Valorisation of immature cereal grains to improve food security in Sub-Saharan Africa:
a case study on *pepeta* in Tanzania

Kulwa Furahisha Miraji

Valorisation of immature cereal grains to improve food security in Sub-Saharan Africa: a case study on *pepeta* in Tanzania

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Propositions

1. The nutritional and health benefits of immature cereal grains are viable trade-offs for grain yield loss.
   (this thesis)

2. Optimised *pepeta* processing conditions cannot be viewed as a *one-size-fits-all solution* for improving both the nutritional quality and functional properties of traditionally processed *pepeta* products.
   (this thesis)

3. An education system that relies only on scholars and teachers is flawed.

4. Food security is undermined by global food standardization.

5. The common opinion that new is better than old, is wrong.

6. The subconscious mind is a undervalued tool.

Propositions belonging to the thesis, entitled

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Kulwa Furahisha Miraji
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To my late uncle, Matthew Frank Mwamsamali

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CHAPTER 1.

General introduction and thesis outline

1.1 Importance of cereals in Sub-Saharan Africa

Cereals constitute an important proportion of the food supply for humans and animals. They are substantial sources of energy, protein, vitamins and minerals for the world population.[1] Cereals such as wheat, rice and maize, are known worldwide and form a major part of the diet in many countries.[2] In Sub-Saharan Africa (SSA), cereals represent the primary food in the tropical and semi-arid areas,[3,4] contributing up to 43% of the total household's food basket (Fig. 1.1) while accounting for 50% of the average daily caloric intake.[5] SSA is the origin and a major producer of sorghum (*Sorghum bicolor*), millets (*Pennisetum* spp.), teff (*Eragrostis tef*), fonio (*Digitaria* spp.) and African rice (*Oryza glaberrima*).[3] These cereals can combat the 'hidden hunger' caused by micronutrient deficiencies due to their high nutritional value. As gluten-free cereals, they have great potential to be formulated into a range of food and beverage products to aid people with celiac disease.[6] Beside their importance to food supplies and health, they can contribute to improved income among the most food-insecure population groups in African countries.[7]

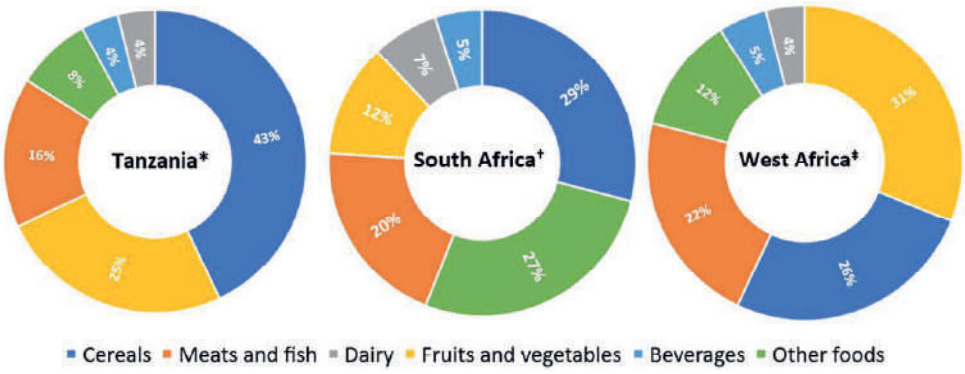

Fig. 1.1: Contribution of cereals and their products to the food basket in SSA
Authors' calculations based on household food consumption data from *Belghith et al.[8] †South Africa Statistics[9] and ‡OECD/FAO[10]

For instance, sorghum and millets (particularly, pearl millet and finger millet) serve as the primary source of dietary energy and protein for millions of people, especially those who live in the African semi-arid and sub-tropical areas, where other cereals such as wheat, maize and rice fail to give substantial yields.[11,12]. Like sorghum and millets, fonio is grown mostly in marginal areas, considered as the *grain of life* due to its consumption as a staple food by millions of the population in West African semi-arid areas when the major crops are yet to reach maturity, and food supply is inadequate.[3,13] High contents of methionine and cysteine make that fonio grains play an important role in human diets.[13] As a major staple crop and native to Ethiopia and Eritrea, teff provides two-thirds of the daily protein intake and 11% of the per capita caloric intake[14,15] while its flour is rich in fibre and bioactive compounds as it is consumed as whole grains because of the small grain size.[16–18]

