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## Nutritional quality and *in vitro* digestion of immature rice-based processed products

 Kulwa F. Miraji, <sup>a,b</sup> Anita R. Linnemann,<sup>b</sup> Vincenzo Fogliano, <sup>b</sup> Henry S. Laswai<sup>c</sup> and Edoardo Capuano <sup>\*b</sup>

Rice is commonly consumed as fully mature grain, but immature rice is considered to have better nutrient and technological properties. This is attributed to changes in content and profile of nutritional and functional compounds during maturation. This study assessed the effect of maturity on nutrient content of rice grains, and *in vitro* digestibility of starch and protein, for immature rice grains of TXD306 and Lawama varieties. The effect of processing of immature rice into so-called pepeta, traditionally produced from immature rice grains and widely consumed in Tanzania, was studied as well. The results showed reductions in lipid, protein, ash, thiamine, nicotinic acid, nicotinamide, and soluble and insoluble dietary fibre contents during rice grain development. However, no effect of maturity on *in vitro* starch and protein digestibility was observed. The contents of protein, ash, lipid, nicotinamide, iron, zinc, and total, soluble and insoluble dietary fibre were higher in pepeta from both varieties than in the corresponding rice grains. Protein digestibility of pepeta flour was 58.9% higher than that of cooked rice for variety TXD306, and 73.8% higher for Lawama. Differential scanning calorimetry indicated that starch of processed immature rice was completely gelatinized whereas its susceptibility to digestion *in vitro* was slightly lower than for cooked rice, possibly due to the higher cellular integrity retained after processing. These results demonstrate that pepeta-type processing improves the nutritional properties of rice and its potential use as a snack or ingredient in cereal-based formulas.

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### 1. Introduction

Rice-based food products are widely consumed worldwide, providing up to 46% and 43% of dietary energy and dietary protein in the sub-Saharan region, respectively.<sup>1,2</sup> Rice is often consumed as fully mature grains in the form of whole kernels after cooking using a particular amount of water or by boiling in excess water.<sup>3</sup> However, several researches reported higher amounts of protein, reducing sugars, calcium, potassium, iron,  $\beta$ -carotene, vitamin C, and vitamin B2, B3 and B6 in immature cereal grains,<sup>4,5</sup> making them nutritionally superior to fully mature grains. These variations in the accumulation of nutrients in developing seeds are related to cellular and physiological changes that occur between different development stages.<sup>6</sup> The proteomic analyses revealed expression patterns (clusters) of proteins which are differentially expressed, and associated with starch synthesis and nutrients accumulation during rice grain development.<sup>7,8</sup>

Besides positive nutritional benefits, immature cereal grains are considered to have better technological properties than fully mature grains due to changes in content and profile of functional compounds during maturation. Lin and Lai<sup>9</sup> reported higher ratios of soluble dietary fibre (SDF) to total dietary fibre (TDF) in immature dehusked unpolished grains ranging from 9.4–17.2% compared to 5.8%–7.4% in fully mature dehusked unpolished grains. Moreover, the content of bioactive compounds, such as phenolics and flavonoids, is significantly higher in developing rice grains than in fully mature seeds.<sup>9,10</sup> Similar studies on immature wheat have reported high contents of total dietary fibre, fructo-oligosaccharides and phytochemicals, such as phenolics and flavonoids.<sup>11,12</sup>

Wholegrain and polished rice are utilized in a very wide range of foods,<sup>13</sup> including flaked rice. Flaked rice is a popular snack in rice-consuming countries. Pepeta, a locally produced flaked product from immature rice grains, is a sought-after snack in Tanzania for its popcorn taste and flavour. The traditional process of making pepeta involves roasting of harvested immature rice grains, dehusking and pounding of grains in a mortar to obtain flattened rice grains and husks, followed by winnowing to remove the husks prior to consumption (Fig. 1). The effect of cooking on the physical and nutritional behaviour of rice kernels is well-documented: swelling

<sup>a</sup>Tanzania Agricultural Research Institute, Ifakara Centre, Ifakara, Tanzania

<sup>b</sup>Food Quality and Design, Wageningen University and Research, Wageningen, The Netherlands. E-mail: edoardo.capuano@wur.nl

<sup>c</sup>Mwenge Catholic University, Moshi, Tanzania



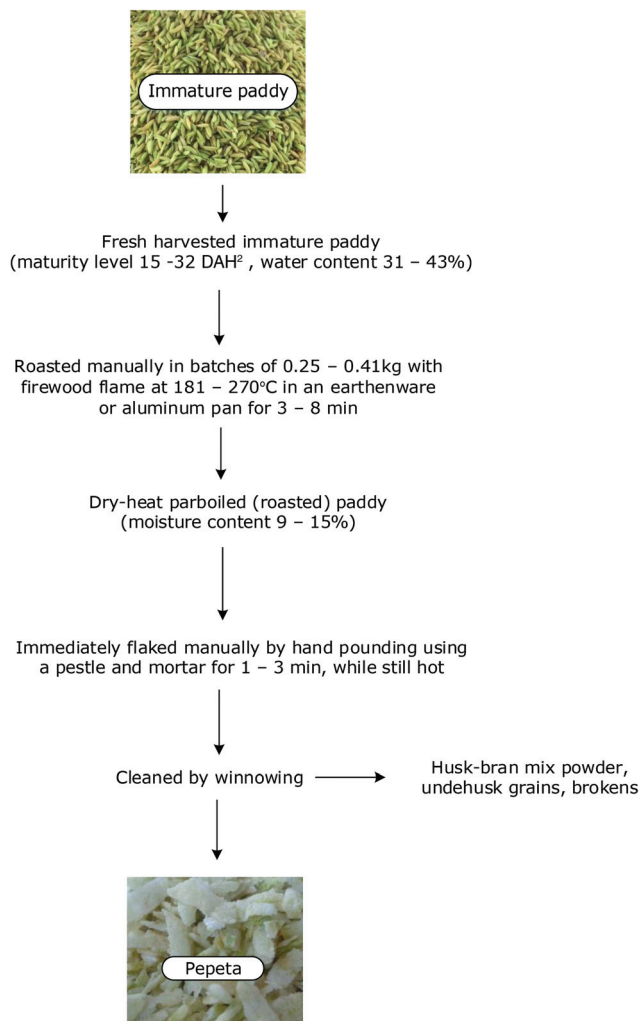


Fig. 1 Pepeta processing flow chart. <sup>a</sup>Days after 50% heading/flowering.

and crack formation due to hydration, starch gelatinization which enhances starch digestibility and the glycaemic index, and leaching of soluble nutrients like starch and vitamin of the B group in water.<sup>14,15</sup> However, in pepeta a dry heating processing step is used, which bears resemblance to dry-heat parboiling (*i.e.* a technique involving rapid roasting of sufficiently soaked paddy).<sup>16</sup> This technique is widely used in processing of flaked, puffed and expanded rice-based snacks.<sup>17</sup> It increases the digestibility of starch in processed products compared to raw products,<sup>16–18</sup> and perhaps the nutrient content as well due an increased total ash in rice- and wheat-based roasted products.<sup>19,20</sup> These positive effects may also apply to pepeta processing, but this still needs to be confirmed by research. This study assessed the effect of maturation on the nutrient content of rice grain in the products of two domestic Tanzanian rice varieties. Effect of maturation on nutritional components on *in vitro* digestibility of pepeta starch and protein during grain development were also examined.

## 2. Materials and methods

### 2.1 Materials

**2.1.1 Rice.** Two rice varieties (*Oryza sativa* L.), namely TXD306 and Lawama, collected from rice farmers at Kilombero and Ulanga districts in Tanzania were used in this study. Both varieties were grown in the same irrigation schemes. Days after 50% heading (DAH) was used as an indicator of maturity level. The heading date (50% heading) was determined when 50 percent of the panicles in the rice field are at least partially visible. Rice grains were harvested based on pepeta processors' and/or farmers' knowledge. TXD306 grains were harvested at 15, 24, 30 and 39 DAH, whereas 19, 24, 29 and 40 DAH for Lawama. Harvested grains were categorized according to modified description of Jiamyangyuen *et al.*:<sup>10</sup> dough grain stage (DGS, 15–21 DAH), mature grain stage (MGS, 22–28 DAH), fully ripe stage (FRS, 29–35 DAH) and over ripe stage (ORS, 36–43 DAH). In this study, rice grains were considered immature at DGS and MGS, since fully mature grains are those above 28 DAH.<sup>10</sup> Harvested rice grains were vacuum sealed in plastic bags (Princess®, S-492967-001, China) in portions of 250 g and stored at 4 °C until use.

**2.1.2 Reagents.** Pepsin from porcine gastric mucosa (P6887, 3200–4500 U mg<sup>-1</sup>), porcine bile extract (B8631), amyloglucosidase from *Aspergillus niger* (10 113, 129.3 U mg<sup>-1</sup>), trypsin from porcine pancreas (T7409, 1000–2000 U mg<sup>-1</sup>), α-chymotrypsin from bovine pancreas (C4129, ≥40 U mg<sup>-1</sup>), α-amylase from porcine pancreas (A4268, 700–1400 U mg<sup>-1</sup>), sodium dodecyl sulfate (SDS), *o*-phthaldialdehyde (OPA), DL-dithiothreitol (DTT), L-serine, thiamine hydrochloride (B1), nicotinic acid (B3, ≥99.5% HPLC), and nicotinamide (B3) were purchased from Sigma-Aldrich Ltd (St Louis, MO, USA). Trichloroacetic acid (CAS 76-03-9) and disodium tetraborate decahydrate (CAS 1303-96-4) were bought from Merck & Co. (Darmstadt, Germany). Assay kits for total starch, resistant starch, amylose/amylopectin, D-glucose (GOPOD) and dietary fibre analyses were acquired from Megazyme Inc. (Wicklow, Ireland). Other chemicals used in this study were of analytical grade.

