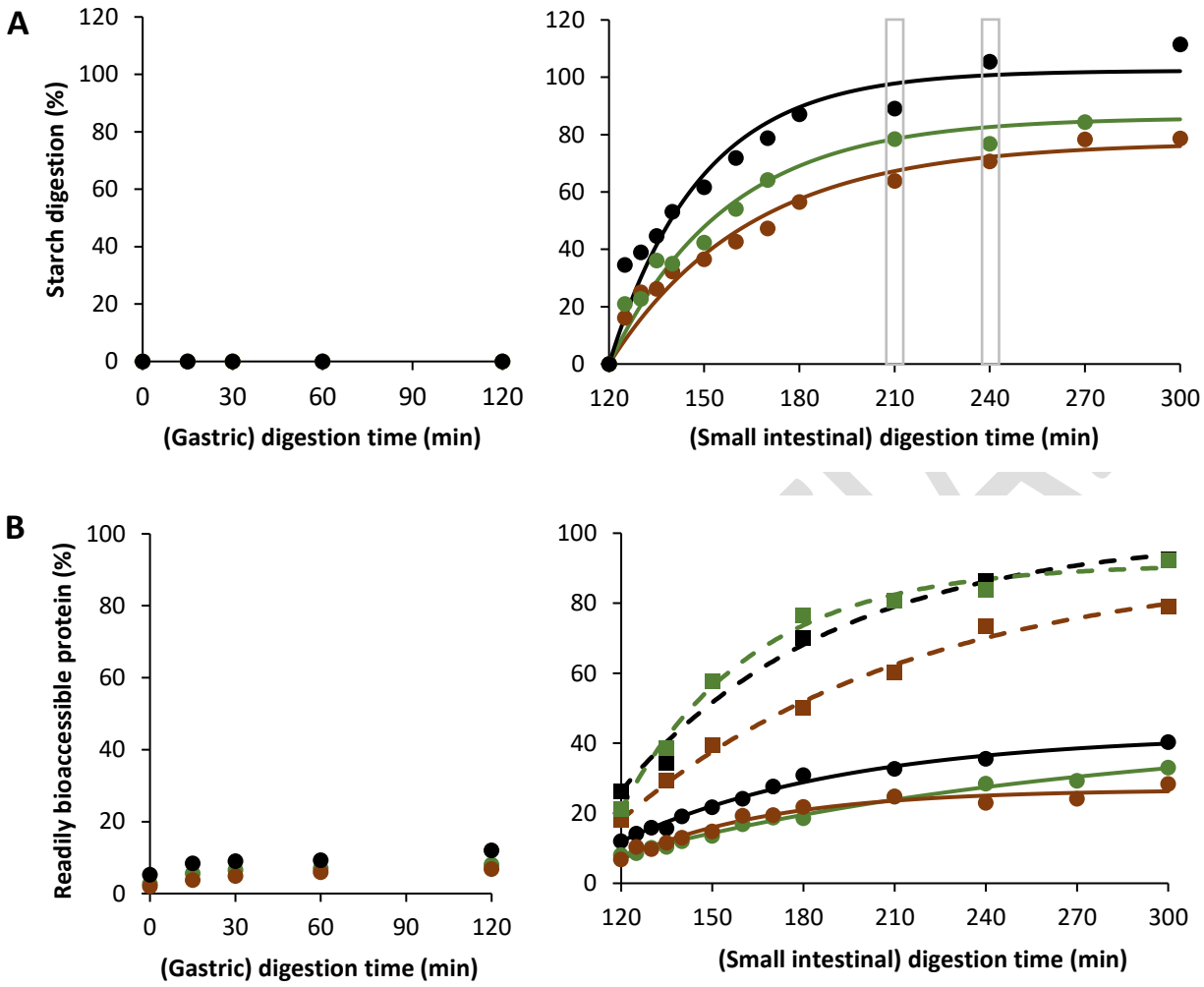


Figure 3: A) In vitro amylolysis of 100%RM (●), 30%ICC (●), and 30%WL (●) pasta cooked to its optimal cooking time. B) In vitro proteolysis of 100%RM (●), 30%ICC (●), and 30%WL (●) pasta cooked to its optimal cooking time in terms of bioaccessible protein (NH_{2TCA}, %, ●) and hydrolyzed bioaccessible protein ($NH_{2TCA,hydro}$, %, ■). Points are experimentally determined data. Lines represent the data modelled using the fractional conversion model. Grey boxes indicate amylolysis levels upon 90 and 120 min of small intestinal digestion.