African rice is native to and cultivated on a small scale in tropical West Africa as a subsistence crop.[3,4] The crop has been crossed with conventional rice (*Oryza sativa* L.) to produce a group of inter-specific hybrid rice varieties called NERICA, which stands for New Rice for Africa.[19] Rice crops, including NERICA and conventional rice, are widely cultivated for dual purposes, namely as a staple food and cash crop in most African countries, accounting for 16% of the cereal calories consumed in Africa. For decades, rice consumption in SSA has increased at about 5.5% per year due to the high population growth rate, rapid urbanisation, and changes in employment patterns.[20,21] Although the increase in rice consumption has led to the detriment of staple cereals such as maize, wheat and other traditional cereals,[3] maize is still the most widely grown staple food crop in SSA, accounting for almost half of the calories and protein consumed in Eastern and Southern Africa, and one-fifth of the calories and protein consumed in West Africa.[21] The average per capita consumption of maize in SSA is 130 g/day, while 16 of the 22 countries where maize contributes the highest percentage of calorie intake, are in Africa.[22,23] On the other hand,

wheat consumption has been steadily increasing and is becoming an important food crop as a result of rapid population growth associated with increased urbanisation and changes in food preferences for precooked food such as bread, biscuits, pasta, noodles and porridge.[21,24]

1.2 Nutritional value of cereals

1.2.1 Macronutrients

Cereals are carbohydrate-rich foods; they contain about 75% carbohydrates,[1] as shown in Table 1.1. Starch, the major component of the cereal, occurs in the endosperm as granules of different sizes depending on cereal type (*e.g.,* a diameter of 5 microns in rice and 25– 40 microns in wheat) and shape (either large, lens-shaped granules or small, spherical granules). Amylose and amylopectin are the main components of the starch granule; their ratio varies depending on the cereal and its variety.[1,4] For instance, about 25–27% of amylose is present in nonwaxy cereals, while in waxy varieties starch is made up mostly of amylopectin. In some cereal products, there is a substantial amount of starch that is not digested and absorbed in the small intestine and can be functionally assimilated to dietary fibre (i.e., resistant starch).[25] Cereals contain low amounts of free sugars, mainly sucrose, up to 2%, and maltose, fructose, and glucose in small quantities.[1]

Proteins in cereals range from 6 – 15%,[26] with the type of stored protein differing among cereal types. For example, prolamins (gliadins and glutenins) are the major storage protein in wheat, albumins and globulins in oats, prolamins (hordeins) and glutelins in barley. At the same time, in rice, glutelin (oryzenin) is the most abundant storage protein, and maize has prolamin (zein).[27] Although cereals provide adequate essential amino acids, sometimes some essential amino acids are limiting. Generally, lysine and tryptophan are the most limiting amino acids for cereals and cereal products.[28] Thus, combining cereals with other plant foods such as legumes and pulses can compensate for these limiting amino acids.

The contribution of cereals to lipids is meagre, the amount ranging from 1–3% in barley, rice, rye and wheat, to 5–10% in maize and oats, with lipid fractions rich in the essential fatty acid linoleic acid (C18:2).[29]

Table 1.1: Proximate composition and energy content of common cereals consumed in Africa

Nutrients (per 100 g)	Cereals							
	Pearl millet*	Finger millet*	Teff*	Fonio*	Sorghum*	Wheat*	Maize†	Brown rice†
Protein (g)	11	7.3	9.6	9.0	7.9	7.8	9.8	7.3
Fat (g)	4.8	1.3	2.0	1.8	2.8	1.1	4.9	2.2
Carbohydrate (g)	70	74.	73	75	73	71	61	71
Fibre (g)	2.3	3.6	3.0	3.3	2.3	2.0	9.0	4.0
Ash (g)	1.9	2.6	2.9	3.4	1.6	1.6	1.4	1.4
Food energy (kJ)	1483	1403	1411	1541	1142	1105	1660	1610

Data adapted from *Belton and Taylor[11] and †Juliano[30]

1.2.2 Dietary fibres

Dietary fibres (DF) are carbohydrate polymers that are not hydrolysed by the endogenous enzymes in the small intestine of humans.[31] Based on solubility in water, DF are classified as soluble (e.g., pectins, gums, oligosaccharides) and insoluble (e.g., cellulose, lignin). Though both types of DF have beneficial effects on human health, their functional values depend on their solubility in water. Soluble DF can increase the viscosity of digesta (associated with slower gastric emptying, slower nutrient absorption, and increased satiety) by increasing the viscosity of the digesta liquid fraction, whereas insoluble DF can increase the viscosity of the digesta by glueing together food particles or by contributing to the insoluble particulate material.[31,32] The most important function of DF is their fermentation by microbiota in the large intestine to produce short-chain fatty acids associated with providing extra energy up to 10% of the diet,[33] decreasing the luminal pH that modifies the microbial population and inhibiting the growth of pathogenic bacteria such as *Salmonella* spp. and *Escherichia coli*,[34,35] decreasing protein fermentation and the level of toxic metabolites produced therefrom,[36] as well as increasing satiety and glucose tolerance,[37] mineral solubility such as of calcium,[38] and