### 2.2 Sample preparation

**2.2.1 Pepeta processing.** A part of the harvested immature paddy rice grains was traditionally processed into pepeta (Fig. 1). Fresh harvested immature paddy (DGS and MGS) at 31–43% moisture content was manually roasted in batches of 0.25–0.41 kg for 3–8 min on an open wood fire at 181–270 °C. The roasted paddy with moisture content of about 9–15% was immediately flaked manually by hand pounding using a pestle and mortar for 1–3 min. Pounding of hot roasted paddy concurrently dehusked, polished and flattened rice grains to produce pepeta. The mixture was then cleaned by winnowing to remove husk-bran mix powder, undehusked and broken grains to obtain clean flattened grains named pepeta. After processing, 3 kg of pepeta was vacuum sealed (Princess®,



S-492967-001, China) in 250 g plastic bags and stored at 4 °C until further analysis.

**2.2.2 Preparation of cooked rice.** First, 500 g of harvested paddy rice grains for each maturity level in both variety were batch dried using a hot air dryer (TG 200, Retsch GmbH, Haan, Germany) at 50 °C and 90 fan power (approximating an air flow of 185 m<sup>3</sup> h<sup>-1</sup>). The drying process ended at a moisture content of 12–13%. Next, the dried samples were left to cool at room temperature for approximately 6 h. A weighed sample (100 g) of dried paddy rice grains was then dehusked in a laboratory sheller (THU, Satake, Tokyo, Japan), and polished to remove the bran layer using a polisher (TP-2, Kett Electric Laboratory, Japan) for 90 s. Head rice grains were handpicked from the milled rice, and cooked in excess water (ratio 1:25) until fully gelatinized. Cooked rice kernels were pressed between two glass slides to check for full gelatinization, *i.e.* when no clear white core was observed anymore. Immediately after cooking, the samples were cryo-milled and stored for further analysis at -20 °C to avoid retrogradation. The remained broken grains after hand picking of head rice grains were stored at 4 °C and used as raw milled white rice samples.

**2.2.3 Grinding.** Pepeta, raw milled white rice and cooked rice samples were cryo-milled (Freezer mill 6875D, Spex Sample Prep) using 2 cycles, 2 min cooling, 5 min grinding and 15 cycles per second (cps). Except for cooked rice, the fraction that passed through a 0.425 mm laboratory sieve is defined as fine ground and used in this study. For preparation of coarse particles, *i.e.* the fraction retained between 2 and 1 mm laboratory sieves, pepeta was ground in the cryo mill set at 1 cycle, 2 min cooling, 15 s grinding and 10 cps. For total protein analysis, samples were freeze dried prior to grinding by placing 50 g in a freeze dryer until a constant weight was obtained.

### 2.3 Proximate analysis

**2.3.1 Dry matter content.** To determine the dry matter content, 2 g of sample flour was dried overnight in an oven at 105 °C and the weight difference calculated.<sup>21</sup>

**2.3.2 Total ash content.** Flour samples (2 g) were combusted in a furnace at 550 °C overnight and the ash content was quantified.<sup>21</sup>

**2.3.3 Total lipid.** Lipid content was determined according to standard methods<sup>21</sup> with the Soxhlet extraction using petroleum ether. The collected fat was expressed as the mass percentage of extracted lipid to the original sample mass (5 g) used in the analysis.

**2.3.4 Total protein.** Nitrogen content was estimated by the Dumas combustion method using an analyzer (EA 1112 NC, Thermo fisher scientific Inc., Waltman, USA) following the manufacturer's protocol. D-Methionine and cellulose were used to prepare the calibration curve and as control, respectively.

A specific conversion factor (Jones' factor for rice products) of 5.95 was used to convert the nitrogen content in the sample to protein.<sup>22</sup>

### 2.4 Crude dietary fibre

Soluble (SDF) and insoluble (IDF) dietary fibre were measured using a commercial Megazyme kit (K-TDFR, Megazyme Int, Wicklow, Ireland). In brief, 1 g of ground sample in MES-TRIS buffer solution was incubated with  $\alpha$ -amylase, protease, and amyloglucosidase enzymes, in series. The mixture was then filtered to obtain the IDF, while SDF was determined by precipitating the filtrate with 95% of hot (60 °C) ethanol. Ash and protein residues were corrected for corresponding SDF and IDF values. Total dietary fibre (TDF) was calculated as the sum of SDF and IDF.

### 2.5 Surface lipid content

Surface lipid content (SLC) was determined to quantify the degree of milling (DOM) according to Matsler and Siebenmorgen,<sup>23</sup> following same procedure as in section 2.3.3. In this analysis, whole kernels of pepeta and milled rice samples were used.

### 2.6 Analysis of Fe and Zn

Fe and Zn were measured by ICP-AES (inductively coupled plasma-atomic emission spectrometry) (Thermo iCAP-6500 DV; Thermo Fisher Scientific, Waltham, USA), following the validated Chemisch Biologisch Laboratorium Bodem (CBLB, Wageningen, the Netherlands) protocol. Briefly, 300 mg of ground sample was digested with concentrated nitric-hydrochloric acid mixture, and hydrogen-peroxide in a microwave digestion system (MarsXpress; CEM Corporation, Matthews, USA), consecutively. After settling of the undissolved silica particles, the supernatant was analysed on ICP-AES. Prior to analysis, samples were dried at 70 °C overnight and ground to 0.425 mm.

### 2.7 Determination of selected vitamin B1 and B3

Determination of thiamine, nicotinic acid and nicotinamide was by a modified procedure of Chen *et al.*<sup>24</sup> The B vitamins were extracted by adding 25 ml milli Q water to 0.5 g ground sample, sonicated at 40 °C for 5 h and then centrifuged at 3000g for 10 min at room temperature. The supernatant was filtered by a 0.2  $\mu$ m membrane filter and 20  $\mu$ L extract was used for HPLC analysis. The mobile phase was 25 mM KH<sub>2</sub>PO<sub>4</sub> (pH3):CNCH<sub>3</sub> in a ratio of 97:3 at a flow rate of 1.0 mL min<sup>-1</sup>. The HPLC system was operated with a Prevail C18 column (5  $\mu$ m, 4.6  $\times$  250 mm) and a UV detector set at 245 and 270 nm at room temperature.

### 2.8 Starch characterisation analysis

**2.8.1 Total starch.** Total starch content was determined according to AOAC Method 996.11 with a Megazyme kit (K-TSTA, Megazyme Int., Wicklow, Ireland). Analysis was according protocol "e" of the Megazyme total starch assay booklet.

**2.8.2 Resistant starch.** Resistant starch (RS) was determined using Megazyme kit (K-STAR, Megazyme Int., Wicklow, Ireland) based on AOAC Method 2002.02. Cooked rice samples



were treated as wet samples due to a higher moisture content (70–80% wet basis).

**2.8.3 Starch isolation.** Starch was isolated from rice grains according to modified wet-milling method of Syahariza *et al.*<sup>25</sup> In summary, 20 g of rice was soaked in sodium metabisulfite (60 mL, 0.45% w/v), for 72 h at 4 °C, and milled for 5 min into rice slurry using a commercial blender. Protein was removed by repeating series of vigorously mixing of slurry with 180 mL NaCl (0.1 M) solution and 20 mL toluene. Starch samples were dried at room temperature for 24 h before storage at –20 °C for further analysis.

**2.8.4 Amylose/amylopectin ratio.** The amylose content in isolated starch was determined according to enzymatic method using the Megazyme kit (K-AMYL, Megazyme Int., Wicklow, Ireland). The amylopectin content was determined indirectly, by subtracting the amylose percentage to the total starch percentage of the samples.

**2.8.5 Particle size distribution.** The particle size distribution of the isolated starch was measured using a Mastersizer 3000 (Malvern Panalytical Ltd, Malvern, UK) following the manufacturer's protocol. Samples were dry dispersed at a pressure of 3 bar, a 3.0 mm hopper gap of 3.0 mm, 1.45 particle refractive index and 0.0001 particle absorption index. Data was calculated according to the Lorenz–Mie theory<sup>26</sup> and presented as mean particle size,  $D \times 10$  (mean particle size of the smallest 10%),  $D \times 50$  (mean particle size of the lower 50%), and  $D \times 90$  (mean particle size below which 90% of the sample is found).

**2.8.6 Differential scanning calorimetry.** Gelatinization behaviour of rice flour and starch samples were assessed with a Perkin Elmer Differential Scanning Calorimetry (DSC) 8000 (Waltham, MA, USA) according to the manufacturer's protocol. Briefly, 20 mg ground sample was weighed in 60  $\mu$ L stainless steel DSC pans (Perkin Elmer) and wetted with water in a ratio 1:3 (w/w) to ensure complete gelatinization of the samples. The samples were left to equilibrate for 5 h at room temperature after immediate hermetical sealing. Both samples and empty reference pans were then heated twice from 10 to 120 °C, with an heating rate of 10 °C min<sup>-1</sup>. Onset ( $T_o$ ), peak ( $T_p$ ) and conclusion ( $T_c$ ) temperatures, and enthalpy change ( $\Delta H$ ) were analysed using Pyris™ software (Version 11, PerkinElmer, Inc. Waltham, USA).

## 2.9 *In vitro* digestion

*In vitro* digestion of starch and protein hydrolysis was performed based on the harmonized INFOGEST protocol<sup>27</sup> with some modifications. The digestion procedure consisted of a gastric and an intestinal phase. Moistened pepeta (1:4 w/v) and cooked rice samples were mixed with pre-warmed simulated salivary fluid (SSF) without salivary  $\alpha$ -amylase, simulated gastric fluids (SGF) and freshly prepared pepsin (2000 U ml<sup>-1</sup>), respectively. Prior to gastric digestion, the pH of the mixture was adjusted to 3 with 1 M HCl and incubated at 37 °C for 2 h. After the gastric phase, the gastric chyme was combined with warmed simulated intestinal fluids (ISF), fresh bile (28.8 mg mL<sup>-1</sup>), and a pancreatic enzyme solution consisting of

$\alpha$ -amylase (200 U ml<sup>-1</sup>), trypsin (100 U ml<sup>-1</sup>) and chymotrypsin (25 U ml<sup>-1</sup>). The pH was adjusted to 7 with 1 M NaOH, after which the mixture was incubated at 37 °C for 2 h to complete intestinal digestion. Sample tubes were placed in a rotator (Multi Rs-60, Biosan, Riga, Latvia) set at 40 rpm throughout the whole digestion procedure. Separate sample tubes were used for aliquot sampling at 0, 60, 120 minutes during the gastric step and at 5, 10, 20, 30, 60 and 120 minutes of the intestinal step for starch and protein analysis, and immediately snap-frozen in liquid nitrogen for 20 s to minimize further enzymatic reactions. After the complete digestion procedure, amylase and protease activity in the aliquot samples (0.4 ml for each starch and protein) were stopped by addition of absolute ethanol (1:4) and 20% TCA (1:2), respectively. Subsequently, the aliquots sample mixtures were centrifuged at 3000g for 10 min at 0 °C, and the supernatant obtained stored at –20 °C until further analysis.

