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The importance of oral processing of bean-based wraps with different microstructures on starch digestion: an *in vivo* and *in vitro* study

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Cellular pulse flours, containing intact cotyledon cells, can be incorporated in various food products to stimulate pulse consumption. This has been shown to slow gastric and small intestinal nutrient digestion compared to traditional, raw-milled flours. Though increasing evidence points to the considerable contribution of the oral phase in the digestion of solid starch-rich food products, this phase has been omitted from most studies. Therefore, this study applied both *in vivo* and *in vitro* oral processes to study their impact on starch digestion in innovative bean-based wraps with distinct microstructures. *In vivo* mastication was performed by 38 participants, who showed large differences in chewing behaviour. However, this variability did not affect bolus microstructure, with intact cells present in all boluses of wraps containing cellular flour. Incorporation of these cells significantly decreased oral starch hydrolysis from 10 to 7%, although large inter-individual differences were seen. This variation probably contributed to the absence of clear correlations between mastication parameters and starch digestibility. All tested *in vitro* oral phase simulations resulted in similar bolus microstructures to *in vivo* observations, with intact cells causing a non-significant decrease in starch digestion. *In vitro* amylolysis levels were within the *in vivo* range, while the exact extent was significantly affected by the applied amylase activity. Moreover, *Bacillus* sp. amylase was shown to be a suitable, cost-efficient alternative to human salivary amylase. As substantial amounts of starch were hydrolysed, this study highlights the importance of considering a relevant oral phase when evaluating the digestibility of starch-rich solid foods.

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1 Introduction

Despite substantial environmental and nutritional benefits, current consumption of pulses, such as common beans, largely falls beneath the recommended intake, especially in the Western world.[1,2] To stimulate the intake of this protein-, fibre- and micronutrient-rich food source, pulse seeds can be milled into flours, which can be used in various, more commonly consumed food products.[3,4] When raw pulse seeds are mechanically disintegrated, the cell walls break open. As a consequence, nutrients in these traditional, raw-milled flours, such as starch and protein, are released from the cotyledon cells.[5–8] However, recent studies have shown the possibility of producing pulse-based flours with intact cotyledon cells, in which intracellular nutrients are bioencapsulated by the cell wall.[5–7,9–11] This can be achieved by hydrothermally treating the pulse seeds before mechanically disintegrating and drying them, which enables cell separation due to solubilisation of pectin in the cell wall and middle lamella.[12] In these innovative cellular flours, the intact cell wall forms a barrier for diffusion of digestion enzymes. Therefore, digestion of nutrients is slowed down, which in turn has been associated with various health benefits, including a lower glycaemic index and prolonged feeling of satiety.[5–7,9,13–17]

Even though they have favourable health-promoting properties, the application of cellular flours in real food products, such as bakery goods, entails specific challenges. Due to the fact that starch and protein are bioencapsulated by the cell wall, they are less able to form a network than when they are freely present, as occurs in products made with traditional, raw-milled flours.[10,11,18,19] As a consequence, the structure of the food product in which these intact cells are incorporated is affected. Therefore, it has been suggested to blend cellular flours with raw-milled pulse flours or traditional wheat flours, to ensure sufficient product quality while retaining potential health benefits.[5,10,11,13,20] The latter contains gluten, important in governing structural properties, and could therefore substantially increase the acceptability of bakery products with cellular flour inclusion. Furthermore, combining a wheat-based and pulse-based flour could be interesting from a nutri-

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tional point of view, due to their complementary essential amino acid composition.[3,21]

Earlier research has evaluated the impact of flour microstructure on the digestive properties of some food applications, such as pasta and bread rolls.[13,20,22–24] These studies primarily focused on gastric and small intestinal digestion, with oral digestion being simplified or not considered. However, increasing evidence points to the significance of the oral phase, especially during digestion of solid, starch-rich food products.[25,26] During oral processing, solid foods are mechanically disintegrated to smaller particle sizes. Moreover, the food is mixed with saliva, which contains α-amylase, and a swallowable bolus is formed. Salivary amylase initiates the hydrolysis of starch into smaller metabolites, such as maltose and maltotriose.[25] Despite the short duration of the oral phase, the contribution of this enzyme to starch digestion is increasingly shown to be substantial, especially since it can remain active for a considerable time in the stomach as well.[25–29] Nevertheless, digestion in the oral phase is currently understudied for starch-rich foods, especially for the case of products containing cellular pulse flour.

The most realistic way to study oral processing is through *in vivo* studies, in which human subjects are asked to chew a certain food product. However, performing *in vivo* mastication is not always so straightforward given volume and ethical constraints, the need for food-grade sample production conditions, and the presence of substantial differences in the chewing behaviour between individuals.[30–32] Therefore, it is essential to develop relevant, standardized *in vitro* oral processing methods, which can be applied in a reproducible way. Nevertheless, to enable the development of such accurate *in vitro* oral phases, information about *in vivo* disintegration patterns is required, which is currently lacking for solid food products with cellular flour inclusion.

In this study, it was evaluated how oral processing impacts the digestion of starch in innovative bean-based wraps with different microstructural properties. More specifically, cellular common bean flour was included in wraps made with either traditional, raw-milled bean flour, or wheat flour. These samples were then compared to a wrap containing only traditional bean flour. Wraps were chosen as a relevant, widely consumed low moisture application, in which the gluten network is of lower importance to govern food structure as compared to for example bread. On the one hand, an *in vivo* mastication study (38 participants) was performed for every sample, during which mastication parameters, such as chewing time and number of chews, of all participants were recorded. On the other hand, with the aim of optimizing an *in vitro* mastication protocol for this food product, the impact of three different *in vitro* oral processing approaches was tested as an alternative to *in vivo* mastication. It was hypothesized that in both *in vivo* and *in vitro* oral processing, the presence of intact cotyledon cells would decrease the level of oral starch digestion. Moreover, it was hypothesized that differences in individual *in vivo* chewing parameters would affect oral starch digestion levels.

2 Materials and methods

2.1 Materials

Red beans (*Phaseolus vulgaris* L.), var. Epic, were grown and harvested in Merelbeke, Belgium, in 2023, and kindly donated by Casibeans (Melsele, Belgium). Dry seeds (moisture content <10%) were manually cleaned and sorted, after which they were stored below their glass transition temperature at −40 °C until use. Reagents were obtained from Merck KGaA (Germany), except for the Total Starch Kit (Megazyme, Bray, Ireland). Regarding the enzymes used for *in vitro* oral processing, pooled human saliva (991-05-P) was obtained from Lee Biosolutions (Maryland Heights, MO, USA), and α-amylase from *Bacillus* sp. (10070, 53.5 U mg^{-1}) was obtained from Merck KGaA. Wheat flour (W_{ref}) and sunflower oil were obtained from a local supermarket (Delhaize, Belgium).

2.2 Production of red bean flours

To produce a food-grade traditional, raw-milled bean flour, also called reference flour (B_{ref}), raw red bean seeds were milled to pass through a 500 μm mesh using a rotor beater mill (SR 300, Retsch, Germany) at the facilities of Food Pilot (Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) & Flanders' Food, Melle, Belgium). Food-grade cellular bean flour (B_{cell}) was produced by firstly soaking raw seeds in standardized water (1 :35 w/v) for 16 h at room temperature. Soaked beans were manually dehulled and cooked in standardized water (1 : 3 w/v) for 90 min at 95 °C, after which the cooked beans were disintegrated in the cooking water for 5 min using a hand blender. The applied hydrothermal process was shown to result in complete cell separation, yielding a maximal proportion of intact cells (data not shown). Finally, the obtained slurry was freeze-dried (48 h, 0.02 mbar) in a food-grade lyophilizer (Epsilon 2-10D LSCplus, Christ, Osterode, Germany) at the facilities of Food Pilot to result in a dry cellular flour.

2.3 Wrap preparation

In an initial test, it was studied how much cellular flour could be included in wraps without substantial quality loss. To this end, 10, 25 and 50% (*w/w*) of cellular bean flour was blended with reference bean flour or wheat flour. Wraps were prepared by mixing 30 mL of water with 50 g of flour (blend), kneading manually to obtain a dough, and rolling out the dough into a thin sheet. Wrap thickness was kept constant over different formulations (approximately 1 mm). Wraps were coated with a standardized amount of sunflower oil and baked in a pan for exactly 2 min on each side until sufficiently baked. Based on qualitative assessment of wrap quality when these proportions of cellular flour were included (section 3.1) and the substantial attenuation in starch digestion observed upon incorporation of low proportions (from 10% on) of cellular bean flour in our previous work on flour model systems,[5] one cellular flour inclusion level was selected for wraps intended for the *in vivo* and *in vitro* study, *i.e.*, 20%. In total, three wrap formulations, which were deemed to result in acceptable quality, were used in the oral processing studies. Wraps consisted of (i) 100% raw-milled, reference red

bean flour ($100B_{ref}$), (ii) 80% reference and 20% cellular red bean flour ($80B_{ref}\_20B_{cell}$), and (iii) 80% wheat flour and 20% cellular red bean flour ($80W_{ref}\_20B_{cell}$).