anti-inflammatory and anti-carcinogenic properties.[39] Generally, all cereals are rich sources of DF and their composition, especially regarding soluble DF, varies among cereal types. For instance, arabinoxylans are the main water-soluble DF in wheat, rye and barley, while β-glucans are the main water-soluble DF in oats. The amounts of β-glucans and arabinoxylans are higher in barley (3-11%), oats (3-7%) and rye (1-2%) than in wheat (<1%).[1,40] However, most of the DF are located in the outer layers of the grains, so refined cereal products have substantially reduced amounts of DF.

1.2.3 Micronutrients

The pericarp, germ and aleurone layers of cereals are rich in vitamins and minerals; thus, their proportion influences the contribution of cereals and cereal products to vitamin and mineral intake. However, refined cereal products lose some nutrients as they are removed during the dehulling and polishing processes. Overall, cereals are an essential source of thiamin, riboflavin and niacin, and vitamin A and β-carotene for yellow maize,[27,41] and appreciable amounts of vitamin E (Table 1.2). Wholegrain cereals and their products provide considerable amounts of iron, magnesium, calcium and zinc, as well as lower levels of many trace elements (Table 1.2). Also, the mineral content of cereals varies depending on the soil mineral content and cereal type.[26,42]

Table 1.2: Vitamin and mineral content of common cereals consumed in Africa

Nutrients (mg / 100 g)	Cereals						
	Brown rice *[β]	Maize*†	Wheat*†	Millet*†	Sorghum*†	Teff[α]	Fonio†
Vitamins							
Vitamin A (RE[#])	0	0.37	0.02	0	10.0	0.01	
Thiamine (Vitamin B_1)	0.29	0.32	0.45	0.63	0.33	0.39	
Riboflavin (Vitamin B_2)	0.04	0.10	0.10	0.33	0.13	0.27	
Niacin (Vitamin B_3)	4.0	1.90	3.70	2.00	3.40	3.36	
Vitamin E	0.8	1.90	1.4	0.07	0.17	0.08	
Minerals							
Calcium, Ca	10.4	10.7	18.1	7.57	9.92	18.0	19.6
Copper, Cu	0.30	0.64	0.74	1.98	0.74		0.82
Iron, Fe	2.01	2.92	2.25	15.3	10.1	7.63	10.0
Magnesium, Mg	120	126	121	155	179	184	157
Manganese, Mn	2.65	1.36	1.86	2.65	3.47		1.84
Phosphorus, P	336	285	243	267	293	429	289
Potassium, K	216	276	112	284	284	427	277
Sodium, Na	1.51	3.74	5.15	2.04	9.04	12	1.85
Zinc, Zn	2.02	0.83	1.23	0.95	1.40	3.63	2.27

[#]Retinol equivalent. Data adapted from *Juliano,[30] [β]Antoine et al.,[43] †Jocelyne et al.,[5] [α]Zhu[18]

1.2.4 Phytochemicals

Phytochemicals are plant secondary metabolites that may have health-promoting effects including antidiabetic, cholesterol-lowering, anti-inflammatory, anticancer and antioxidant properties.[1,26] Cereals contain a range of these compounds (Table 1.3), thus being a major contributor to overall antioxidant activity.[44] Whole grain cereals and their products contain a higher amount of phytochemicals compared to refined counterpart products as most phytochemicals are concentrated in the bran fraction.[45] Common phytochemicals in cereals include phenolic acids, flavonoids, and carotenoids, as well as lignans, tannins, polycosanols, phytosterols, stilbenes, and phenolamides.[26,44–47] Although the amount of phytochemicals is lower than that in fruits and vegetables,[48,49] cereals can be an important source because of the large quantities consumed daily.