The effect of particle size in the rate and extent of starch and protein digestion was also evaluated. For this analysis, coarse particles from pepeta samples, the fractions retained between 2 and 1 mm sieves, were used.

**2.9.1 Determination of starch hydrolysis.** An aliquot of 0.1 ml of the supernatant obtained after addition of ethanol and centrifugation was mixed with an amyloglucosidase solution (27.17 U ml<sup>-1</sup>) in 0.1 M sodium acetate buffer (pH 4.8) and incubated at 37 °C for 1 h. The amount of glucose was then quantified using a Megazyme D-glucose assay kit (GOPOD FORMAT, K-GLUC, Megazyme Inc., Bray, Ireland). To obtain corresponding amount of starch, the glucose content was multiplied by a factor of 0.9 and the results expressed as g of hydrolyzed starch per 100 g of dry starch. The kinetics of starch and protein digestion were described by fitting the experimental data to a first-order equation:

$$C_t = C_\infty(1 - e^{-kt})$$

where  $C_t$ ,  $C_\infty$ , and  $k$  represent the hydrolysed starch % at time  $t$ , the maximum degree of starch hydrolysis in % (at infinite time), and the hydrolysis rate constant, respectively.

**2.9.2 Determination of protein hydrolysis.** The concentration of free amino groups (NH<sub>2</sub>) in TCA samples was determined using the *ortho*-phthalaldehyde (OPA) method.<sup>28</sup> In order to quantify the total content of NH<sub>2</sub> groups in the samples, non-digested samples were hydrolyzed with 6 M HCl, incubated at 110 °C for 24 h, after which the free amino groups were estimated by OPA. Degree of hydrolysis (DH) was estimated using the following equation:

$$\text{DH}(\%) = \frac{\text{NH}_2(\text{DS}) - \text{NH}_2(t=0)}{\text{NH}_2(\text{total}) - \text{NH}_2(t=0)} \times 100$$

where: NH<sub>2</sub> (DS), free amino groups from digested sample; NH<sub>2</sub> ( $t = 0$ ), free amino groups from samples at time 0 of digestion; NH<sub>2</sub> (total), maximum amount of NH<sub>2</sub> present in the sample.



### 2.10 Confocal laser scanning microscopy

The endosperm cell wall morphology of non-digested raw rice, cooked rice and pepeta samples was visualized using a Zeiss 510 inverted microscope (Carl Zeiss microscopy, Oberkochen, Germany). For this, raw rice and pepeta samples were moistened between water-wetted tissue paper for 3 h. Cooked rice samples were freshly prepared in excess water (ratio of 1 : 25) to full gelatinization. All samples were manually sectioned into thin cross section slices, stained with 0.02% calcofluor-white dye and left to incubate for 5 min before being excited at 405 nm. Images were taken using 40× (N.A. 1.3 oil immersion) objective lenses.

### 2.11 Statistical analysis

One-way ANOVA analysis was performed with SPSS version 25 (IBM Statistics, Armon, USA) to evaluate the effects of maturity level for the individual varieties. When significant effects were observed, Tukey's test ( $p < 0.05$ ) was used for multiple comparison. A paired-sample  $t$  test was used to assess the effect of pepeta processing method between rice and pepeta for each maturation level and variety, whereas independent  $t$  test was computed to evaluate statistical difference between pepeta samples in the same variety. Data are presented as means  $\pm$  standard deviation of at least two replicates.

## 3. Results and discussion

### 3.1 Proximate analysis

Table 1 summarizes the proximate composition of rice grains harvested at different grain development stages. The total lipid content of milled white rice grains ranged from 0.44 (FRS)–1.17 (MGS) g per 100 g in TXD306 and 0.80 (DGS)–0.99 (ORS) g per 100 g in Lawama, showing lower amounts than in previous research<sup>29</sup> where paddy, *i.e.* whole rice grains, were used. In Lawama, the total lipid content increased as rice grains matured from DGS to ORS, while showing an inconsistent trend in TXD306. The observed total lipid trend could be due to variations in degree of milling (DOM) among maturity levels as a similar trend for surface lipid content (SLC) was observed in both varieties during rice maturation. In this study, SLC was used to measure the DOM, *i.e.* the amount of bran and germ remains after milling (section 2.5), as rice bran contains about 20% lipid.<sup>30</sup> The protein content was 6.54 (DGS)–9.74 (MGS) g per 100 g in TXD306 and 6.19 (DGS)–8.33 (MGS) g per 100 g in Lawama, and showed significant differences among growth stages in both varieties. The results are within the range of values reported from fully mature rice.<sup>31</sup> However, the protein content increased from DGS to MGS, decreased at FRS before increasing again at ORS for both varieties. This trend is in contrast to Chen-Ming *et al.*<sup>5</sup> who reported a general decrease in protein content during rice grain development. A significant change in ash content was observed; immature grains showing higher amounts in TXD306 (1.03 g per 100 g, DGS) and Lawama (0.96 g per 100 g, DGS) than in fully matured grains in FRS (0.41 g per 100 g) and ORS (0.41 g per 100 g) respect-

ively. Similar results have been reported for other rice varieties during their development.<sup>5</sup>

Fig. 2 (panels a and b) reports the results of SDF, IDF and TDF. The amounts of TDF are in the same range as reported in previous work on rice maturation, and so is the ratio between SDF and IDF.<sup>9</sup> The SDF content slightly increased during grain development in both varieties. However, the IDF showed an inconsistent trend in both varieties, whereas high values were observed at ORS and MGS for TXD306 and Lawama, respectively. The content of TDF, which is the sum of SDF and IDF, ranged from 2.90 (DGS)–5.04 (ORS) % in TXD306 and 2.64 (DGS)–3.19 (MGS) % in Lawama.

Pepeta products prepared from immature rice grains at DGS and MGS were also analysed to evaluate the effect of pepeta processing (Table 1 and Fig. 2 (panels c–h)). This study found more protein, TDF, IDF and SDF in pepeta compared to rice, though the values did not differ significantly. Pepeta processing significantly increased total lipid content in TXD306 and total ash content in Lawama at MGS. These results are likely because of the bran residue.<sup>30</sup> The results indicate that hand pounding and dehusking, *i.e.* an intermediate processing step in pepeta, is less efficient in removing bran and germ compared to mechanical milling. This caused pepeta to have significantly higher amount of SLC compared to milled rice. Further assessment of pepeta samples within variety showed significance differences between DGS and MGS for total protein, total lipid and total ash content in TXD306, and only total protein content in Lawama. The higher values were observed at DGS except for protein content in both variety, possibly due to associated trend with SLC.

### 3.2 Microstructural characterization

Confocal laser scanning microscopy (CLSM) was employed to visualize the morphology of endosperm cells of raw rice, cooked rice and pepeta in three different cell regions, *i.e.* the outer kernel layers, the central starchy endosperm and the crease region of the kernel sections (Fig. 3). Staining of the cell walls (light blue) in the micrographs of raw rice (Fig. 3 panels a–c), clearly shows different shapes of the cells in different regions of the endosperm. The endosperm cell wall profile elongates inwards when moving towards the inner region of the kernel sections, in line with previous findings.<sup>32,33</sup> CLSM micrographs of pepeta (Fig. 3 panels d and e) resemble those of raw rice, indicating a structural organization of endosperm cell walls even after pepeta processing. However, the cell profile of the outer kernel layers in pepeta was partially disrupted as compared to those of raw rice, probably due to physical damage during pounding. Pepeta processing also involves roasting of paddy grain (dry heat), which apparently produced limited damage on cell wall structure compared with cooking (wet heat). A previous study<sup>34,35</sup> reported that cell expansion and disruption in cooked rice depend on the extent of starch gelatinization, amount of water absorption and temperatures close to 100 °C. This is also the case in our study: cooked rice micrographs (Fig. 3 panels g–i), showed endosperm cell wall organization was lost after cooking with no visible intact cells.



Note that rice grains were cooked in excess boiling water until fully gelatinized (section 2.2.2).

### 3.3 Vitamins and minerals

Table 1 shows the concentration of selected water-soluble B vitamins, *i.e.* thiamine, nicotinic acid and nicotinamide, measured in this study. Nicotinic acid (16.1 (FRS)–49.7 (DGS) mg per 100 g in TXD306 and 21.3 (ORS)–46.06 (MGS) mg per 100 g in Lawama) was abundant, followed by nicotinamide (1.21 (FRS)–2.79 (DGS) mg per 100 g in TXD306 and 1.67 (ORS)–2.32 (MGS) g per 100 g in Lawama). The amount of nicotinic acid and nicotinamide significantly decreased during grain development; TXD306 at FRS and Lawama at ORS contained the lower amounts. Significantly higher thiamine contents, 1.88 mg per 100 g for TXD306 and 0.65 mg per 100 g for Lawama were detected at DGS and MGS, respectively. Thiamine (except for TXD306 at DGS) and nicotinamide were in range with a study<sup>5</sup> on two Korean rice varieties during maturation. Other studies<sup>5,36,37</sup> reported decreases in vitamins B contents and/or their conjugates during maturation of cereal grains. The decrease could be due to the biochemical function of the B vitamins, which are cofactors and precursors in regulating plant metabolism.<sup>38–40</sup>

Pepeta processing showed no significant effect on nicotinic acid and nicotinamide content (except at the MGS of TXD306) when comparing pepeta flour with rice flour (Table 1). In both varieties, pepeta contained higher levels of nicotinamide (2.45 (MGS)–3.13 (DGS) mg per 100 g in TXD306 and 2.87 (MGS)–3.43 (DGS) mg per 100 g in Lawama) than the corresponding rice grains. This trend is opposite for the nicotinic acid content. Though pepeta processing significantly affected thiamine content in TXD306 and Lawama, no consistent changes were observed. Except for thiamine in TXD306, no significant differences between DGS and MGS was observed on thiamine, nicotinic acid and nicotinamide in pepeta samples. The results indicate that maturity has very limited effect on analysed vitamin Bs composition in pepeta samples.