2.4 Compositional analysis

Of all produced flours and wraps, the moisture content was determined following freeze-drying (24 h, 0.1 mbar, Alpha 2–4 LSC plus, Christ, Osterode, Germany). The starch, protein, lipid, and ash content were analysed exactly as described before.[5] Furthermore, the fiber-rich residue, consisting of, among others, cell wall polysaccharides and different sugars, was calculated as the difference between the total composition and the determined nutrient levels.

2.5 *In vivo* mastication study

To assess the impact of wrap microstructure on *in vivo* oral starch digestion, a mastication study was conducted, with a setup based on Pallares Pallares *et al.* (2019).[31] Three mastication sessions were held with a minimum of 1 week in between, with one wrap type assessed per session (first the $100B_{ref}$ wrap, then the $80B_{ref}\_20B_{cell}$ wrap, and finally the $80W_{ref}\_20B_{cell}$ wrap). Exclusion criteria for participation were incomplete dentition or any known allergy to common bean or wheat. In total, 38 participants were recruited, between the ages of 20 and 52 years old. The mastication study was approved by the Social and Societal Ethics Committee (SMEC) of KU Leuven, Belgium (file number G-2024-8566). In accordance with the guidelines provided by the Declaration of Helsinki for performing human trials, all participants were provided with detailed information on the targets and methodology of the study, and were asked to sign an informed consent form.

Participants were instructed not to consume any food or beverages, with the exception of water, for at least 1.5 h before the start of each mastication session. Participants were separated in sensory booths and asked to chew ten equal, bite-sized portions of wrap, each weighing 6 g. This setup is visualised in SI 1. The first two portions could be swallowed and were used as training sequences to identify the participants' swallowing limit. Participants subsequently chewed four portions until reaching their swallowing threshold, and expectorated the obtained boluses one by one into a single plastic container on ice. During these four sequences, participants were asked to record the time necessary to chew each portion. To ensure the collection of all bolus particles after chewing 4 portions, participants were instructed to rinse their mouths using 10 mL of water, which was also expelled into the same plastic container. After this, the remaining four portions were chewed and expectorated into the same plastic container, followed by another rinsing step with water, which was also collected together with the other expectorated portions (8 portions in total + 20 mL water). During these final four sequences, participants were not only asked to record the chewing duration, but also the number of chews needed to reach the swallowing threshold. For each participant, an average chewing time and number of chews were calculated for each wrap type. In the end, for each participant, one pooled bolus was obtained, which included all 8 expectorated wrap portions of 6 g and 20 mL of water. This entire bolus was weighed and divided into separate smaller portions, depending on the subsequent analysis. For analysis of microstructure, the bolus was frozen immediately using liquid nitrogen and stored at −80 °C until use. To evaluate oral starch digestion, salivary amylase activity was stopped by addition of 1 mL of 1M HCl to 6 g of bolus before freezing and storing as before.[30]

2.6 *In vitro* oral processing

Different *in vitro* oral processing strategies were applied to mimic *in vivo* mastication, which were each performed two independent times to test their reproducibility. Two different salivary amylase activities were tested to span *in vivo* observed variability.[30,33–36] Firstly, saliva was diluted to obtain an activity of 150 U mL^{-1}, in accordance with the INFOGEST procedure.[37] Secondly, pure, commercially obtained saliva which had an activity of 302 U mL^{-1} was used. Next to these two activities, amylase from *Bacillus* sp. was tested as a cost-efficient alternative to human saliva. More specifically, in accordance with the standardized INFOGEST procedure on semi-dynamic *in vitro* digestion, oral fluids were added to all wraps in a 1 : 1 (*w*/*v*) ratio of wrap dry weight to fluids.[37] These oral fluids consisted of either (i) 80% electrolyte-containing simulated salivary fluid (SSF, pH 7) and 20% $CaCl_2$ (7.5 mM) in which pooled human saliva was diluted, (ii) undiluted human saliva, or (iii) 80% SSF and 20% $CaCl_2$ in which *Bacillus* sp. amylase was dissolved to achieve an activity of 150 U mL^{-1}. Fluids were added to the wraps in the correct ratio and the mixture was mashed manually using mortar and pestle for 2 min.[37,38] Similar to the *in vivo* mastication study, enzymatic activity in the obtained boluses was stopped by addition of 1M HCl.

2.7 Microstructural analysis of boluses

The microstructure of *in vivo* and *in vitro* generated boluses was characterized following wet-sieving using a vibratory sieve system (AS200, Retsch, Germany).[6,31] Five sieves with different mesh sizes (3.1–1.4–0.125–0.056–0.020 mm) were stacked. Boluses were placed on top, and two runs of 30 s were performed, during which samples were sieved with a vibratory amplitude of 2.5 mm through a continuous flow of demineralized water. The fractions remaining on each sieve were analysed using light microscopy (Axio Observer 3 with LED-Driver LEDD1B and digital camera Axiocam 305 color (Carl Zeiss, Zaventem, Belgium)). Additionally, unsieved boluses were examined macroscopically by taking photographs.

2.8 Determination of starch digestion

The amount of reducing sugars released from starch through activity of amylase was determined using the dinitrosalicylic acid (DNS) method, which has a high throughput and can thus easily be used to analyse a large amount of samples.[6,39,40] Firstly, 1 g of *in vivo* or *in vitro* generated bolus was weighed in triplicate, and 4 mL of cold demineralized water was added. All samples were centrifuged (1000*g*, 10 min, 4 °C)[30] and the obtained supernatant was used for DNS analysis. In short, 150 μL of diluted supernatant was added to 75 μL DNS reagent

in duplicate, followed by centrifugation (1000*g*, 2 min, 20 °C) and incubation at 100 °C for 15 min. After cooling, samples were diluted with 675 μL demineralized water, and the absorbance was measured at 540 nm. Using a maltose calibration curve (0–2 mg mL^{-1}), the absorbance was converted to a concentration of reducing sugars per gram of analysed sample. Using the determined bolus dry matter, this value was converted to a concentration per gram of dry bolus. From this, the concentration of reducing sugars in the entire bolus obtained after *in vivo* mastication or *in vitro* oral processing was calculated by multiplying this value with the total bolus dry weight. This concentration was converted to starch equivalents using a conversion factor of 0.947. Finally, to account for differences in starch content between the different wrap types, which could influence the amount of metabolites released, the obtained concentration was divided by the total starch present in the wrap portions. This calculation, shown in eqn (1), reflects the degree of starch digestion during oral processing.

$$\text{Starch hydrolysis } (\%) = \frac{\text{Maltose eq. in bolus} \times 0.947}{\text{Starch content wraps}} \times 100 \quad (1)$$

2.9 Statistical analysis

A *post hoc* test to determine the power of the *in vivo* study when using 38 participants was performed using *G* × power. At this sample size, a power of 0.99 was obtained, showing that a sufficient number of participants were recruited to investigate the impact of wrap microstructure on oral digestion. Boxplots of mastication parameters (bolus weight, chewing time and number of chews) and release of starch metabolites upon *in vivo* mastication were constructed using JMP Pro 17 (SAS Institute Inc., Cary, NC, USA). To evaluate differences in average values of these parameters between different sessions of the *in vivo* mastication study, one-way ANOVA and Tukey tests ($p < 0.05$) were employed after assessing the normality of the data. Differences in oral starch digestion extent across wraps when applying varying *in vitro* mastication conditions were studied in the same way. To check if repeated measurements of the same individual affected results by introducing intra-individual variability, a mixed model was applied, with participant as a random effect. Nevertheless, no impact of intra-individual variability was observed, with inter-individual differences having a much larger influence on results. Therefore, this model will not be discussed further. Finally, correlations between different parameters of the *in vivo* mastication study, both within each session as well as between all sessions, were assessed using Pearson correlation coefficients. These correlations were visualized in a heat map using JMP Pro 17.

3 Results and discussion

3.1 Incorporation of cellular flour in wraps

Due to the hydrothermal treatment applied in the production of cellular flour, intact cotyledon cells, in which nutrients are bioencapsulated, formed its predominant microstructural fraction, compared to abundant free starch granules visible in the reference bean flour (SI 2). As mentioned before, the absence of free biopolymers reduces the network-forming potential of cellular flour, thereby limiting its maximum degree of incorporation in food products.[10,11,18,19] Even more so, it is possible that the presence of cotyledon cells disrupts network formation by free starch and protein coming from the reference bean flour or wheat flour, thereby potentially decreasing dough and product cohesiveness. Therefore, a preliminary study of the maximal incorporation degree of cellular flour yielding acceptable wrap quality was performed.