Table 1.3: Phytochemical and anti-nutrients of common cereals consumed in Africa

Compounds (mg / 100 g dry matter)	Cereals						
	Brown rice†[β]	Maize*†	Wheat*	Millet*†	Sorghum*†	Teff[α]	Fonio*
Phytochemicals							
Phenolic acids	103	174	137	173	107	140	134
Flavonoids	98.3	5.07	4.76	2.40	1.51		3.79
Anti-nutrients							
Phytates	290	852	792	651	620	682	19.5
Oxalates	3.6	116	85.0	131	137		80.1
Tannins	14.3	48.6	23.7	78.3	66.7	16.0	34.7

Data adapted from *Jocelyne et al.,[5] †Amalraj and Pius,[46] [α]Baye,[50] [β]Gong et al.[51]

1.2.5 Anti-nutrients

Although cereals are rich in starch and contain a substantial amount of protein and minerals, their utilisation for normal body functions (i.e., bioaccessibility and bioavailability), is limited by the relatively high amount of anti-nutrients such as phytate.[1,46] The amount of phytates in cereals is present in a wide range, depending on the type of cereal (Table 1.3). In most cereals, phytates are concentrated in the aleurone layer and germ.[52] This means that dehulling and polishing to remove the bran and germ from cereals subsequently reduce the phytate content, enhancing the bioavailability of minerals such as iron, zinc, and calcium.[51] However, the content of minerals and some vitamins of these refined cereals is simultaneously reduced. Given the heavy reliance of low-income populations on cereals as a food source, methods that reduce the phytate content of cereals without altering the amount of micronutrients are of paramount importance. These methods include soaking, fermentation, and germination/ malting, which can reduce the phytate content of unrefined cereals such as maize, rice, millet, and sorghum by 20 – 90 %, depending on the cereal type, pH, as well as length and conditions of the processing method.[53,54] Other anti-nutrients commonly found in cereals include tannins (e.g., in sorghum) and trypsin and protease inhibitors (e.g., in pearl millet and rye) that impair protein digestibility but are of little concern to humans as they are either reduced during

processing (e.g., by germination, soaking and treatment of the cereal with calcium oxide, potassium carbonate, ammonium bicarbonate or sodium bicarbonate) or destroyed by thermal processing (e.g., cooking, boiling, steaming or baking), particularly trypsin and protease inhibitors.[1,53,54]

1.3 Contribution of cereals and cereal products to the diets of Africans

Cereals and cereal products are sources of essential nutrients for most African populations. Table 1.4 shows the contribution of cereals and cereal products to the SSA diet. This food group represents the major source of dietary energy, protein, and most B vitamins, especially thiamin, riboflavin and niacin, and appreciable amounts of minerals such as iron, magnesium, sodium, and zinc for millions of people in the SSA region. Many cereals are consumed as wholegrain foods or processed into attractive and nutritious traditional food products commonly consumed by resource-limited communities in the countries.

1.3.1 Cereal-based products consumed as whole grains

Consumption of cereal grains (i.e., intact cereal grains) as dehulled or polished grains is common in SSA, varying in processing methods, food products, and forms of consumption among countries and cereal types. For instance, for some cereals such as rice, dehusked or polished grain meals are the main forms of consumption due to a non-edible hard outer layer (husk). In contrast, dehusking is an optional process for cereal grains that contain soft outer layers (hull) such as maize, wheat, and sorghum, while it is not feasible for small grains such as millets, fonio and teff. Parboiled grains are cereal products prepared by roasting (dry heat parboiling) or steaming (wet heat parboiling) of soaked or wet grains to improve their nutritional and dehusking or milling qualities.[55–57]

Table 1.4: Average contribution of cereals and cereal products to the nutrient intake in SSA

Nutrient	% contribution of cereals and their products to the average intake of nutrients		
	*East Africa	†West Africa	‡Southern Africa
Energy	27	20	30
Protein	12	10	17
Carbohydrate	44	41	49
Fat	11	6	14
Dietary fibre	22	18	29
Vitamin A	1	2	1
β-carotene equivalent	2	1	0
Thiamin (Vitamin B_1)	13	18	36
Riboflavin (Vitamin B_2)	12	7	13
Niacin (Vitamin B_3)	17	10	25
Pyridoxine (Vitamin B_6)	18	17	16
Folate (Vitamin B_9)	-	13	27
Cobalamin (Vitamin B_{12})	2	1	-
Vitamin C	2	1	14
Vitamin D	-	0	10
Vitamin E	-	5	27
Calcium (Ca)	8	4	12
Iron (Fe)	16	18	19
Magnesium (Mg)	19	16	29
Phosphorus (P)	17	16	23
Potassium (K)	10	7	11
Sodium (Na)	14	5	12
Zinc (Zn)	16	16	12
Copper (Cu)	-	10	13

Authors' calculations based on food composition tables for *Kenya,[58] †West Africa,[59] and ‡Lesotho[60]

1.3.2 Flour and bakery products: bread and cookies

Cereal flour is prepared from whole, dehulled or polished cereal grains, which can be used as a basic material for producing different cereal-based products such as leavened and semi-leavened breads, couscous, dumplings and other fermented and non-fermented cereal

products. Generally, the milling of grain flour can be carried out in dry or wet conditions: in the dry-milling process by direct grinding of the cereal grains, and in the wet milling process by first steeping the cereal grains in water before milling with water to form a slurry, pouring the slurry into a thick cloth bag and centrifuging to remove the free water before drying.[61] Note that the wet-milling process is laborious, and has higher costs associated with flour loss, water consumption, wastewater treatment and energy consumption than dry-milling.[62] Dry-milling does not generate wastewater and consumes less energy, but the quality of dry-milled rice flour is not adequate for some food items, such as rice noodles, as the flour produced is too coarse.