Supplementary material 1

Table S1: Indicated and values measured via ICP-OES (mean ± stdev) of certified reference materials BCR-129 and BCR-679.

	Indicated values (mg/g)		Measured values (mg/g)	
	Hay powder BCR-129	Cabbage powder BCR-679	Hay powder BCR-129	Cabbage powder BCR-679
Fe	0.055	0.114	0.0433 ± 0.0063	0.0847 ± 0.0032
Mg	1.362	1.45	1.192 ± 0.053	1.269 ± 0.023
Zn	0.0797	0.0321	0.0723 ± 0.0031	0.0245 ± 0.0019
Ca	7.768	6.4	6.537 ± 1.170	5.467 ± 0.2721

Supplementary material 2

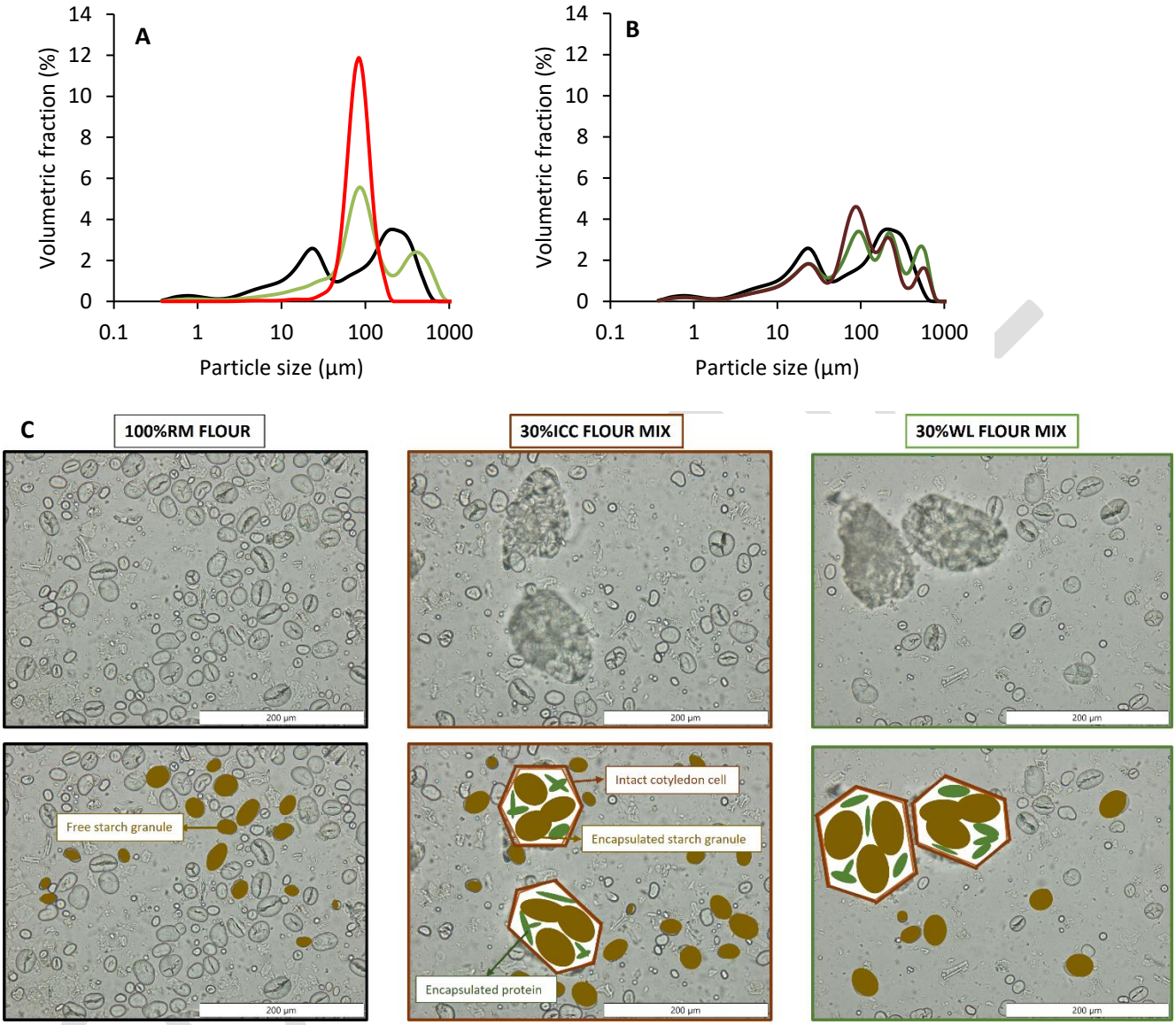


Figure S2: Particle size distributions (PSDs) and representative micrographs of lentil flours with different microstructures and their mixtures. A) PSDs of raw-milled (–), isolated cotyledon cell (ICC) (–), and whole precooked lentil (WL) (–) powders; B) PSDs of raw-milled (–) lentil flour, 30% ICC (–), and 30% WL (–) flour mixtures. C) representative micrographs of raw-milled flour (–), 30% ICC (–), and 30% WL (–) flour mixtures. (magnification 40x, scale bar 200 µm), the bottom row includes the same pictures, but with a schematical representation of the free and bio-encapsulated nutrients present in the flour mixes.