The amount of iron ranged from 0.31–1.27 mg per 100 g for TXD306 and 0.58–2.05 mg per 100 g for Lawama during rice maturation (Table 1), with the highest amounts at ORS for TXD306 and MGS for Lawama. The amount of zinc significantly increased as rice grains developed, with the highest levels at ORS of 2.11 and 2.14 mg per 100 g for TXD306 and Lawama, respectively. These results differ from previous work,<sup>5</sup> possibly due to the use of different rice varieties and cultivation conditions.<sup>41,42</sup>

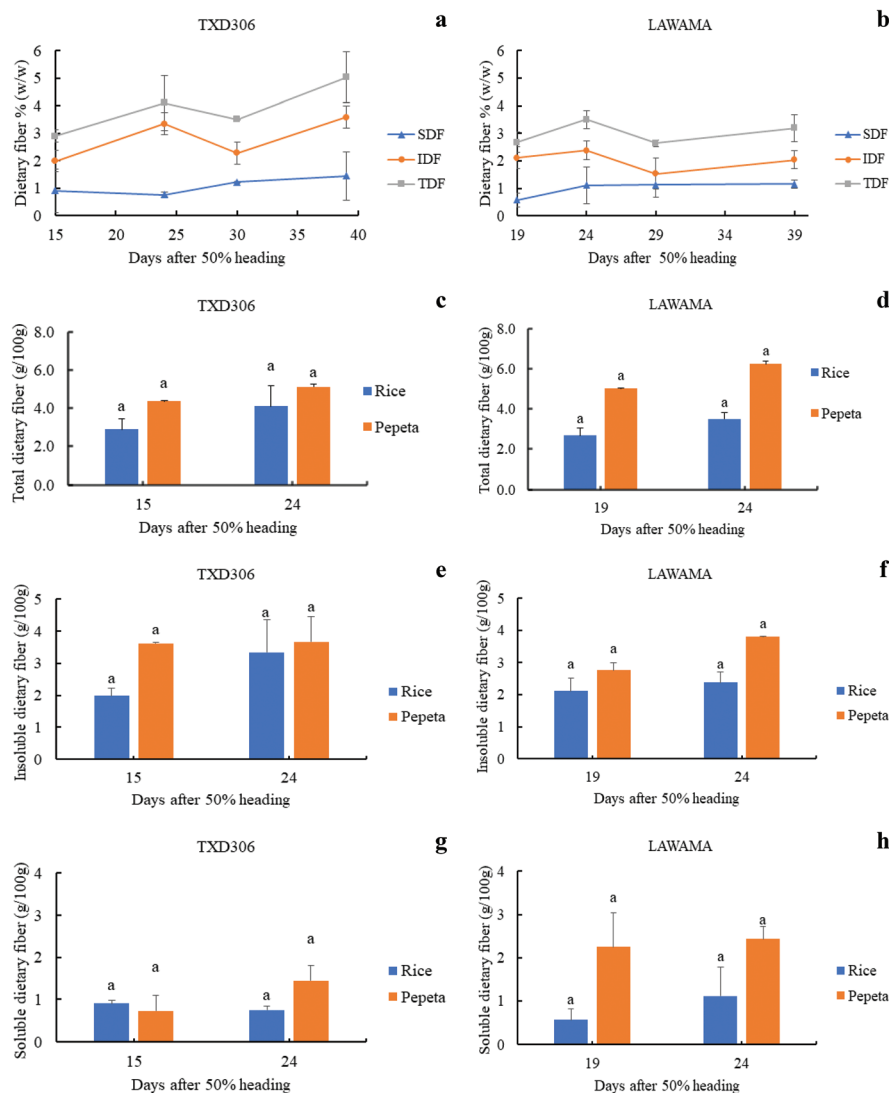
For pepeta processing, no significant difference was found for Lawama in the iron content between pepeta and the rice grains, although pepeta contained a higher amount than the grains. In TXD306, pepeta at MGS had a significantly higher iron content. Zinc content significantly increased during pepeta processing at DGS (1.88 mg per 100 g) and MGS (1.77 mg per 100 g) in TXD306, and at DGS (1.65 mg per 100 g) in Lawama. These results are attributed to the high amount of bran and germ remains in pepeta (section 3.1). Maturity level significantly affect iron and zinc composition of pepeta in TXD306,

**Table 1** Nutritional content of TXD306 and Lawama rice varieties as a function of maturity level and processing conditions