Wheat flour is probably the most widely used cereal flour in the bakery industry because of its gluten protein, i.e., the viscoelastic protein network responsible for flour processing characteristics in the bakery industry and textural properties of the finished bread.[63] Rice and teff flour offer many benefits, and are used to make products for celiac patients.[64,65] Use of other cereals such as maize, sorghum, millet and fonio to produce cereal flour for the preparation of traditional cereal-based products such as stiff porridge (*ugali*), bread, sourdough and beverages, is common in SSA.[66,67]

1.3.3 Cereal beverages: non-alcoholic and alcoholic drinks

Local alcoholic and non-alcoholic beverages made from cereal flour and flour of the germinated cereal grains (malt) are widespread in SSA. They play an essential role in the people's daily social, economic, nutritional, and cultural lives as they are relatively cheap to prepare, and hence a viable alternative for low-income consumers who cannot afford imported or industrially processed beverages.[68] Although the names of the beverages and their production differ from one region to another, the actual production involves the malting, brewing and fermentation of cereals such as millet, maize, rice, sorghum and fonio.[69] Women

typically manage the production of these beverages at the household level or on a small scale, involving either lactic acid fermentation for non-alcoholic beverages or lactic acid fermentation and alcoholic fermentation for alcoholic beverages.[69,70] Non-alcoholic starchy and starchy saccharified beverages made from cereal crop flour and malt flour (such as *togwa, mawe, gowe, maheu*, *mangisi*, *munkoyo*, and *kunun-zaki*[69,71,72]) are consumed by all populations as popular breakfast items, refreshments, weaning food, patients food, and energy drinks for farmers at working sites.[73,74] Haggblade & Holzapfel,[75] documented the production procedures of some of the most common local alcoholic beverages in Africa. Some of these traditional alcoholic beverages such as *chibuku, burukutu, busaa, dolo, otika, pito, tchapalo, tchoukoutou*, *mbege*, *komomi, ikigage, thobwa, kachasu, ingwebu,* and *pungwe* [69,76–80] are commercially available in the cities of Africa.

1.3.4 Ready–to–eat snack foods: flakes, puffed, popped and extruded products

Consumers' demand for convenient, nutritious, healthy, ready-to-eat processed food with a satisfying taste is rising because of urbanisation and increased women's employment in industrial and public sectors worldwide,[81] including SSA. This necessitates SSA countries to further strengthen the capacity to venture into this opportunity. Staple cereals such as maize, rice, wheat and sorghum are widely used as raw materials for pre-cooked ready-to-eat food, either directly in the production of expanded, popped and flaked products or in flour form for extruded products, noodles and pasta.[82,83]

Popped and puffed products are prepared by sudden release and expansion of water vapour generated inside the grains by instantaneous heating when exposed to a high temperature for a short time.[83] Flaked cereals are manufactured by roasting or steam cooking cereal grains and then pressing/pounding or slicing the grains into flakes.[84] Similarly, the extrusion process involves the forced passing of pre-cooked dry coarse cereal flour through perforated plates

(extruder).[85] Convenient snack foods such as popcorn, popped and puffed rice, popped sorghum and popped wheat, roasted, flaked rice, cornflakes, and as well as extruded flour, noodles, and pasta are examples of popular products worldwide.[83–86] As the simplest, inexpensive and quickest methods of heat application that impart good taste and desirable aroma to the products, popping, puffing, flaking and extrusion cooking have been used as the basis for the development of supplementary foods.