Supplementary material 3

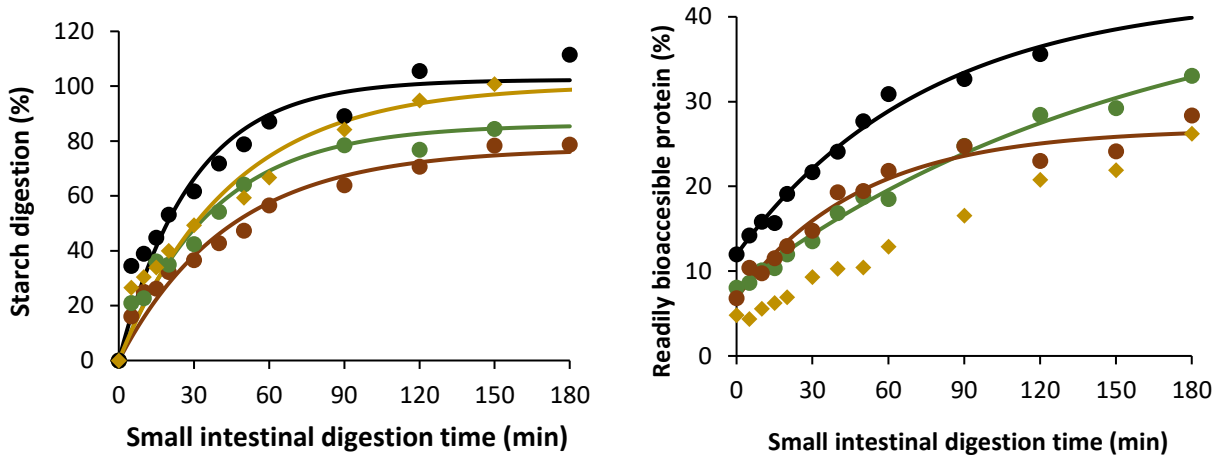


Figure S3: In vitro hydrolysis of starch and protein (in terms of readily bioaccessible protein, NH2TCA) of 100%RM (●), 30%ICC (●), and 30%WL (●) pastas cooked to their optimal cooking time (OCT), compared to a commercial 100% wheat-based spaghetti (◆) prepared according to the manufacturer's guidelines. Points are experimentally determined data. Lines represent the data modelled using the fractional conversion model, except for proteolysis of the wheat-based pasta, which did not follow this model.

Supplementary material 4

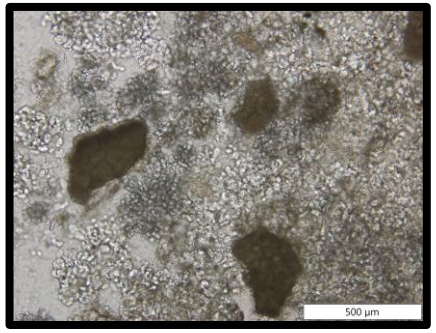
Table S4: Bioaccessible Ca, Mg, Fe, and Zn concentration (mg/100g cooked pasta, wet matter) and contribution to average daily requirement (ADR) of a reference portion of 100 g cooked pasta for 100%RM, 30%ICC, and 30%WL lentil spaghetti. Within one mineral type (column), different letters indicate statistical differences between the mean values (Tukey's range test, $p<0.5$).