In Fig. 1, wraps made with reference bean flour (top row) or wheat flour (bottom row) supplemented with 0, 10, 25 and 50% (*w*/*w*) cellular bean flour are shown. Pulse-based flours have been successfully used as (partial) replacement of wheat flour in various bakery products, going from breads to cakes and biscuits.[3,13,41,42] In the current study, wraps made with 100% reference bean flour (Fig. 1A) were also deemed to be of high quality, although denser and smaller than their wheat-based counterpart (Fig. 1E), in line with reported observations for various bakery products.[3,42] This can probably be attributed to the absence of gluten, which are important in determining product structure. It was expected that the presence of such gluten in the wheat-based wrap would increase the percentage of cellular bean flour that could be included without significantly affecting product quality. Nevertheless, upon incorporation of cellular flour, the structure of both the completely bean-based as well as the wheat-based wrap was substantially affected, especially at the highest inclusion level (50%). At this substitution level, the structure of the wrap turned more brittle and crumbly, and the edges became irregular (Fig. 1D and H). In contrast, lower inclusion levels (10 and 25% of cellular flour) resulted in a structure more similar to wraps without cellular flour addition, with more smooth edges (Fig. 1A–C and E–G). Based on this qualitative assessment, it was decided that sufficient product quality could be ensured at a maximum incorporation level of 25% of cellular flour in both the bean-based and wheat-based wraps. In an earlier study, 60% of chickpea cells was included in wheat-based bread rolls, however, additional gluten had to be added in this case to guarantee appropriate quality.[13] As it was opted not to supplement gluten in the current study, ultimately, an inclusion percentage of 20% cellular flour was selected for both the bean-based and wheat-based wraps ($80B_{ref}\_20B_{cell}$ and $80W_{ref}\_20B_{cell}$, respectively), to guarantee their acceptability during the *in vivo* mastication study. These wraps were then compared to a bean-based wrap without any intact cells present ($100B_{ref}$, section 2.3). It was shown in our previous work that very low proportions of cellular bean flour (from 10% on) could decrease the rate of starch digestion in model systems.[5] Therefore, it was hypothesized that 20% of intact cells would be sufficient to substantially affect the hydrolysis of starch during oral processing. This inclusion percentage is similar to what was reported before for incorporation of lentil and chickpea cells (30%) in pasta[22,24] and bread rolls[13] with impacted starch digestibility or glycaemic responses.

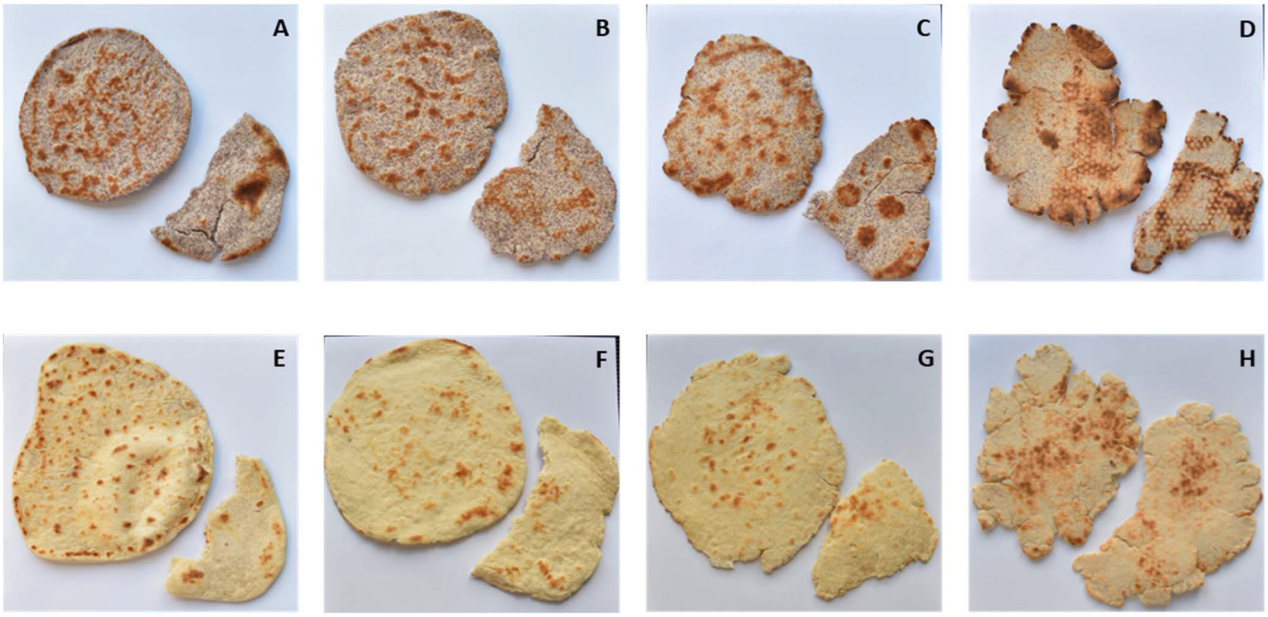

Fig. 1 Wraps produced with raw-milled, reference Epic red bean flour (top row, A–D) or wheat flour (bottom row, E–H) with incorporation of 0 (A and E), 10 (B and F), 25 (C and G) or 50% (D and H) (*w/w*) cellular red bean flour.

3.2 Wrap composition

Based on the results of the previous section, bean- and wheat-based wraps containing 20% of cellular bean flour ($80B_{ref}\_20B_{cell}$ and $80W_{ref}\_20B_{cell}$, respectively) were made, and compared to a bean-based wrap without cellular flour inclusion ($100B_{ref}$). The composition of these different wrap types is depicted in Table 1. While the completely bean-based wraps ($100B_{ref}$ and $80B_{ref}\_20B_{cell}$) showed a similar composition, the wrap in which wheat and bean flour were combined ($80W_{ref}\_20B_{cell}$) contained substantially higher levels of starch (approximately 47% *vs.* 25–27%). Additionally, although inclusion of pulse flour has been shown to substantially increase the protein content of bakery products,[3] the protein level of the composite wrap was lower than the fully bean-based wraps (approximately 9% *vs.* 16%). Nevertheless, the combination of wheat and common beans could be interesting in terms of essential amino acid intake. As cereals and pulses have a complementary amino acid pattern, combining them has been put forward as an effective way to enhance protein quality in terms of amino acid score.[3,21,43–46] Due to constraints in product quality, more specific product structure and cohesiveness (section 3.1), the composite wrap in the current study contained a lower level of bean-based flour than the optimal combination ratio of 40 : 60 cereals : pulses.[21,44] However, it can be hypothesized that including 20% of bean flour already substantially increased protein quality by providing a more balanced essential amino acid composition.

The lipid content of all wraps was comparable, but the wheat-based wrap consisted of substantially less ash and fiber-rich residue than the bean-based wraps. The reference bean flour was produced by milling raw bean seeds (section 2.2), meaning that the seed coat was retained in the flour, and thus also present in the wraps. This hull is rich in dietary fiber, such as different cell wall polysaccharides, and also contains various minerals (*e.g.*, calcium and iron).[47] In contrast, the wheat flour used for producing the $80W_{ref}\_20B_{cell}$ wrap was refined, contributing to its lower ash and fiber content, even though wheat bran as such also consists of large amounts of dietary fiber and minerals.[48]

Table 1 Average proximate composition with standard deviation of wraps made with 100% raw-milled, reference red bean flour ($100B_{ref}$), 80% reference and 20% cellular red bean flour ($80B_{ref}\_20B_{cell}$) or 80% wheat flour and 20% cellular bean flour ($80W_{ref}\_20B_{cell}$), expressed in g/100 g wrap

Wrap type	Moisture	Starch	Protein	Lipid	Ash	Fiber-rich residue[a]
$100B_{ref}$	23.67 ± 4.81	25.70 ± 0.31	16.18 ± 0.16	7.53 ± 0.09	3.23 ± 0.25	23.69 ± 4.83
$80B_{ref}\_20B_{cell}$	24.69 ± 4.58	27.41 ± 0.65	16.35 ± 0.31	6.67 ± 0.56	3.26 ± 0.13	21.61 ± 4.67
$80W_{ref}\_20B_{cell}$	24.96 ± 0.49	46.85 ± 1.92	8.82 ± 0.21	8.84 ± 0.02	1.02 ± 0.04	9.49 ± 1.99

[a] Calculated as 100 – moisture – starch – protein – lipid – ash, contains fibers and sugars such as oligosaccharides.