1.3.5 Complementary foods

In SSA, cereals form the basis for complementary foods (i.e., nutritious meals which also serve as weaning food), prepared as thin gruels or porridges.[87] Unfortunately, low protein content and the deficiency of certain essential amino acids, such as lysine, and the presence of anti-nutrients, such as phytic acid, tannins, polyphenols, and the coarse nature of the grains makes these complementary foods inferior compared to animal products.[88] To ameliorate this problem, cereal flours, such as maize, rice, sorghum and millet flour, are mixed with locally available legumes, nuts and fruits to produce composite flour for complementary foods. For instance, *soy-ogi* (a blend of soya bean and maize), *eko ilera* (a modified maize dough prepared by the addition of toasted cowpea flour, red palm oil and sugar), *Weanimix* (made from 75 - 80% maize, 10 - 15% soya bean or cowpeas and 10% groundnuts), and *kenkey* (a modified maize dough prepared by addition of 20% cowpea flour) are common complementary foods developed to improve the protein quality of maize-based foods in West Africa.[89–93] Similarly, in Eastern Africa, soya bean was included in the production of *ugali* (a stiff maize porridge) to enhance content of protein and available lysine.[94]

1.4 Uses of immature cereals as food

1.4.1 Nutritional relevance of immature cereals

Immature cereals have been reported to contain higher amounts of several nutrients compared to their mature counterparts (Table 1.5). Generally, the chemical and nutritional composition of cereal grains changes substantially during maturation. Previous studies found the reduction of DF such as fructooligosaccharides and fructans, during the ripening of wheat and barley grains.[95–98] Also, the amount of sugar was higher in green wheat, while starch quantities increased during maturation.[98–100] Similar to wheat grains, the highest amounts of total sugars, glucose and fructose were found in immature rice and maize grains, while starch quantities increased with maturity.[98,101–104] Contrary to other grains (i.e., wheat, rice, maize and sorghum), the amount of total sugar increased as barley grain matured.[105] Protein content decreased during maturation of maize[103] and sorghum,[106] whereas it increased in wheat and barley during maturation.[95,107] Even though the amount of protein was lower for green wheat and barley, the content of some essential amino acids such as lysine, methionine and threonine for wheat and histidine and lysine for barley was significantly higher than in their mature grains counterparts.[98,107]

Micronutrients such as potassium, phosphorus, magnesium, manganese and iron decreased during maturation, whereas calcium and zinc were relatively stable.[108,109] The proportion of total zinc in the embryo of maize compared to endosperm zinc increased with maturation. However, total levels of zinc did not significantly change.[110] Besides, the amount of iron, copper, manganese and nickel decreased during a late stage of milky maturation of maize grains.[111] A similar trend was observed in barley, where minerals such as phosphorus, calcium and magnesium decreased during maturation.[105] Furthermore, immature wheat and rice have been reported to contain a higher amount of vitamin C, niacin and B group vitamins, as well as bioactive compounds such as provitamin A, α- and γ-tocopherol, α- and γ-tocotrienol, γ-

Table 1.5: Changes in content of nutrients and bioactive compounds of several cereal grains during maturation (assessed on the basis of the concentration of a compound per 100 g)*

		Cereals					
Nutrient group	Compound	Rice	Wheat	Maize	Barley	Sorghum	Reference
Lipids	Lipids		↓	↑			103,107
Carbohydrates	Starch	↑	↑	↑	↑	↑	98–100,103–105,116,117
	Sugars	↓	↓	↓	↓	↑	
	Fibre	↓	↓		↓		95–98
	Fructo-oligosaccharides		↓				
	Beta-glucans				↑		
	Fructans				↓		
Proteins and amino acids	Protein		↑	↓	↑	↓	95,103,106,107
	Lysine		↓		↓		98,107
	Histidine				↓		
	Methionine		↓				
	Threonine		↓				
	Cysteine				↑		
	Leucine		↑		↑		
	Phenylalanine		↑				
Phenolic Compounds	Antioxidant activity	↓		↑			112–114,118
	Phenolic compounds			↓			
	Anthocyanidins*			↑↓			
	Proanthocyanidins	↓					
	Tocochromanols	↓					
	Catechin	↓					
Carotenoids	Lutein			↓			114,115
	Zeaxanthin			↓			
	Cryptoxanthin			↓			
Vitamins	Vitamin B_1	↓			↓		99,107,116
	Vitamin B_2	↓	↓				
	Vitamin B_3	↓	↓		↓		
	Vitamin B_6	↓	↓				
	Vitamin C	↓	↓				
	Tocopherols	↓	↓				
	Tocotrienols	↓					
	Provitamin A	↓	↓				
Minerals	Potassium		↓				105,108–111
	Phosphorus		↓			↓	
	Magnesium		↓			↓	
	Manganese		↓	↓			
	Iron		↓	↓			
	Nickel			↓			
	Copper			↓			

* An downward pointing arrow indicates a higher amount of a compound in the immature grain compared to the mature grain, and an upward pointing arrow indicates a higher amount of a compound in the mature grain.

tocochromanols, proanthocyanidins, catechin and antioxidant activity compared to their mature counterparts.[99,101,107,112,113] A variation in B group vitamins was observed during barley grain development; thiamine increased while niacin decreased, whereas riboflavin

remained relatively constant during maturation.[99] The most prevalent carotenoids in maize (i.e., lutein, zeaxanthin and cryptoxanthin) and anthocyanin and phenolics decreased during maturation.[114,115]