Nutritional compound	Sample	TXD306						Lawama						
		DGS (15–21 DAH)	MGS (22–28 DAH)	FRS (29–35 DAH)	ORS (36–43 DAH)	DGS (15–21 DAH)	MGS (22–28 DAH)	FRS (29–35 DAH)	ORS (36–43 DAH)	DGS (15–21 DAH)	MGS (22–28 DAH)	FRS (29–35 DAH)	ORS (36–43 DAH)	
<i>Proximate</i>														
Surface lipid content (g per 100 g, db)	Rice	0.76 ± 0.09 <sup>ab</sup>	0.75 ± 0.07 <sup>ab</sup>	0.26 ± 0.03 <sup>b</sup>	0.68 ± 0.15 <sup>a</sup>	0.44 ± 0.06 <sup>ab</sup>	0.41 ± 0.03 <sup>ab</sup>	0.58 ± 0.06 <sup>ab</sup>	0.66 ± 0.01 <sup>b</sup>	1.10 ± 0.10 <sup>ab</sup>	1.37 ± 0.03 <sup>ab</sup>	1.37 ± 0.03 <sup>ab</sup>	1.37 ± 0.03 <sup>ab</sup>	0.99 ± 0.03 <sup>a</sup>
	Pepeta	1.66 ± 0.06 <sup>ab</sup>	1.32 ± 0.05 <sup>ab</sup>	0.44 ± 0.02 <sup>b</sup>	0.85 ± 0.27 <sup>ab</sup>	0.80 ± 0.18 <sup>ab</sup>	0.89 ± 0.20 <sup>ab</sup>	0.92 ± 0.14 <sup>a</sup>	0.99 ± 0.03 <sup>a</sup>	1.65 ± 0.09 <sup>ab</sup>	1.65 ± 0.09 <sup>ab</sup>	1.65 ± 0.09 <sup>ab</sup>	1.65 ± 0.09 <sup>ab</sup>	7.12 ± 0.02 <sup>c</sup>
Total lipids (g per 100 g, db)	Rice	1.17 ± 0.02 <sup>ab</sup>	1.18 ± 0.04 <sup>ab</sup>	0.44 ± 0.02 <sup>b</sup>	0.85 ± 0.27 <sup>ab</sup>	0.80 ± 0.18 <sup>ab</sup>	0.89 ± 0.20 <sup>ab</sup>	0.92 ± 0.14 <sup>a</sup>	0.99 ± 0.03 <sup>a</sup>	1.65 ± 0.09 <sup>ab</sup>	1.65 ± 0.09 <sup>ab</sup>	1.65 ± 0.09 <sup>ab</sup>	1.65 ± 0.09 <sup>ab</sup>	7.12 ± 0.02 <sup>c</sup>
	Pepeta	2.14 ± 0.12 <sup>ab</sup>	1.55 ± 0.00 <sup>ba</sup>	6.63 ± 0.73 <sup>a</sup>	9.53 ± 0.14 <sup>b</sup>	6.19 ± 0.08 <sup>ab</sup>	6.19 ± 0.08 <sup>ab</sup>	6.30 ± 0.18 <sup>a</sup>	7.12 ± 0.02 <sup>c</sup>	8.33 ± 0.29 <sup>ba</sup>	8.33 ± 0.29 <sup>ba</sup>	8.33 ± 0.29 <sup>ba</sup>	8.33 ± 0.29 <sup>ba</sup>	0.41 ± 0.01 <sup>c</sup>
Total protein (g per 100 g, db)	Rice	6.54 ± 0.09 <sup>ab</sup>	9.74 ± 0.21 <sup>ba</sup>	0.41 ± 0.01 <sup>c</sup>	0.45 ± 0.01 <sup>d</sup>	6.58 ± 0.03 <sup>ab</sup>	6.58 ± 0.03 <sup>ab</sup>	0.96 ± 0.04 <sup>bb</sup>	0.41 ± 0.01 <sup>c</sup>	8.36 ± 0.17 <sup>ba</sup>	8.36 ± 0.17 <sup>ba</sup>	8.36 ± 0.17 <sup>ba</sup>	8.36 ± 0.17 <sup>ba</sup>	0.96 ± 0.02 <sup>a</sup>
	Pepeta	7.33 ± 0.51 <sup>ba</sup>	9.66 ± 0.16 <sup>ba</sup>	0.41 ± 0.01 <sup>c</sup>	0.45 ± 0.01 <sup>d</sup>	6.58 ± 0.03 <sup>ab</sup>	6.58 ± 0.03 <sup>ab</sup>	0.96 ± 0.04 <sup>bb</sup>	0.41 ± 0.01 <sup>c</sup>	8.36 ± 0.17 <sup>ba</sup>	8.36 ± 0.17 <sup>ba</sup>	8.36 ± 0.17 <sup>ba</sup>	8.36 ± 0.17 <sup>ba</sup>	0.96 ± 0.02 <sup>a</sup>
Total ash (g per 100 g, db)	Rice	1.03 ± 0.01 <sup>ab</sup>	0.76 ± 0.01 <sup>ba</sup>	0.41 ± 0.01 <sup>c</sup>	0.45 ± 0.01 <sup>d</sup>	1.03 ± 0.03 <sup>ab</sup>	1.03 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	0.41 ± 0.01 <sup>c</sup>	1.09 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	0.41 ± 0.01 <sup>c</sup>
	Pepeta	1.10 ± 0.03 <sup>ab</sup>	0.86 ± 0.03 <sup>ba</sup>	0.41 ± 0.01 <sup>c</sup>	0.45 ± 0.01 <sup>d</sup>	1.03 ± 0.03 <sup>ab</sup>	1.03 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	0.41 ± 0.01 <sup>c</sup>	1.09 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	0.41 ± 0.01 <sup>c</sup>
<i>Vitamins</i>														
Thiamine (mg per 100 g, db)	Rice	1.88 ± 0.17 <sup>ab</sup>	0.46 ± 0.01 <sup>bb</sup>	0.47 ± 0.05 <sup>b</sup>	0.43 ± 0.01 <sup>b</sup>	0.51 ± 0.00 <sup>ab</sup>	0.65 ± 0.00 <sup>ba</sup>	0.60 ± 0.03 <sup>b</sup>	0.56 ± 0.02 <sup>a</sup>	0.52 ± 0.02 <sup>ab</sup>	0.55 ± 0.06 <sup>ab</sup>	0.55 ± 0.06 <sup>ab</sup>	0.55 ± 0.06 <sup>ab</sup>	0.56 ± 0.02 <sup>a</sup>
	Pepeta	1.48 ± 0.03 <sup>ba</sup>	2.49 ± 0.03 <sup>ba</sup>	16.1 ± 0.16 <sup>b</sup>	18.5 ± 3.47 <sup>b</sup>	34.2 ± 7.30 <sup>ab</sup>	46.04 ± 4.45 <sup>ab</sup>	28.06 ± 4.09 <sup>ab</sup>	21.3 ± 4.04 <sup>b</sup>	49.7 ± 3.92 <sup>ab</sup>	45.2 ± 3.97 <sup>ab</sup>	45.2 ± 3.97 <sup>ab</sup>	45.2 ± 3.97 <sup>ab</sup>	21.3 ± 4.04 <sup>b</sup>
Nicotinic acid (mg per 100 g, db)	Rice	49.7 ± 3.92 <sup>ab</sup>	45.2 ± 3.97 <sup>ab</sup>	16.1 ± 0.16 <sup>b</sup>	18.5 ± 3.47 <sup>b</sup>	34.2 ± 7.30 <sup>ab</sup>	46.04 ± 4.45 <sup>ab</sup>	28.06 ± 4.09 <sup>ab</sup>	21.3 ± 4.04 <sup>b</sup>	49.7 ± 3.92 <sup>ab</sup>	45.2 ± 3.97 <sup>ab</sup>	45.2 ± 3.97 <sup>ab</sup>	45.2 ± 3.97 <sup>ab</sup>	21.3 ± 4.04 <sup>b</sup>
	Pepeta	32.6 ± 4.81 <sup>ab</sup>	21.7 ± 0.43 <sup>ab</sup>	1.21 ± 0.00 <sup>b</sup>	1.48 ± 0.22 <sup>b</sup>	1.95 ± 0.17 <sup>ab</sup>	2.32 ± 0.75 <sup>ab</sup>	2.06 ± 0.37 <sup>a</sup>	1.67 ± 0.24 <sup>a</sup>	32.6 ± 4.81 <sup>ab</sup>	31.0 ± 8.51 <sup>ab</sup>	31.0 ± 8.51 <sup>ab</sup>	31.0 ± 8.51 <sup>ab</sup>	1.67 ± 0.24 <sup>a</sup>
Nicotinamide (mg per 100 g, db)	Rice	2.79 ± 0.09 <sup>ab</sup>	1.22 ± 0.06 <sup>ab</sup>	1.21 ± 0.00 <sup>b</sup>	1.48 ± 0.22 <sup>b</sup>	1.95 ± 0.17 <sup>ab</sup>	2.32 ± 0.75 <sup>ab</sup>	2.06 ± 0.37 <sup>a</sup>	1.67 ± 0.24 <sup>a</sup>	2.79 ± 0.09 <sup>ab</sup>	2.32 ± 0.75 <sup>ab</sup>	2.32 ± 0.75 <sup>ab</sup>	2.32 ± 0.75 <sup>ab</sup>	1.67 ± 0.24 <sup>a</sup>
	Pepeta	3.13 ± 0.35 <sup>ab</sup>	2.45 ± 0.19 <sup>ab</sup>	1.21 ± 0.00 <sup>b</sup>	1.48 ± 0.22 <sup>b</sup>	1.95 ± 0.17 <sup>ab</sup>	2.32 ± 0.75 <sup>ab</sup>	2.06 ± 0.37 <sup>a</sup>	1.67 ± 0.24 <sup>a</sup>	3.13 ± 0.35 <sup>ab</sup>	2.87 ± 0.61 <sup>ab</sup>	2.87 ± 0.61 <sup>ab</sup>	2.87 ± 0.61 <sup>ab</sup>	1.67 ± 0.24 <sup>a</sup>
<i>Mineral</i>														
Iron (mg kg <sup>-1</sup> , db)	Rice	0.59 ± 0.44 <sup>ab</sup>	0.64 ± 0.17 <sup>ab</sup>	0.31 ± 0.06 <sup>a</sup>	1.27 ± 0.37 <sup>a</sup>	0.58 ± 0.03 <sup>ba</sup>	2.05 ± 0.02 <sup>ab</sup>	0.89 ± 0.45 <sup>b</sup>	1.06 ± 0.23 <sup>ab</sup>	0.82 ± 0.14 <sup>ba</sup>	1.85 ± 0.17 <sup>ba</sup>	1.85 ± 0.17 <sup>ba</sup>	1.85 ± 0.17 <sup>ba</sup>	1.06 ± 0.23 <sup>ab</sup>
	Pepeta	0.82 ± 0.14 <sup>ba</sup>	1.66 ± 0.05 <sup>ba</sup>	1.73 ± 0.07 <sup>b</sup>	2.11 ± 0.13 <sup>a</sup>	1.34 ± 0.44 <sup>ab</sup>	1.85 ± 0.17 <sup>ba</sup>	0.96 ± 0.05 <sup>c</sup>	2.14 ± 0.00 <sup>a</sup>	0.75 ± 0.00 <sup>cb</sup>	1.53 ± 0.08 <sup>ba</sup>	1.53 ± 0.08 <sup>ba</sup>	1.53 ± 0.08 <sup>ba</sup>	2.14 ± 0.00 <sup>a</sup>
Zinc (mg kg <sup>-1</sup> , db)	Rice	0.75 ± 0.00 <sup>cb</sup>	0.93 ± 0.01 <sup>cb</sup>	1.73 ± 0.07 <sup>b</sup>	2.11 ± 0.13 <sup>a</sup>	0.62 ± 0.05 <sup>db</sup>	1.53 ± 0.08 <sup>ba</sup>	0.96 ± 0.05 <sup>c</sup>	2.14 ± 0.00 <sup>a</sup>	1.88 ± 0.00 <sup>ab</sup>	1.77 ± 0.01 <sup>ba</sup>	1.77 ± 0.01 <sup>ba</sup>	1.77 ± 0.01 <sup>ba</sup>	2.14 ± 0.00 <sup>a</sup>
	Pepeta	1.88 ± 0.00 <sup>ab</sup>	1.77 ± 0.01 <sup>ba</sup>	1.73 ± 0.07 <sup>b</sup>	2.11 ± 0.13 <sup>a</sup>	1.65 ± 0.05 <sup>ba</sup>	1.78 ± 0.01 <sup>ba</sup>	0.96 ± 0.05 <sup>c</sup>	2.14 ± 0.00 <sup>a</sup>	1.88 ± 0.00 <sup>ab</sup>	1.77 ± 0.01 <sup>ba</sup>	1.77 ± 0.01 <sup>ba</sup>	1.77 ± 0.01 <sup>ba</sup>	2.14 ± 0.00 <sup>a</sup>

Values in each row (small letter) and column (capital letter) bearing different superscripted letters are statistically different ( $p \leq 0.05$ ) for each rice variety. DGS – dough grain stage, MGS – mature grain stage, FRS – fully ripe stage, ORS – over ripe stage, DAH – days after 50% heading, db – dry basis, nd – not detected. Data expressed as mean ± standard deviation.





**Fig. 2** Dietary fibre contents of two rice varieties (TXD306 and Lawama) as affected by maturity level (a and b) and effect of processing into pepeta (c–h). TDF – total dietary fibre, IDF – insoluble dietary fibre, SDF – soluble dietary fibre, rice – raw rice flour, Pepeta – locally prepared rice flake. Labels with different letters within maturity level indicate a statistically significant difference in fibre content ( $p \leq 0.05$ ).

high values found at MGS and DGS respectively. However, no significant difference was observed in Lawama, possibly due to observed concomitant SLC trend.

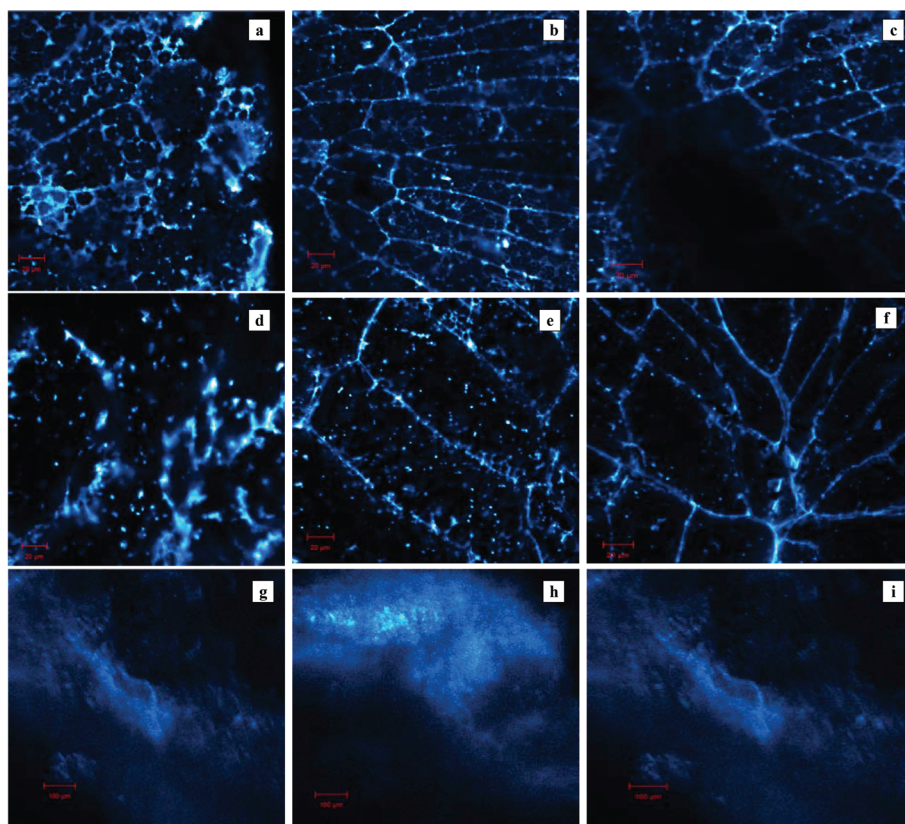
### 3.4 Starch characterisation

Total starch of rice grain ranged from 79.0 (ORS) to 82.8 (DGS) g per 100 g in TXD306 and from 80.9 (FRS) to 84.0 (DGS) g per 100 g in Lawama (Table 1), similar to previous research.<sup>43</sup> Grain maturation did not significantly affect the total starch content in both varieties. Previous studies showed a constant level or small increase in starch content after 18 DAH during rice grain development.<sup>4,44</sup> However, we observed a slight decrease of starch content during maturation, which may be associated with a concomitant slight increase in accumulation of protein observed during maturation (section 3.1).