3.3 *In vivo* mastication

3.3.1 Mastication parameters. An *in vivo* study was performed during which three wrap types were masticated: a bean-based wrap without cellular flour inclusion ($100B_{ref}$), and bean and wheat wraps in which 20% cellular flour was incorporated ($80B_{ref}\_20B_{cell}$ and $80W_{ref}\_20B_{cell}$, respectively). For all 38 participants of the mastication study, the total bolus weight, average chewing time and average number of chews necessary to reach the swallowing threshold for 1 portion of wrap (6 g) were recorded, as depicted in Fig. 2. In Table 2, average values of mastication parameters across all participants are shown. Between different wrap types, no significant differences could be discerned ($p > 0.05$). This indicates that wrap formulation did not substantially affect chewing parameters or bolus weight, as also visualized in the boxplots in Fig. 2, which are all situated around similar values for each mastication parameter. While over the different wrap types, intra-individual differences in mastication parameters were small (*i.e.*, constant behaviour, independent of wrap type), large inter-individual differences were observed. This is shown by the large standard deviations of the mastication parameters reported in Table 2, and by the large spread of the boxplots in Fig. 2. For example, within one wrap type, bolus weight, obtained after pooling all expectorated wrap portions (8 chewed portions of 6 g + 2 × 10 mL rinsing water), differed from approximately 60 to over 130 g. It should be noted that for some participants, the obtained bolus weight was lower

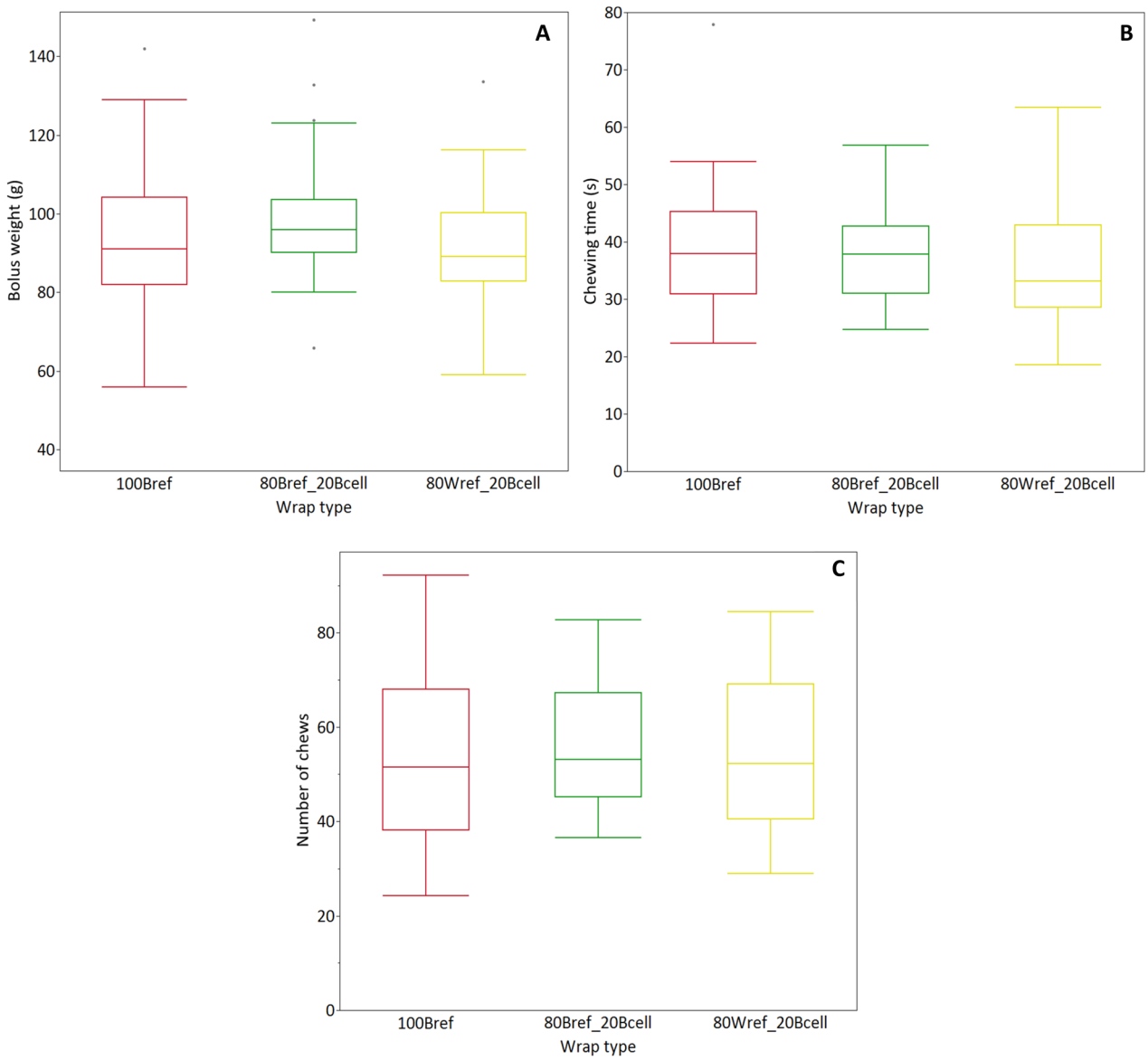

Fig. 2 *In vivo* mastication parameters bolus weight (A), chewing time (B) and number of chews (C) of 38 participants after chewing of wraps consisting of 100% raw-milled, reference red bean flour ($100B_{ref}$, red), 80% reference and 20% cellular red bean flour ($80B_{ref}\_20B_{cell}$, green) or 80% wheat flour and 20% cellular bean flour ($80W_{ref}\_20B_{cell}$, yellow).

Table 2 Mastication parameters and starch hydrolysis during *in vivo* mastication by 38 participants of wraps consisting of 100% reference, raw-milled red bean flour ($100B_{ref}$), 80% raw-milled and 20% cellular red bean flour ($80B_{ref}\_20B_{cell}$) or 80% wheat flour and 20% cellular bean flour ($80W_{ref}\_20B_{cell}$). Within each parameter, significant differences between average values are indicated by different superscript letters (Tukey test, $p < 0.05$). Minimum and maximum values measured across participants are depicted within brackets.

Wrap type	Bolus weight (g)	Chewing time (s)	Number of chews	Starch hydrolysis (%)
$100B_{ref}$	93.94 ± 17.29^{a} (56.00–141.92)	39.17 ± 11.50^{a} (22.40–77.88)	54 ± 18^{a} (24–92)	9.52 ± 4.52^{a} (0.62–20.26)
$80B_{ref}\_20B_{cell}$	98.34 ± 15.30^{a} (65.84–149.27)	38.03 ± 8.69^{a} (24.72–56.88)	56 ± 15^{a} (37–83)	7.09 ± 3.85^{b} (0.45–16.88)
$80W_{ref}\_20B_{cell}$	92.16 ± 13.89^{a} (59.10–133.60)	35.45 ± 9.54^{a} (18.53–63.50)	54 ± 17^{a} (29–85)	7.11 ± 3.13^{b} (1.71–15.27)

than expected, based on the weight of administered wrap portions and rinsing water. It can be hypothesized that some wrap particles remained stuck between these participants' teeth, or that they partially swallowed some wrap pieces. Furthermore, due to the fact that the bolus weight also includes 20 mL of water used to rinse mouths after mastication (section 2.5), it does not solely correspond to the wrap weight and incorporation of saliva. Nevertheless, as this rinsing water was kept constant over all participants, the large variation in bolus weight suggests a substantial difference in saliva uptake by the bolus, depending on the individual. Based on earlier reports, saliva flow rate largely depends on, for example, age, sex, and body profile,[49,50] which could have contributed to the observed differences. This considerable difference in saliva uptake is also reflected in Fig. 3, which shows boluses of participants with the lowest (A–C) and highest (D–F) bolus weight for all