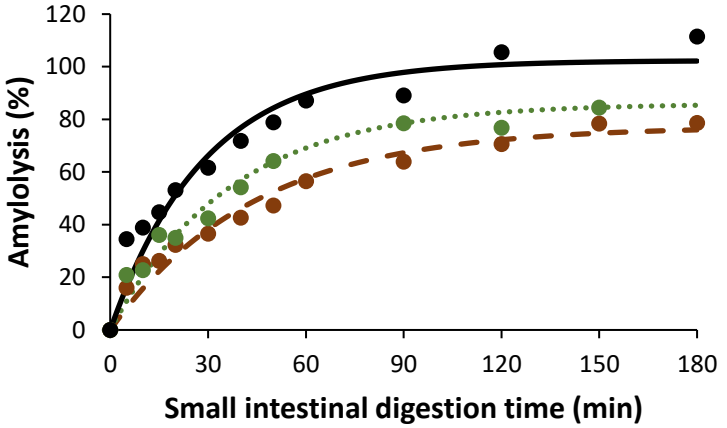
Lentil pasta		Ca	Mg	Fe	Zn
100%RM	Bioaccessible mineral concentration (mg/100g cooked pasta, wet matter)	33.3 ± 1.9[a]	46.6 ± 5.1[a]	0.9 ± 0.2[a]	0.9 ± 0.1[a]
	Contribution to ADR (%)[1]	4.76	13.3	14.8	9.3
30%ICC	Bioaccessible mineral concentration (mg/100g cooked pasta, wet matter)	41.0 ± 9.8[a]	34.0 ± 2.2[b]	0.7 ± 0.1[a]	0.5 ± 0.1[b]
	Contribution to ADR (%)[1]	5.8	9.7	11.8	5.16
30%WL	Bioaccessible mineral concentration (mg/100g cooked pasta, wet matter)	31.4 ± 2.9[a]	45.9 ± 4.6[a]	0.7 ± 0.1[a]	0.8 ± 0.1[a]
	Contribution to ADR (%)[1]	4.5	13.1	12.2	7.8

[1]Contribution to the average daily requirement (ADR), based on average daily requirements of 700, 350, 6, and 10 mg/day for Ca, Mg, Fe, and Zn, respectively (EFSA, 2017).

100%RM: lentil pasta made of 100% raw-milled lentil flour; 30%ICC: lentil pasta made of 70% raw-milled flour and 30% isolated cotyledon cell powder; 30%WL: lentil pasta made of 70% raw-milled and 30% whole precooked lentil powder.

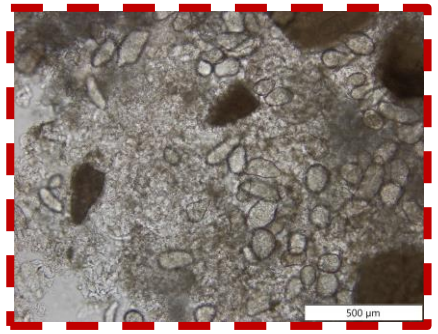
100% raw-milled flour

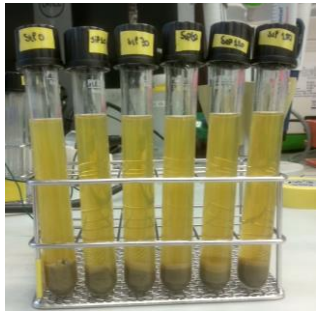




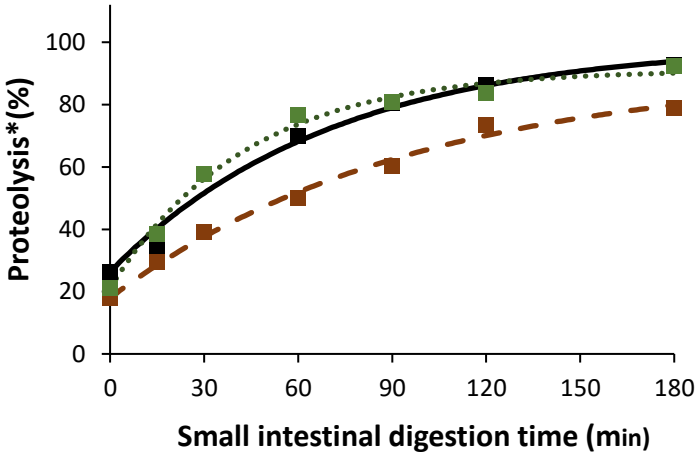
Lentil pasta

30% isolated cotyledon cell powder





In vitro digestion
INFOGEST 2.0



**hydrolyzed readily bioaccessible protein*

30% whole precooked lentil powder