1.4.2 Traditional immature grain-based products

Immature cereals also form an important food source for the resource-limited communities in Africa. Immature grains are used to break hunger when no food is available in the household while crops in the field are not mature. Additionally, immature grain-based food products are preferred by communities due to the desirable nutritional and sensorial benefits they offer over counterpart food products. Generally, harvested immature whole or dehusked grains at the milky or dough stage are processed into several food products such as roasted or boiled, steamed or parboiled and fermented green cereal products commonly consumed in these countries (Table 1.6).

Table 1.6: Some traditional African immature cereal-based food products

Product	Product and process description	Processing techniques	Origin	References
Pepeta	Immature rice that is soaked, roasted and pounded	Soaking, roasting, pounding	Tanzania	Miraji et al.[119]
Wotelo	Broiled unripe spikes of cereals at milky and dough stage	Roasting over flame	Tanzania	Bekele et al.[120]
Freekeh, or Frikeh	Green wheat that is sun-dried, roasted and rubbed	Roasting, sun-drying, threshing	North Africa	Bayram[121]
Green barley spikes	Awns are removed from the spikes, which are crushed between the palms and consumed. These unripe spikes can also be green roasted over a fire	Crushing, roasting	Ethiopia	Asfaw[122]
Eshet	Maize harvested at milk or doughy stage which is then roasted or boiled	roasting over flame/boiling	Tanzania	Bekele et al. [120]
Green mealie (maize) bread	Wet milling of green maize kernels to a thick dough on a grinding stone and adding salt for taste. The dough is placed over a steamer to cook	Wet milling, grinding, steaming	Lesotho	Nkhabutlane et al.[67]
Senkhoana	Green sorghum bread. Wet milling of green Sorghum kernels to a thick dough on a grinding stone and adding salt for taste. The dough is placed over a steamer to cook	Wet milling, grinding, steaming	Lesotho	Nkhabutlane et al.[67]

1.4.3 Immature grains as food ingredients

Immature grains can be used in food products as functional ingredients, where their composition could enhance the nutritional quality of food products. Table 1.7 shows some cereal-based and dairy products enhanced with immature cereal grains to improve taste, colour, flavour, and nutritional quality. Immature cereals grains, as ingredients, add a substantial amount of vitamins, minerals and bioactive compounds to cereal-based and dairy products. For instance, immature wheat and rice flour increase the amount of DF (such as fructooligosaccharides and fructans), ferulic acid, free polyphenols and flavonoid , as well as

ascorbate, glutathione and antioxidant activity in breads,[123–125] and pasta[96,126,127] products without a considerable alteration of sensory and physical properties such as appearance, crust colour and thickness, crumb structure, volume and elasticity.[123] Additionally, enhancing yoghurt with immature cereal grains or flour increases volatile aromatic acids, acetaldehyde, minerals, insoluble DF, α-tocopherol and γ-oryzanol, phenolic compounds and antioxidant activity.[128–131] These examples demonstrate the possibilities to use immature grains to improve the nutritional quality of cereal-based products, thereby increasing their valorisation. Using immature grains also fits the current trend of consumers wanting more healthy foods containing health-promoting properties.

Table 1.7: Examples of food products formulated with (part of) ingredients from immature grains and changes in nutritional and sensorial properties