Reportedly, an increase in protein content in cereal grains negatively correlates with amylose accumulation, a component of starch.<sup>45,46</sup> In addition, a rice proteomic study<sup>7,8</sup> showed that considerably upregulated proteins were involved in starch synthesis and accumulation in developing grains. Though pepeta contained a slightly lower total starch content, no significant effect was observed between pepeta and its corresponding rice grains in both varieties. We attribute this to the high amount of bran residues in pepeta (section 3.1).

Amylose content ranged from 17.1 (FRS) to 20.2 (ORS) g per 100 g in TXD306 and 15.2 (ORS)–20.1 (DGS) g per 100 g in Lawama, similar to previous work,<sup>47</sup> which reported 1.6–21.7% amylose in polished rice samples of three different varieties. Except for TXD306 at ORS, the amylose content showed a decreased trend as rice grains matured, even though the differ-





**Fig. 3** Confocal images of raw rice (a–c), pepeta (d–f) and cooked rice (g–i) in different area of the cross section of the kernel; outer kernel layer (a, d and g), central starchy endosperm (b, e and h), and inner region of kernel (c, f and i). Cell walls were stained in light blue. Micrographs a–f were taken using 40 $\times$  objective lens while g–i 20 $\times$ .

ences were not significant. This is in line with previous study,<sup>48</sup> suggested the accumulation of amylose in rice during the mid-stage of grain filling. Rice grains are classified according to amylose content.<sup>49</sup> The varieties in this study are regarded as low amylose rice (10–19%).

RS contributes to gastrointestinal health as part of dietary fibre, reported to considerably reduce the postprandial blood glucose level.<sup>50</sup> For a realistic comparison between the RS content of pepeta and rice, cooked rice grains were used as rice is normally eaten cooked,<sup>51</sup> and the results were expressed per 100 g total starch (Table 2). The RS in cooked rice ranged from 0.28 to 0.54 g per 100 g in TXD306 and 0.05 to 0.40 g per 100 g in Lawama, the lowest values in both varieties recorded at FRS. The results corroborate previous research that reported less than 1% RS in cooked low-amylose rice varieties.<sup>52</sup> Cooking (wet heat) and pepeta (dry heat) processing methods show no significant difference in RS content for both varieties. Dutta *et al.*<sup>16</sup> and Sagum *et al.*<sup>53</sup> also reported no significant difference in RS between boiling and pressure-cooking, and among dry heat parboiling methods in low amylose rice varieties, respectively. TXD306 and Lawama, like other low amylose varieties, both exhibit very low levels of RS when cooked and processed into pepeta.<sup>16,52,53</sup>

In both varieties, particle size distribution analysis of rice starch granules showed no significant effect for mean particle

size,  $D \times 10$ ,  $D \times 50$  and  $D \times 90$  as rice grains matured (Table 2), indicating that rice starch granules are fully developed at DGS. The mean particle size ranged from 5.24 (MGS) to 5.54 (FRS)  $\mu\text{m}$  in TXD306 and 5.16 (MGS) to 5.23 (ORS)  $\mu\text{m}$  in Lawama, which was similar as reported for six other rice varieties.<sup>54</sup>

DSC was conducted on raw rice flour of DGS and MGS, their corresponding starch and pepeta flour to investigate the effect of maturity, food matrix and pepeta processing on the gelatinization behaviour of starch (Table 2). Significant effects were found between DGS and MGS on  $T_p$ ,  $T_p$  and  $T_p$  of rice flour and rice starch samples. However, there was no significant effect between maturity levels for the  $\Delta H$  associated with gelatinization. The  $T_o$ ,  $T_p$ , and  $T_c$  values shifted to lower temperatures in all samples (rice flour and rice starch) as rice grain developed from DGS to MGS. The results suggest possible differences in the way the polysaccharides are organized in the cell during grain development, which exhibit variation in thermal stability and crystallinity.<sup>55,56</sup> In addition, we observed significant differences in  $T_o$ ,  $T_p$  and  $T_c$  in both varieties, and  $\Delta H$  of Lawama variety for rice flour as compared with rice starch samples. The  $T_o$ ,  $T_p$  and  $T_c$  values in all varieties shifted towards lower temperatures in rice starch samples, likely due to absence of cell walls, limiting heat and water transfer to starch. The removal of proteins and lipids may also contribute to the shift as in Ye *et al.*<sup>57</sup> who reported an increase in suscep-



**Table 2** *In vitro* digestion, starch particles size distribution and gelatinization behaviour of TXD306 and Lawama rice varieties at different maturity level and processing conditions