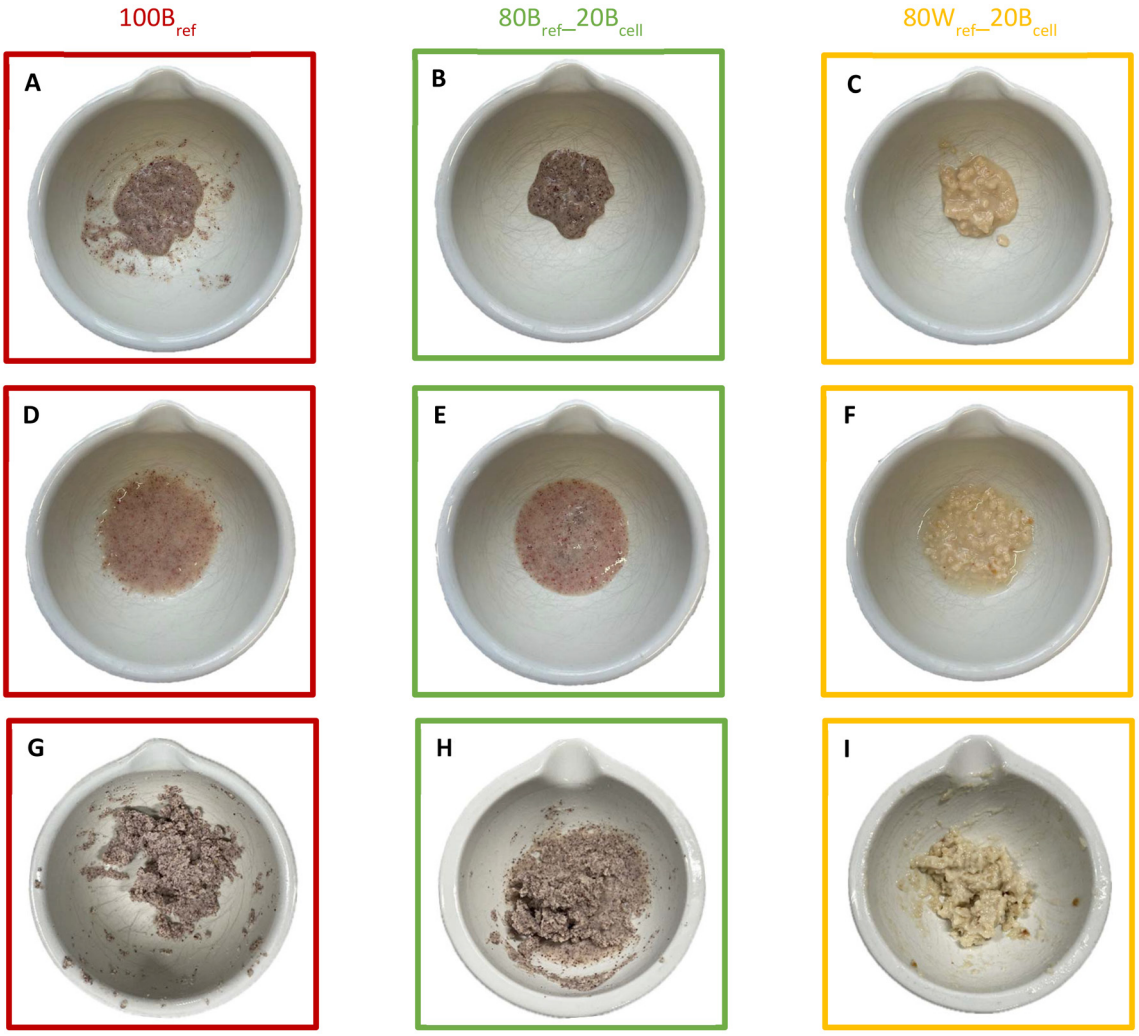

Fig. 3 Selected unsieved boluses obtained after *in vivo* mastication by the participant with the lowest (A–C) and highest (D–F) bolus weight, and after *in vitro* processing (G–I) of wraps made with 100% raw-milled, reference Epic red bean flour ($100B_{ref}$, red), 80% reference and 20% cellular bean flour ($80B_{ref}\_20B_{cell}$, green), and 80% wheat flour and 20% cellular bean flour ($80W_{ref}\_20B_{cell}$, yellow).

three wrap types. It is clear that the consistency of the expectorated boluses is vastly different between the two individuals, with samples being much more hydrated in the participant with the highest bolus weight. The physical properties of boluses at the swallowing threshold were thus substantially different between participants. Similar to bolus weight, chewing time and number of chews showed a large interpersonal variability, while not being affected by wrap type. The fact that substantial differences existed in the chewing behaviour of different participants is in line with previous reports for different food products, such as beans, rice and pasta.[30–32,51]

3.3.2 ***In vivo* bolus microstructure.** For all three wrap types, between 80 and 90% of the *in vivo* generated bolus particles were smaller than 1.4 mm, based on the weight of the sample maintained on different sieves following wet-sieving (data not shown). This indicates that, independent of wrap formulation, chewing resulted in similar ratios of larger to smaller particles. This is similar to earlier reports on differently processed bean samples.[30] In contrast to their comparable particle size distribution, bolus microstructure differed vastly between all wrap types. This is visualized in Fig. 4, which depicts microscopic pictures of the 56–125 μm fraction obtained *via* wet-sieving of boluses of one representative participant. This size fraction was studied in more detail due to the potential presence of intact cotyledon cells, based on their previously reported size of approximately 100 μm.[5,9,52,53] The $100B_{ref}$ wrap, made with 100% reference, raw-milled bean flour, mostly showed small seed coat particles and some free nutrients released from the cotyledon cells during milling, such as starch granules and protein bodies. In contrast, microscopic pictures of boluses of the other two wrap types, $80B_{ref}\_20B_{cell}$ and $80W_{ref}\_20B_{cell}$, clearly showed the presence of intact cells, stemming from the incorporation of cellular flour in the wrap. This means that these cotyledon cells not only (at least partially) persisted during the wrap-making process, including dough preparation and baking, but also during mastication. Similar, earlier studies have shown intact pulse cells to persist during the manufacturing of pasta, both without and with extrusion,[22,24] bread rolls[13] and biscuits.[20] Moreover, it was shown that cotyledon cells form a prominent microstructural fraction in boluses obtained after mastication of beans.[30,31]

3.3.3 Oral starch hydrolysis. Boxplots visualizing the extent of oral starch digestion upon *in vivo* mastication of the three different wrap types are depicted in Fig. 5. Average values across all 38 participants are shown in Table 2. Starch hydrolysis values ranged from 1 to 20%, 1 to 17%, and 2 to 15% for the $100B_{ref}$, $80B_{ref}\_20B_{cell}$, and $80W_{ref}\_20B_{cell}$ wrap, respectively. This is in line with earlier reports of *in vivo* oral amylolysis in starch-rich products such as rice, pasta, bread, and chickpeas, which reached up to approximately 20%, depending on the exact food and the individual participant.[27,32,34,54,55] Moreover, it highlights the importance of the oral phase in initiating amylolysis, as a substantial part of starch was already hydrolysed in this first digestion phase. Additionally, as salivary amylase was shown to remain active during the beginning of the gastric phase, when the *in vivo* pH has not yet

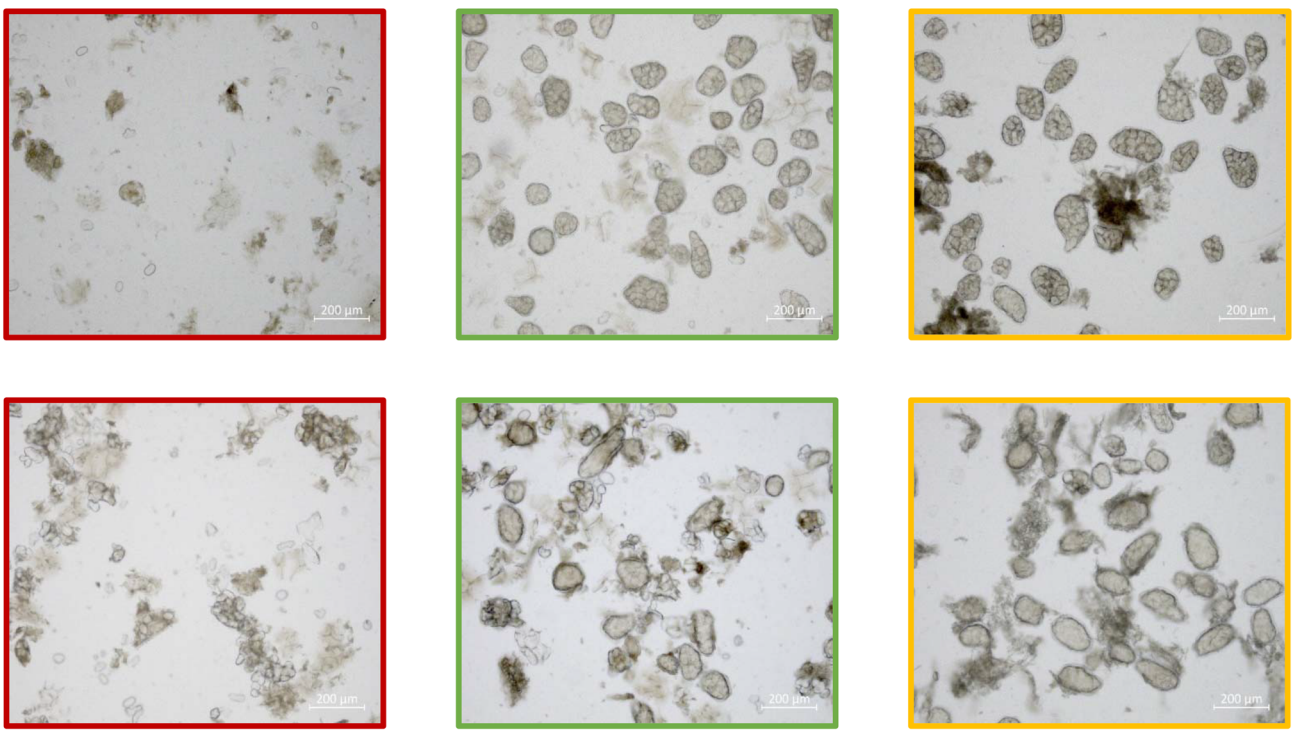

Fig. 4 Microscopic pictures of the 56–125 μm fraction obtained through wet-sieving of selected boluses after *in vivo* (top row) and *in vitro* (bottom row) mastication of wraps made with 100% raw-milled, reference Epic red bean flour ($100B_{ref}$, red), 80% reference and 20% cellular bean flour ($80B_{ref}\_20B_{cell}$, green), and 80% wheat flour and 20% cellular bean flour ($80W_{ref}\_20B_{cell}$, yellow). Scale bar represents 200 μm.

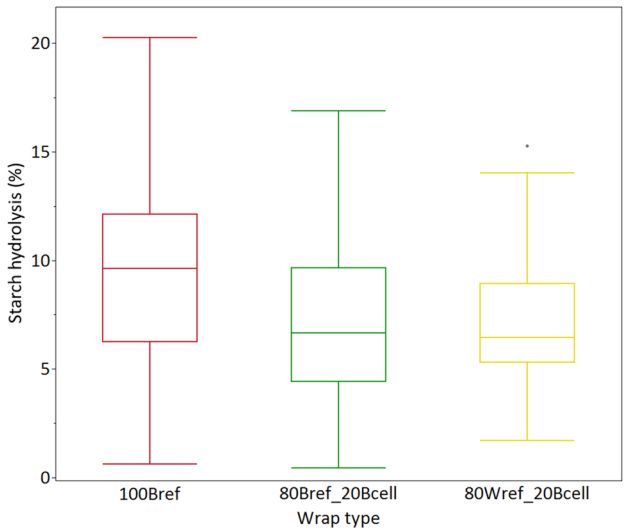

Fig. 5 *In vivo* starch digestion during mastication by 38 participants of wraps consisting of 100% raw-milled, reference red bean flour ($100B_{ref}$, red), 80% reference and 20% cellular red bean flour ($80B_{ref}$–$20B_{cell}$, green) or 80% wheat flour and 20% cellular bean flour ($80W_{ref}$–$20B_{cell}$, yellow).

decreased substantially, its contribution to starch digestion could be even more important due to its action beyond the oral phase.[26–29] Within each wrap type, large differences in *in vivo* oral amylolysis could be discerned. This inter-individual variation is confirmed by both the large standard deviations shown in Table 2 and the spread of the boxplots in Fig. 5. One potential explanation for these observed differences is the substantial reported variation in salivary amylase level and activity between individuals.[30,33–36] In this context, a higher activity of salivary amylase has been linked to a greater release of sugars from starch.[33,34,54,56] Although not measured, differences in enzymatic activity could have thus substantially affected the degree of oral starch digestion in the different participants. Furthermore, in section 3.3.1, it was discussed how mastication parameters across all participants vastly differed, which may have contributed to the observed variability in amylolysis. It can be hypothesized that links between these chewing parameters and starch digestion can exist, and this is studied in section 3.3.4.

Despite the large variability in oral starch digestion between the participants of the mastication study, a clear effect of wrap type was observed. This is in contrast to the earlier mentioned mastication parameters, such as chewing time, which were shown to remain constant, independent of wrap formulation (section 3.3.1). More specifically, Fig. 5 shows a reduction in median starch hydrolysis extent in the $80B_{ref}$–$20B_{cell}$ and $80W_{ref}$–$20B_{cell}$ wraps compared to the $100B_{ref}$ wrap. Even more so, Table 2 shows that upon inclusion of cellular flour in the wraps, the average amylolysis level decreased slightly but significantly from approximately 9 to 7%. In line with this, the intact cotyledon cells in cellular pulse-based flours have been shown multiple times to retard or reduce digestion of starch in model systems or food products.[5,7,9,13,17,22] This is caused by the intact cell wall and protein matrix, which both form a barrier for enzyme diffusion, thereby decreasing the accessibility of digestive enzymes to starch.[14,15,57] Moreover, the cell wall has been shown to be able to bind amylase, thereby also reducing its diffusion into the cell.[57,58] As mentioned earlier (section 3.1), our previous research observed a retarded starch digestion, even when very low proportions of cellular bean flour (from 10% on) were included in flour model systems.[5] This coincides with the current results, showing that including 20% of cellular flour into a food product can decrease digestion of starch.

While a reduced starch digestion in intact cotyledon cells compared to free nutrients has been observed both *in vitro* as well as *in vivo*, previous studies mostly focussed on small intestinal amylolysis. Interestingly, from the present study, it is clear that cellular integrity also affects the susceptibility of starch to salivary amylase, thereby already impacting amylolysis early on, in the oral phase of digestion. Similarly, it was shown before that *in vivo* oral starch digestion of bread was decreased upon inclusion of coarse semolina containing intact cell clusters.[59]

3.3.4 Correlation between mastication and oral starch digestion. To evaluate whether mastication parameters and *in vivo* starch digestion were correlated, a heat map relating these properties was constructed, as shown in Fig. 6. This heat map depicts Pearson correlation coefficients, with a red colour indicating a positive correlation. The more bright this red, the stronger the correlation, while lighter red or grey colours imply little to no correlation exists between different parameters. The exact correlation coefficients and corresponding *p*-values are given in SI 3. Within each mastication parameter, positive correlations were observed over the different wrap types (r = 0.74–0.88, $p < 0.0001$; 0.42–0.69, $p < 0.01$ and 0.61–0.79, $p < 0.0001$ for bolus weight, chewing time and numbers of chews, respectively). This means that individual chewing behaviour remained consistent over the different wrap types, which is in accordance with the non-significant differences in average values of these parameters (section 3.3.1). Furthermore, not only mastication parameters of each individual were consistent over the different wrap types, amylolysis levels were as well. This can be derived from the strong correlations in starch hydrolysis extents over the three wrap types (r = 0.72–0.81, $p < 0.0001$). In section 3.3.3, large inter-individual variations in starch digestion were observed, attributable to multiple parameters such as differences in the level and activity of salivary amylase.[30,33–36] In contrast to these substantial inter-individual variations, the observed correlations suggest that within each individual, the efficiency of salivary amylase in hydrolysing starch was similar over the different mastication sessions. Earlier research indicates that the activity of salivary amylase not only varies between people, but also within an individual, depending on for example the earlier consumption of starch or the time of day.[54] However, it was tried to minimize these effects by instructing participants not to eat or drink anything 1.5 h before the mastication study, and by organizing each session around the same time of the day.

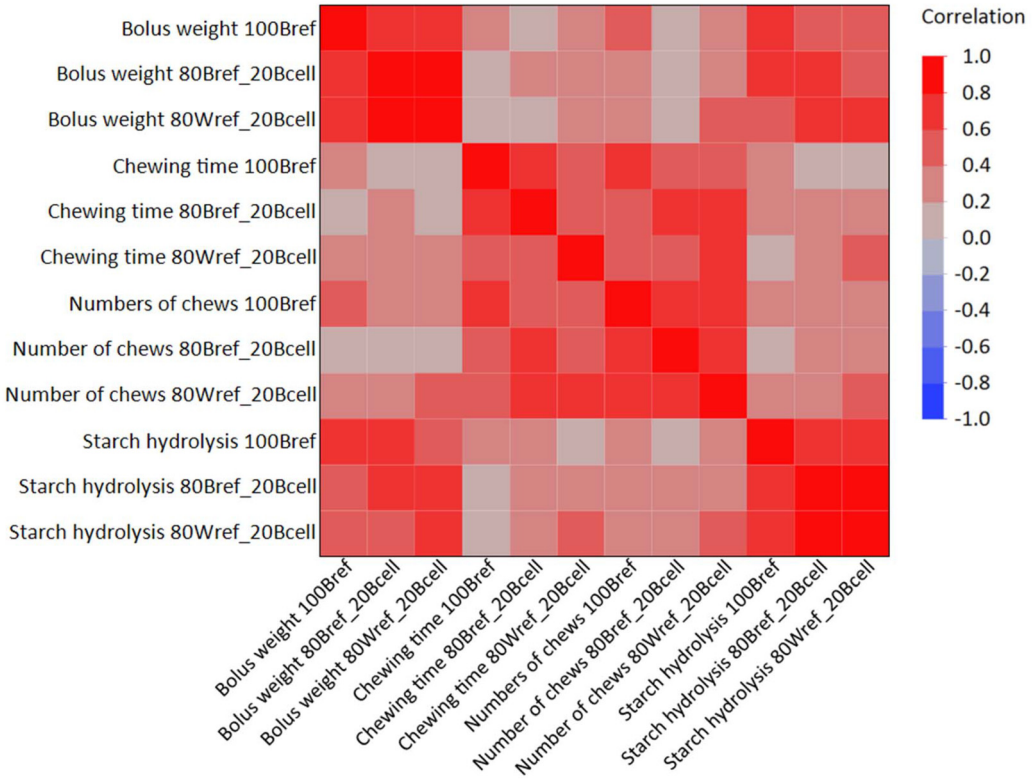

Fig. 6 Heat map visualizing Pearson correlation coefficients between bolus weight, chewing time, number of chews and level of starch hydrolysis upon *in vivo* mastication of wraps consisting of 100% raw-milled, reference red bean flour ($100B_{ref}$), 80% reference and 20% cellular red bean flour ($80B_{ref}\_20B_{cell}$) or 80% wheat flour and 20% cellular bean flour ($80W_{ref}\_20B_{cell}$).