Nutritional component	Sample	Lawama							
		TXD306	DGS (15–21 DAH)	MGS (22–28 DAH)	FRS (29–35 DAH)	ORS (36–43 DAH)	DGS (15–21 DAH)	MGS (22–28 DAH)	FRS (29–35 DAH)
<b>Starch characterization</b>									
Total starch (g per 100 g, db)	Rice	82.8 ± 2.30 <sup>ab</sup>	81.4 ± 2.85 <sup>ab</sup>	81.2 ± 2.84 <sup>a</sup>	79.0 ± 0.14 <sup>a</sup>	84.0 ± 2.20 <sup>ab</sup>	81.5 ± 5.57 <sup>ab</sup>	80.9 ± 2.22 <sup>a</sup>	81.4 ± 2.51 <sup>a</sup>
	Pepeta	78.2 ± 2.31 <sup>ab</sup>	77.1 ± 1.18 <sup>ab</sup>			78.7 ± 1.20 <sup>ab</sup>	78.2 ± 2.31 <sup>ab</sup>		
Resistant starch (g per 100 g starch db) <sup>a</sup>	Rice	0.54 ± 0.09 <sup>ab</sup>	0.41 ± 0.01 <sup>ab</sup>	0.28 ± 0.00 <sup>b</sup>	0.33 ± 0.05 <sup>ab</sup>	0.35 ± 0.05 <sup>ab</sup>	0.40 ± 0.13 <sup>ab</sup>	0.05 ± 0.01 <sup>b</sup>	0.24 ± 0.09 <sup>ab</sup>
	Pepeta	0.35 ± 0.13 <sup>ab</sup>	0.34 ± 0.21 <sup>ab</sup>			0.33 ± 0.11 <sup>ab</sup>	0.21 ± 0.07 <sup>ab</sup>		
Amylose (g per 100 g, db)	Rice	19.2 ± 1.35 <sup>a</sup>	18.6 ± 3.16 <sup>a</sup>	17.1 ± 2.43 <sup>a</sup>	20.2 ± 0.03 <sup>a</sup>	20.1 ± 0.81 <sup>a</sup>	18.2 ± 2.74 <sup>a</sup>	19.1 ± 2.97 <sup>a</sup>	15.2 ± 2.03 <sup>a</sup>
<b>Particle size distribution</b>									
Mean particle size (µm)	Rice	5.35 ± 0.20 <sup>a</sup>	5.24 ± 0.29 <sup>a</sup>	5.54 ± 0.06 <sup>a</sup>	5.35 ± 0.07 <sup>a</sup>	5.23 ± 0.19 <sup>a</sup>	5.16 ± 0.05 <sup>a</sup>	5.21 ± 0.22 <sup>a</sup>	5.23 ± 0.04 <sup>a</sup>
<i>D</i> × 10 (µm)	Rice	3.68 ± 0.05 <sup>a</sup>	3.49 ± 0.09 <sup>a</sup>	3.73 ± 0.08 <sup>a</sup>	3.55 ± 0.02 <sup>a</sup>	3.62 ± 0.08 <sup>a</sup>	3.42 ± 0.14 <sup>a</sup>	3.55 ± 0.12 <sup>a</sup>	3.49 ± 0.09 <sup>a</sup>
<i>D</i> × 50 (µm)	Rice	5.17 ± 0.18 <sup>a</sup>	5.02 ± 0.18 <sup>a</sup>	5.34 ± 0.03 <sup>a</sup>	5.12 ± 0.05 <sup>a</sup>	5.05 ± 0.18 <sup>a</sup>	4.94 ± 0.08 <sup>a</sup>	5.03 ± 0.02 <sup>a</sup>	5.04 ± 0.05 <sup>a</sup>
<i>D</i> × 90 (µm)	Rice	7.21 ± 0.37 <sup>a</sup>	7.20 ± 0.75 <sup>a</sup>	7.56 ± 0.24 <sup>a</sup>	7.38 ± 0.18 <sup>a</sup>	7.02 ± 0.33 <sup>a</sup>	7.10 ± 0.06 <sup>a</sup>	7.06 ± 0.35 <sup>a</sup>	7.26 ± 0.01 <sup>a</sup>
<b>Starch hydrolysis</b>									
<i>C</i> <sub>∞</sub> (g per 100 g, db) <sup>a</sup>	Cooked rice	98.45 ± 2.35 <sup>ab</sup>	91.28 ± 8.15 <sup>ab</sup>	95.85 ± 1.39 <sup>a</sup>	99.07 ± 0.03 <sup>a</sup>	88.47 ± 1.90 <sup>ab</sup>	85.11 ± 1.92 <sup>ab</sup>	94.48 ± 5.11 <sup>a</sup>	93.80 ± 0.51 <sup>a</sup>
	Finely ground	82.99 ± 2.13 <sup>ab</sup>	76.69 ± 3.97 <sup>ab</sup>			85.04 ± 2.30 <sup>ab</sup>	83.81 ± 6.79 <sup>ab</sup>		
	Coarsely ground	81.81 ± 3.73 <sup>ab</sup>	79.32 ± 0.91 <sup>ab</sup>			79.78 ± 1.57 <sup>ab</sup>	76.05 ± 0.81 <sup>ab</sup>		
	pepeta								
<i>k</i> (min <sup>-1</sup> )	Cooked rice	0.32 ± 0.02 <sup>ab</sup>	0.33 ± 0.06 <sup>ab</sup>	0.29 ± 0.00 <sup>a</sup>	0.25 ± 0.02 <sup>a</sup>	0.35 ± 0.02 <sup>ab</sup>	0.32 ± 0.00 <sup>ab</sup>	0.24 ± 0.02 <sup>a</sup>	0.26 ± 0.16 <sup>a</sup>
	Finely ground	0.29 ± 0.02 <sup>ab</sup>	0.31 ± 0.02 <sup>ab</sup>			0.34 ± 0.05 <sup>ab</sup>	0.27 ± 0.00 <sup>ab</sup>		
	Coarsely ground	0.20 ± 0.00 <sup>ab</sup>	0.18 ± 0.05 <sup>ab</sup>			0.25 ± 0.04 <sup>ab</sup>	0.20 ± 0.01 <sup>ab</sup>		
	pepeta								
<b>Protein hydrolysis</b>									
Gastric digestion (g per 100 g, db)	Cooked rice	19.17 ± 6.16 <sup>ab</sup>	05.98 ± 0.98 <sup>ab</sup>	13.41 ± 5.53 <sup>a</sup>	09.42 ± 0.80 <sup>a</sup>	13.77 ± 4.74 <sup>ab</sup>	08.34 ± 2.36 <sup>ab</sup>	12.04 ± 7.60 <sup>a</sup>	14.75 ± 8.57 <sup>a</sup>
	Finely ground	31.02 ± 8.85 <sup>ab</sup>	26.92 ± 18.4 <sup>ab</sup>			12.94 ± 2.67 <sup>ab</sup>	20.94 ± 0.69 <sup>ab</sup>		
	pepeta								
	Coarsely ground	08.60 ± 3.96 <sup>ab</sup>	08.74 ± 3.25 <sup>ab</sup>			21.07 ± 13.4 <sup>ab</sup>	11.86 ± 4.18 <sup>ab</sup>		
	pepeta								
Intestinal digestion (g per 100 g, db)	Cooked rice	71.66 ± 14.2 <sup>ab</sup>	46.87 ± 4.54 <sup>ab</sup>	68.92 ± 11.1 <sup>a</sup>	61.44 ± 0.94 <sup>a</sup>	73.10 ± 8.50 <sup>ab</sup>	54.36 ± 5.88 <sup>ab</sup>	70.47 ± 0.39 <sup>a</sup>	63.97 ± 19.3 <sup>a</sup>
	Finely ground	84.06 ± 3.60 <sup>ab</sup>	74.50 ± 9.74 <sup>ab</sup>			79.47 ± 15.6 <sup>ab</sup>	94.48 ± 4.50 <sup>ab</sup>		
	pepeta								
	Coarsely ground	75.77 ± 8.66 <sup>ab</sup>	50.77 ± 16.1 <sup>ab</sup>			77.51 ± 7.32 <sup>ab</sup>	63.68 ± 3.74 <sup>ab</sup>		
	pepeta								
<b>Gelatinization behaviour</b>									
<i>T</i> <sub>0</sub> (°C),	Rice	68.53 ± 0.37 <sup>ab</sup>	68.14 ± 0.25 <sup>ab</sup>			67.17 ± 0.18 <sup>ab</sup>	65.74 ± 0.17 <sup>ba</sup>		
	Rice starch	64.90 ± 0.26 <sup>ab</sup>	63.99 ± 0.12 <sup>bb</sup>			64.07 ± 0.17 <sup>ab</sup>	61.99 ± 1.12 <sup>ba</sup>		
	Pepeta	nd	nd			nd	nd		
<i>T</i> <sub>p</sub> (°C)	Rice	75.75 ± 0.18 <sup>ab</sup>	75.27 ± 0.22 <sup>ab</sup>			74.19 ± 0.03 <sup>ab</sup>	72.47 ± 0.17 <sup>ba</sup>		
	Rice starch	71.32 ± 0.29 <sup>ab</sup>	70.71 ± 0.41 <sup>ab</sup>			70.32 ± 0.04 <sup>ab</sup>	67.95 ± 0.99 <sup>ab</sup>		
	Pepeta	nd	nd			nd	nd		
<i>T</i> <sub>c</sub> (°C)	Rice	85.36 ± 0.30 <sup>ab</sup>	83.06 ± 0.18 <sup>ba</sup>			84.21 ± 0.01 <sup>ab</sup>	81.41 ± 0.22 <sup>ba</sup>		
	Rice starch	80.05 ± 0.69 <sup>ab</sup>	78.93 ± 0.36 <sup>ab</sup>			79.26 ± 0.06 <sup>ab</sup>	75.57 ± 0.94 <sup>ba</sup>		
	Pepeta	nd	nd			nd	nd		
$\Delta H$ (J g <sup>-1</sup> )	Rice	11.21 ± 1.15 <sup>ab</sup>	11.04 ± 0.26 <sup>ab</sup>			11.67 ± 0.15 <sup>ab</sup>	11.21 ± 0.06 <sup>ab</sup>		
	Rice starch	14.80 ± 1.27 <sup>ab</sup>	15.82 ± 1.22 <sup>ab</sup>			15.33 ± 0.58 <sup>ab</sup>	15.12 ± 0.27 <sup>ab</sup>		
	Pepeta	nd	nd			nd	nd		

<sup>a</sup> Expressed in g per 100 g of total starch, values in each row (small letter) and column (capital letter) bearing different superscripted letters are statistically different ( $p \leq 0.05$ ) for each rice variety. DGS – dough grain stage, MGS – mature grain stage, FRS – fully ripe stage, ORS – over ripe stage, DAH – days after 50% heading, db – dry basis, nd – not detected,  $D \times 10$  – average particle size of smallest 10%,  $D \times 50$  – average particle size of the smallest 50%,  $D \times 90$  – average particle size of the smallest 90%,  $C_{\infty}$  – equilibrium hydrolysis,  $k$  – rate constant,  $T_0$  – onset temperature,  $T_p$  – peak temperature,  $T_c$  – conclusion temperature,  $\Delta H$  – enthalpy change. Data expressed as mean ± standard deviation.

tibility of starch granules to gelatinization when lipids and/or protein were removed from the rice flour. In this study, no endothermic peak was observed in the DSC scans of pepeta flour from both varieties, indicating that pepeta processing conditions (initial moisture content of rice grains, and roasting temperature and duration) are sufficient to fully gelatinize pepeta starch, with very limited retrogradation.

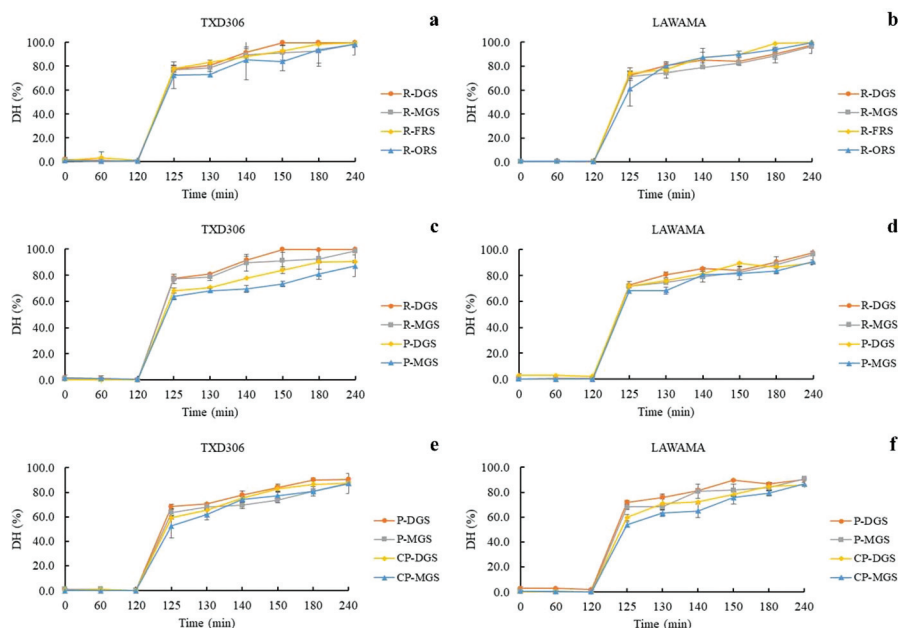
### 3.5 *In vitro* digestibility of starch and proteins

**3.5.1 Starch hydrolysis.** Fig. 4 reports the kinetics of *in vitro* starch digestion in cooked rice grains and pepeta at different development stages and particle size. The oral phase was not simulated during *in vitro* digestion as the action of  $\alpha$ -amylase during the limited digestion time (only 2 min) of the oral phase before amylase is inactivated by the acidic medium of the gastric phase is reported to be very limited.<sup>58,59</sup> In both varieties more than 60% of starch was hydrolysed after the first 5 min of intestinal digestion, which would be expected because digestion of starch is known to be fast and complete in rice.<sup>57,60</sup>

According to Fig. 4 panels a and b, the ORS in TXD306 and MGS in Lawama had slightly lower digestibility under simulated conditions. The  $C_{\infty}$  and  $k$ , estimated by fitting the experimental data to the first order kinetic equation (section 2.9), showed an inconsistent trend for maturation for both varieties (Table 2). The  $C_{\infty}$  (91.28 (MGS)–99.07 (ORS) % in TXD306 and 85.11 (MGS)–94.48 (FRS) % in Lawama) and  $k$  (0.25 (ORS)–0.33 (MGS)  $\text{min}^{-1}$  in TXD306 and 0.24 (FRS)–0.32 (DGS)  $\text{min}^{-1}$  in

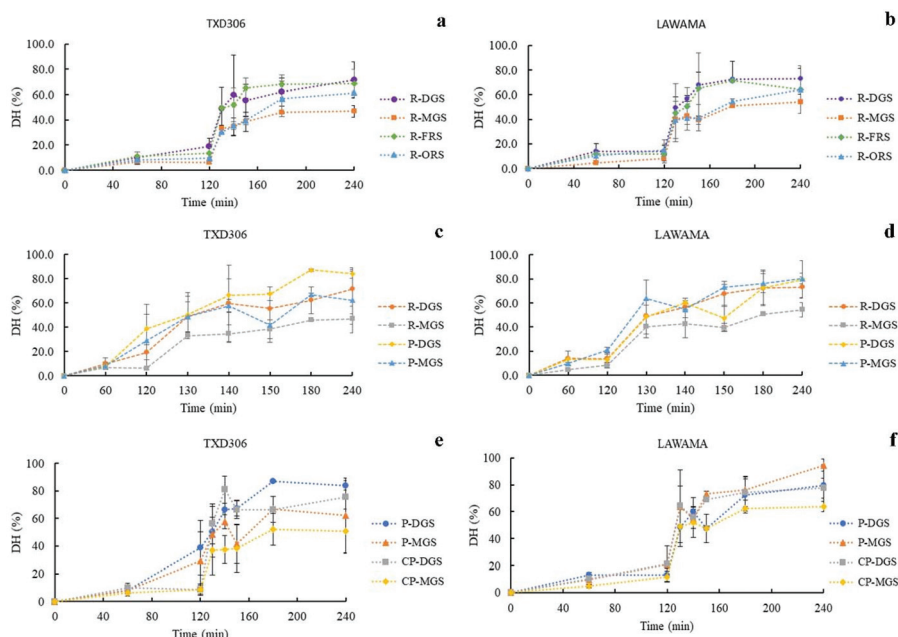
Lawama) values for cooked rice are in line with previous work.<sup>31,57,61</sup> However, no significant effect on  $C_{\infty}$  and  $k$  values were observed in both varieties as rice grain developed from DGS to FRS. These results suggest that the susceptibility of cooked rice starch to *in vitro* digestion is not influenced by maturity level. The digestibility of rice starch by enzymatic hydrolysis has been extensively studied,<sup>16–18</sup> but there appear to be relatively few reports concerning the susceptibility of immature rice starch as affected by maturation. It is well known that intrinsic characteristics such as granule size and shape, size and amount of amylose and amylopectin in the granules, molecular and supramolecular structure (crystallinity, growth rings, packing in cell), and amount of lipids and proteins and their interactions with starch granules may change during grain maturation, and are important factors affecting *in vitro* starch digestibility.<sup>56</sup> However, none of these factors seems to play a role when starch is fully gelatinized (section 2.2.2) as in our study.

Digestograms (Fig. 4 panels c and d) clearly show the low digestibility of pepeta starch as compared to cooked rice in both varieties. No significant difference existed for estimated hydrolysis parameters (except  $C_{\infty}$  at DGS in both varieties and  $k$  of Lawama variety at MGS) between cooked rice and pepeta. Slightly lower  $C_{\infty}$  and  $k$  values were observed in pepeta as compared to cooked rice (Table 2). Chitra *et al.*<sup>17</sup> reported lower rice starch digestion after a dry heat treatment as compared to a wet heat treatment (parboiling), suggesting RS formation in dry heat products to be the reason for low digestibility.



**Fig. 4** *In vitro* hydrolysis of starch in two rice varieties, TXD306 and Lawama during different stages of development (a and b) and as a function of processing method (c and d) and particles sizes (e and f). R-DGS – cooked rice at dough grain stage (15–21 days after 50% heading (DAH)), R-MGS – cooked rice at mature grain stage (22–28 DAH), R-FRS – cooked rice at fully ripe stage (29–35 DAH), R-ORS – cooked rice at over ripe stage (36–43 DAH), P-DGS – pepeta powder ( $\leq 0.425$  mm) prepared at dough grain stage, P-MGS – pepeta powder ( $\leq 0.425$  mm) prepared at mature grain stage, CP-DGS – pepeta coarse (2–1 mm) prepared at dough grain stage, an CP-MGS – pepeta coarse (2–1 mm) prepared at mature grain stage.





**Fig. 5** Digestograms of protein enzymatic hydrolysis *in vitro* for TXD306 and Lawama rice varieties during different stages of development (a and b), and as a function of processing method (c and d) and particles sizes (e and f). R-DGS – cooked rice at dough grain stage (15–21 days after 50% heading (DAH)), R-MGS – cooked rice at mature grain stage (22–28 DAH), R-FRS – cooked rice at fully ripe stage (29–35 DAH), R-ORS – cooked rice at over ripe stage (36–43 DAH), P-DGS – pepeta powder ( $\leq 0.425$  mm) prepared at dough grain stage, P-MGS – pepeta powder ( $\leq 0.425$  mm) prepared at mature grain stage, CP-DGS – pepeta coarse (2–1 mm) prepared at dough grain stage, an CP-MGS – pepeta coarse (2–1 mm) prepared at mature grain stage.

However, in the present study, both cooked rice and pepeta samples contained similar, and very small amounts of RS, and starch was completely gelatinized (section 2.2.2 and 3.4). Therefore, the observed difference may be due to a more extensive disruption of cell walls by excess water during cooking (wet heat) compared to pepeta processing (dry heat) (section 3.2). It must be noted that the difference in starch digestibility between pepeta and cooked rice could even be higher should the cooked rice flour been passed through the 0.425 mm sieve. Starch digestibility in rice is known to be inversely proportional to the rice particle size.<sup>62,63</sup>

The effect of food bolus (particle size) on susceptibility of starch to *in vitro* enzymic hydrolysis was also evaluated in pepeta samples. Coarsely ground pepeta (CGP, 1–2 mm), which is an estimation of the swallowed particle size of pepeta,<sup>64</sup> and fine ground pepeta (FGP,  $<0.425$  mm) were compared for their *in vitro* starch digestibility. Fig. 4 (panels e and f) show that CGP had a slightly low digestibility than FGP under *in vitro* simulated digestion conditions, which was expected as digestion of grain flours is controlled by diffusion of enzymes through the milled grain fragments.<sup>65</sup> Though no significant decrease in  $C_{\infty}$  was observed in both varieties,  $k$  values significantly increased when the particle size of pepeta was increased, which indicates that the integrity of the rice matrix, and of the cell walls in particular (section 3.2), has an effect on pepeta starch digestibility, as repeatedly reported for other cereals.<sup>66–68</sup> The slightly lower starch digestibility in

pepeta is therefore possibly related to the higher level of structural integrity as compared to cooked rice.

**3.5.2 Protein hydrolysis.** Fig. 5 panels a and b present digestograms for protein *in vitro* hydrolysis in cooked rice samples during maturation. Immature rice at MGS showed a lower gastric and intestinal digestibility than FRS and ORS rice, with an inconsistent trend in both varieties (Table 2). The fact that immature grains contain less digestible protein than fully mature grains may be responsible. Previous studies<sup>7,8</sup> revealed proteome (*i.e.* a set of expressed proteins) changes at molecular level for different functions (storage, structural/metabolic and protective proteins) during rice grain development. Thereby storage proteins involved in proteolysis as a nitrogen source for germinating seedlings increased in fully mature grains. A similar pattern of protein digestion was observed for fine pepeta samples (Fig. 5 panels c and d). In both varieties, the digestograms of finely ground pepeta were higher than of cooked rice from immature (DGS and MGS) and fully mature grains (FRS and ORS), and coarsely ground pepeta samples. Pepeta processing showed no significant effects on gastric and intestinal digestion values (except at mature stage in Lawama) of pepeta protein as compared with corresponding cooked rice (Table 2). However, processing into pepeta increased the intestinal digestion of rice protein up to 58.9% in TXD306 and 73.8% in Lawama for the product at mature stage. This indicates that the *in vitro* digestibility of rice protein became more susceptible when processed into



pepeta. Previous work<sup>53,69,70</sup> found that different rice processing methods inhibit protein digestibility by exposing hydrophobic amino acids that form hydrophobic aggregates, and/or restructuring of intermolecular disulphide bridges. However, the type, size and amount of protein aggregates formed varies with processing conditions, and their susceptibility to proteolytic hydrolysis depends on rice variety, enzyme concentration and assay technique.<sup>70,71</sup> In this study, protein aggregation and formation of intermolecular disulphide bridges may have occurred differently between cooked rice and pepeta samples.

The *in vitro* protein digestion also decreased with an increase in particle size in CGP (Fig. 5 panels e and f). As expected, low gastric and intestinal digestion values were observed in CGP compared to FGP due to likely hindrance of digestive enzymes by cell wall fragments in the larger particle sizes (section 3.2). The results are consistent with previous studies on the effect of particle size on *in vitro* digestibility of legume and cereal flours.<sup>28,65</sup> However, differently from what observed with starch digestion, the presence of a higher degree of cellular integrity in CGP and FGP did not result in a reduced protein digestibility compared to cooked rice.

## 4. Conclusion

The present study investigated the changes in nutritional quality of rice grains during maturation. Immature rice represents an important source of micro- and macronutrients. This study demonstrates that a mild processing step such as the one used for pepeta can improve the nutritional potential of rice and its use as a food ingredient. The results showed a reduction in most nutritive components of rice grains during ripening. The highest levels of SLC, ash, thiamine, nicotinic acid and nicotinamide contents were observed at dough grain stage, whereas that of lipid, protein, SDF and IDF contents at mature stage. No effect of maturity on *in vitro* starch and protein digestibility was observed. On the other hand, conversion of rice into pepeta increased the content of some nutrients: pepeta contained up to twice as much iron and zinc, and three times more SDF compared to rice grains. Ash, lipid, nicotinamide, IDF and TDF contents in pepeta were increased by about 80%. The *in vitro* protein digestibility of pepeta was higher than for rice, indicating pepeta as a good source of high digestible protein. Pepeta starch susceptibility to hydrolytic enzymes *in vitro* was slightly lower than for cooked rice although the starch was completely gelatinized. Further investigations are necessary to understand the effects of processing conditions to optimize the nutritional quality of food products made from immature cereal grains, such as pepeta, and develop rice-based products with a more favourable glycaemic response than cooked rice.

## Conflicts of interest

The authors declare that they have no conflict of interest.

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