When assessing different mastication parameters within each wrap type, strong correlations (r = 0.73–0.76, $p < 0.0001$) can be observed between chewing time and numbers of chews. If longer chewing was required to reach the swallowing limited for a portion of wrap, this thus naturally resulted in a larger number of chews. In contrast, longer chewing or an increased number of chews did not result in a higher saliva uptake, as depicted by the absence of a correlation between these parameters and bolus weight for each wrap (r = 0.17–0.41, $p >$ 0.009). This is in contrast to most previous research, which has shown that a longer oral residence time or higher number of chews increases saliva secretion and uptake.[30,34,60,61] However, earlier literature often only evaluated a limited number of participants, frequently below 20, while in the current study, mastication behaviour of 38 people was assessed. As a consequence, previous studies could have failed to capture the large variability between individuals. Nevertheless, these large inter-individual differences in the current study, which were discussed more in detail in section 3.3.1, probably prevented the observation of correlations between bolus weight on the one hand and chewing time or number of chews on the other hand. Furthermore, some previous studies show correlations between mastication duration and bolus microstructure, with longer chewing resulting in more small particles.[30,32,34] However, such relations were not observed here, with between 80 and 90% of bolus particles being smaller than 1.4 mm for all participants, independent of mastication behaviour (data not shown). This means that, in contrast to being largely affected by wrap formulation as mentioned in section 3.3.2, bolus microstructure was not impacted by individual chewing behaviour. Similar, *in vivo* mastication of common beans showed that bolus particle size distribution was not impacted by individual oral processing, but only by process-induced seed hardness.[31]

Additionally, while it was hypothesized that longer chewing would lead to a greater level of oral amylolysis, no correlations were detected between chewing time or number of chews and the extent of starch digestion (r = 0.25–0.37, $p > 0.01$). This is opposed to various previous studies on food products such as chickpeas, beans and rice, which found relations between food-saliva contact time and starch hydrolysis.[30,34,60,61] However, this can once again possibly be linked to the large inter-individual variability in chewing parameters and oral starch digestion (sections 3.3.1 and 3.3.3), with the latter potentially being the result of, among others, substantial differences in salivary amylase activity.[30,33–36] In contrast, clear positive relations were found between bolus weight and starch digestion within each wrap type (r = 0.58–0.68, $p \leq 0.0001$). This is in line with earlier reports on *in vivo* oral processing of rice, in which saliva uptake and oral starch hydrolysis were strongly correlated.[32] It is possible that, independent of chewing time or number of chews, the addition of a higher

saliva volume led to a better mixing between salivary amylase and the food matrix. While personal amylase activities may have differed, this better mixing may have improved the contact between the enzyme and starch, thereby leading to a greater amylase efficiency, and thus higher levels of amylolysis. This can also partially clarify the earlier observed correlations between starch digestion levels over the different wrap types. Individual bolus weight, and thus saliva uptake, was shown to remain stable over the different samples. People with a constant higher volume of saliva could thus potentially systematically show better mixing with the food product, and thus, independent of the wrap type, digest more starch in the oral phase compared to individuals with less efficient mixing.

3.4 Can *in vivo* mastication be simulated *in vitro*?

While an *in vivo* mastication process is the most realistic representation of the oral phase of digestion, section 3.3 clearly showed that there is a substantial variability between human subjects, which complicates standardization. Furthermore, performing an *in vivo* oral phase, for example as a start for further *in vitro* gastric and intestinal digestion, is not always straightforward. Consequently, there is a large need for *in vitro* oral processing methods, which can be performed in an easy and consistent way, and are representative for the *in vivo* process. Therefore, three different *in vitro* oral processing strategies were tested as alternatives for the observed *in vivo* mastication process. Based on the INFOGEST standardized procedure, a salivary amylase activity of 150 U mL^{-1} was applied in a first step.[37] To cover the wide range of salivary amylase activities that can occur *in vivo*,[30,33–36] which may have contributed to the large variability in oral starch digestion observed in section 3.3.3, undiluted human saliva with an activity of 302 U mL^{-1} was applied in a second step. In a final step, amylase from *Bacillus* sp. was used as a cost-effective substitute to human saliva. The impact of these three approaches on bolus structure and starch digestion was tested.

3.4.1 *In vitro* bolus microstructure. In Fig. 3, boluses generated with the first *in vitro* mastication simulation are visualized. Furthermore, microscopic pictures of the 56–125 μm fraction obtained after wet-sieving are shown in the bottom row in Fig. 4. Because the employed disintegration step was equal for all evaluated conditions (section 2.6), the structural properties of boluses of every wrap type across different oral processing strategies were identical (data not shown). As a result, the selected pictures are representative for the other applied oral phase simulation conditions as well. From Fig. 3, it is clear that addition of oral fluids in a 1 : 1 (*w*/*v*) ratio resulted in a bolus consistency more closely related to what was observed for the participant with the lowest bolus weight in the *in vivo* mastication study. Fig. 4 shows that, similar to *in vivo* samples, boluses of wraps with cellular flour ($80B_{ref}\_20B_{cell}$ and $80W_{ref}\_20B_{cell}$) contained intact cells, proving again that these cotyledon cells are retained during wrap preparation and mastication. In contrast, the $100B_{ref}$ did not show any intact cells, but primarily consisted of free starch granules and seed coat fragments, due to the breakage of cell walls during milling of the raw bean seeds.[12] In general, *in vitro* oral processing resulted in a comparable bolus (micro)structure to that obtained with *in vivo* mastication.

3.4.2 Starch digestion. Fig. 7 shows the extent of starch digestion upon applying the three different *in vitro* oral processing strategies to all wrap types twice. As earlier studies report large inter-individual variations in *in vivo* salivary amylase activity,[30,33–36] different activity levels were tested, *i.e.*, diluted and undiluted commercially obtained saliva. A rise in salivary amylase activity from 150 to 302 U mL^{-1} caused a significant increase in starch digestion in all wrap types. Even more so, the level of starch hydrolysis more than doubled, from approximately 3 to 8%, 2 to 7%, and 2 to 6% for the $100B_{ref}$, $80B_{ref}\_20B_{cell}$, and $80W_{ref}\_20B_{cell}$ wrap, respectively. Although limited in number, previous *in vitro* studies also observed a clear impact of (salivary) amylase activity on oral or small intestinal starch hydrolysis.[33,62] Moreover, as mentioned before and in line with expectations, *in vivo* studies focussing on oral digestion clearly showed amylolysis to be increased with higher enzyme activities.[33,34,54,56]

Not only different amylase activities were tested during *in vitro* oral processing, also different amylase sources were evaluated. Although human saliva is the most realistic when trying to simulate *in vivo* physiology, its high cost results in a need for appropriate alternatives. These may include pancreatic amylase, but this enzyme has been reported to also have proteolytic activity, which decreases its suitability for studying oral starch digestion. Moreover, microbial amylases, such as from *Bacillus* sp., can be applied, but it is essential to study their suitability in mimicking salivary amylase activity.[63–67] In Fig. 7, it is shown that the use of *Bacillus* sp. amylase resulted in comparable starch digestion levels to when human salivary amylase with the same activity was used, with no observed significant differences. In accordance with our results, a study on potato starch also observed a similar release of maltose upon application of amylase from both human saliva and *Bacillus* sp.[63] Although this indicates that the former may be an appropriate substitute, these results should be interpreted with caution. *Bacillus* sp. amylase was reported to also contain proteolytic activity, which is evidently not present in human saliva.[63] While not tested in the current study, this should be taken into account when deciding to apply this alternative enzyme during *in vitro* oral processing, as any unintended protein digestion may affect amylolysis, thereby decreasing result accuracy. Furthermore, it is possible that both enzymes have varying hydrolysis patterns, releasing metabolites from starch in a different way. However, such patterns were not evaluated in the current study. Moreover, the potential of this *Bacillus* sp. amylase to remain (partially) active in subsequent digestion phases, such as during gastric digestion, should also be studied and compared to human salivary amylase.

Overall, for all three applied *in vitro* oral processing methods, the level of amylolysis varied between approximately 1.5 to 8%, which is within the *in vivo* obtained range (section 3.3.3). Though in earlier studies, similar *in vitro* oral starch hydrolysis extents have been observed, the exact obtained

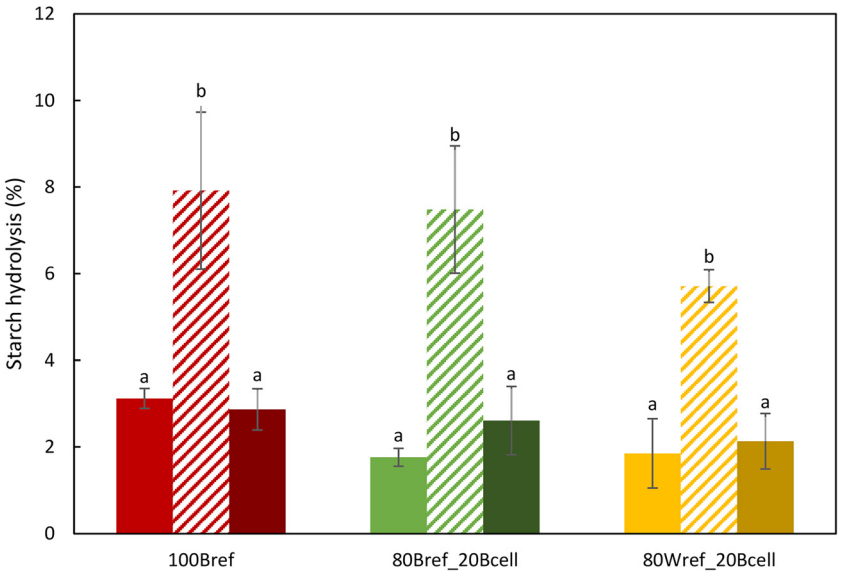

Fig. 7 *In vitro* oral starch digestion in wraps consisting of 100% raw-milled, reference red bean flour ($100B_{ref}$, red), 80% reference and 20% cellular red bean flour ($80B_{ref}\_20B_{cell}$, green) or 80% wheat flour and 20% cellular bean flour ($80W_{ref}\_20B_{cell}$, yellow) upon *in vitro* oral processing with 150 U mL^{-1} salivary amylase (light solid fill), 302 U mL^{-1} salivary amylase (striped fill) or 150 U mL^{-1} *Bacillus* sp. amylase (dark solid fill). Significant differences in average amylolysis extents between different wrap types or oral phase simulation approaches are indicated by different letters (Tukey test, $p < 0.05$).

results vary largely, depending on not only the food product under consideration, but also the applied oral phase simulation.[26,27,68–70] Nevertheless, in the current study, a considerable amount of starch was hydrolysed during *in vitro* oral processing. Therefore, these results emphasize the importance of including an oral phase when performing *in vitro* digestion experiments, especially when gradual acidification is applied in the gastric phase and the contribution of oral amylase increases due to its prolonged activity.[26–29] If salivary amylase is not included in the oral phase, this may result in underestimation of starch digestion. Generally, the three applied methods may be deemed suitable for simulating *in vivo* mastication, based on the obtained bolus microstructure and the starch digestion extents, which both resemble *in vivo* obtained data. Nevertheless, the results also highlight that the way *in vitro* oral processing is performed largely impacts digestion levels. The significant effect of amylase activity emphasizes that it is essential to standardize enzyme activity when simulating *in vitro* oral digestion. The INFOGEST standardized procedure suggests to use 150 U mL^{-1}, which is a well-considered average activity.[37] While other activities may also be representative of the *in vivo* situation, the applied activity should be kept constant over different samples. Moreover, although leading to similar results in the current study, the choice of amylase source should be well-thought-out, and adjusted to the research objective. For example, *Bacillus* sp. amylase may be suitable for studying oral starch digestion, but its impact during subsequent digestion phases should be carefully considered.

Finally, no significant differences were observed between the different wrap types upon application of equal *in vitro* oral processing conditions. Nevertheless, the wraps containing intact cells ($80B_{ref}\_20B_{cell}$ and $80W_{ref}\_20B_{cell}$) consistently showed lower starch digestion levels compared to the $100B_{ref}$ wrap. This indicates that also during *in vitro* oral simulations, the presence of only a small fraction (20%) of cotyledon cells, visible in Fig. 4, forms a barrier for amylase and reduces its ability to efficiently hydrolyse starch, similar to what was reported before for *in vitro* small intestinal digestion.[5–7,9,14,15] These limited differences between samples have the potential to become more pronounced during the gastric phase when a dynamic pH profile is considered, and of course during the small intestinal phase. Furthermore, it was shown in section 3.3.3 that *in vivo* mastication with 38 participants also resulted in a lower amylolysis level in wraps containing intact cells. This once again indicates that the developed *in vitro* oral processing methods are appropriate for mimicking an *in vivo* oral phase for the food product under consideration. However, it should be noted that, although a similar bolus microstructure and extent of amylolysis could be obtained using *in vitro* methods, they do not capture the large variability in mastication behaviour that occurs *in vivo*. Nevertheless, these inter-individual differences can be thought to affect digestion in subsequent phases, *i.e.*, stomach and intestine.

4 Conclusions

The impact of *in vivo* and *in vitro* oral processing of bean-based wraps with different microstructures was investigated. It was shown that during *in vivo* mastication, large differences in bolus weight, chewing time and number of chews existed between individuals. Despite this large variation, mastication parameters were not affected by wrap type. In contrast, wrap

type substantially affected the microstructure of boluses, while individual chewing behaviour did not. When cellular bean flour was incorporated in wraps, boluses clearly contained intact cotyledon cells. The presence of these intact cells caused a significant decrease in *in vivo* oral amylolysis compared to wraps not containing these cellular ingredients, although large inter-individual differences were observed. Mastication parameters over different wrap types were constant for all participants, shown by clear positive correlations, which suggests individual chewing behaviour remained constant. Surprisingly, no clear correlations were found between mastication parameters and the extent of starch digestion. This was attributed to the large inter-individual variation, and emphasizes the importance of taking this variability into account when performing *in vivo* mastication studies.

Application of different *in vitro* oral processing approaches resulted in similar microstructural properties as what was observed *in vivo*. A higher activity of human salivary amylase more than doubled amylolysis levels in all wraps. The use of a more cost-efficient alternative to human saliva, amylase from *Bacillus* sp., resulted in similar starch digestion extents as upon application of salivary amylase, indicating it could be an appropriate substitute, at least in the simulated oral phase. Its persisted activity in subsequent phases, such as the gastric phase, should also be studied and compared to saliva. Moreover, in all three *in vitro* oral phase simulations, incorporation of cellular flour in wraps decreased starch hydrolysis, although differences were not significant. Overall, it was shown that it is essential to consider an appropriate *in vivo* or *in vitro* oral phase when studying the digestibility of a solid, starch-rich food product.

While product quality was assessed qualitatively, future work should evaluate the sensorial acceptability of the prepared wraps in a more quantitative way. The impact of *in vivo* mastication of more well-known reference foods, *e.g.*, 100% wheat-based wraps, could also be investigated and compared to the current results, to get even more insight into the effect of wrap microstructure and formulation on digestion. Moreover, while the ethical approval did not permit measurements of individuals' salivary amylase activity, it would be interesting to link this parameter to *in vivo* oral starch hydrolysis extents, and investigate whether stronger correlations are obtained as compared to those observed here. Furthermore, the current work highlighted the importance and significant contribution of the oral phase to the digestive process, but it would also be interesting to study gastric and small intestinal digestion of the prepared wraps after an *in vivo* or *in vitro* oral phase. This way, it can be evaluated whether the impact of wrap microstructure persists. Additionally, although oral starch digestion was not correlated with chewing time or number of chews, it is possible that inter-individual differences in *in vivo* chewing behaviour affect subsequent gastrointestinal release of starch metabolites. It is thus possible that more clear correlations could be observed between, for example, chewing time and small intestinal amylolysis, which is why these subsequent phases should be studied as well. Finally, future work could also consider oral processing of other food products in which cellular pulse flours are included.

Author contributions

Esther Staes: writing – original draft, visualization, validation, methodology, investigation, formal analysis, data curation, conceptualization. Dorine Duijsens: writing – review & editing, supervision, methodology, conceptualization. Serafien Lefever: investigation, formal analysis, data curation. Lieza Theuwissen: investigation, formal analysis, data curation. Masha Mikhalski: methodology, conceptualization. Ann Van Loey: project administration, funding acquisition, conceptualization. Tara Grauwet: writing – review & editing, supervision, project administration, funding acquisition, conceptualization.

Conflicts of interest

There are no conflicts to declare.

Data availability

The complete dataset will be available from the corresponding author upon reasonable request. De-identified datasets, used to generate the figures in this manuscript, are publicly available in Zenodo (10.5281/zenodo.20391801).

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d6fo00876c.

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