Product	Description	Improved nutritional and sensory properties	Source
Semolina	Green durum wholemeal flour added to semolina from mature wheat at 0, 20, 40, 60, 80 and 100 w/w% proportions.	Fructans, ascorbate, glutathione, antioxidant activity and dough tenacity.	Paradiso et al.[96]
Pasta	Pasta enriched with 30% immature wheat grain dried at to 13% moisture and milled	DF, glycemic index, colour and cooking quality.	Casiraghi et al.[126]
Bread	Bread made from wheat flour enhanced with 10/20 w/w% immature wheat flour	Exopolysaccharide and fructo-oligosaccharide.	Pepe et al.[123]
Madeleine (pastry)	Madeleine made from immature and mature wheat flour	Ferulic acid and sensory perception.	Kim and Kim[127]
Set-type yoghurts	Set-type yoghurt enriched with immature wheat flour at 0, 1, 2, and 3 w/w%	Acetaldehyde content, aromatic volatile acids and taste	Göktepe and Akın[128]
Sourdough bread	Sourdough bread made with immature durum and wheat grain	Free flavonoids, DF and polyphenol.	Saa et al.[124]
Sourdough bread	Sourdough bread enhanced with immature wheat flour (10 w/w%)	Total DF, hardness and volume	Çetin-Babaoglu et al.[132]
Set-type yoghurts	Set-type yoghurt enriched with immature wheat flour at 0, 1, 2, and 3 w/w%	Antioxidative activity, total phenolic and textural properties	Demirci et al.[129]
Sourdough bread	Sourdough bread made with immature durum and wheat grain	DF and bioactive compounds	Saa et al.[125]
Syrup from immature rice	Syrup from immature rice sugar using solid-state fermentation of Aspergillus oryzae TISTR 3102.	Reducing sugars	Chuayjum et al.[133]
Gluten-free bread	Gluten-free bread with infrared stabilised immature rice grain at 0, 30, 50, 70, 100 w/w% proportions	Insoluble DF, Mg, Mn, K, Fe, Tocopherols, γ-oryzanol, hardness, adhesiveness and chewiness.	Özer et al.[131]
Green Spelt Tempeh	Tempeh made by fermenting green spelt	DF, free amino acids, peptides, antioxidant capacity and phenolic acid and compounds.	Starzyńska-Janiszewska et al.[134]
Wholemeal Spelt Bread	Wholemeal spelt bread enriched with green spelt grain, in proportions of 4, 8 and 12 w/w%.	P, Mg, Ca, Zn, amino acids, lipids, mono- and polyunsaturated fatty acids.	Kraska et al.[135]

1.5 Knowledge gap and research rationale

In the SSA region, cereals are important staples that are critical to the daily survival of millions of people, where most of the cereals are consumed as refined products (dehusked or polished) to improve their sensory properties. The refining process removes the bran and germ of cereal grains, which are rich in important nutrients beneficial to health, including DF, vitamins, minerals and phytochemicals.[2,49,136] As such, the micronutrient deficiency incidences are still high in the SSA region, associated with the consumption of low-quality food.[137,138] This poses a challenge to develop cereal-based food products with improved nutritional quality, meeting consumer expectations. Immature cereals and their products are shown to have potential nutritional benefits (section 1.4.2 and 1.5). Thus, utilisation of immature cereal-based products such as *pepeta*, a locally prepared immature rice-based food product, is among the alternatives to pursue the goal of better quality cereal-based nutrition.

Pepeta is widely consumed in Tanzania where rice is the second most important food and commercial crop after maize, providing employment, income, and food security for farming households.[139] Almost all rice produced in Tanzania is consumed as milled white rice, with limited economic usage of rice-based products.[139] The indigenous *pepeta* processing knowledge and the product itself are unique and very different from other existing rice thermal processing technologies such as parboiling, flaking and puffing. The *pepeta* preparation process involves roasting of the immature paddy rice grains harvested at the milk or grain filling stage, followed by immediate hand pounding of the grains using mortar and pestle to obtain flattened rice grains mixed with husks, and then cleaning by winnowing to remove the husks, after which the product is ready for consumption.

Traditionally prepared *pepeta* has potential nutritional benefits because of its sole ingredient and the applied processing technique – the immature rice grains and thermal processing of the paddy grain. To date, the information about the effect of *pepeta* processing conditions on the

nutritional and sensory properties and the bioavailability of nutrients in immature rice grains and their products, such as *pepeta*, is limited. Similar to most traditional immature cereal-based products in SSA (Table 1.6), *pepeta* processing is laborious and expensive, and the factors that affect its quality and hence the prospects for further product development, as well as the hygienic conditions associated with *pepeta* preparation, are scarce. This lack of knowledge and understanding of underlying principles hinders the full utilisation of immature cereal grains and their products, including *pepeta*, despite their nutritional, sensory and functional potential benefits. To be able to develop a nutritious, immature cereal-based product of high sensorial quality, the factors influencing the quality, including the nutritional, physicochemical, rheological, and aroma properties, need to be studied and understood.

Therefore, this thesis assesses the traditional knowledge of the *pepeta* product and its quality to improve the processing conditions and product's nutritional, sensory, and digestibility properties. As a result, the outcomes could encourage the purposeful use of immature rice grains for improved nutritional and enhanced economic values by creating new markets in the cities and towns in the country and outside Tanzania. Additionally, the findings are expected to benefit the use of other immature cereal grains and SSA countries as well.

The thesis project aimed to valorise potentially nutritious immature cereals and their food products to improve the nutritional and sensory quality of cereal-based food products for enhanced food security in SSA, using *pepeta* as a case study. To achieve this, four main research objectives were identified.

a) Gain insight into the *pepeta* processing knowledge and assess variations in the processing conditions and parameters across the main production areas.
b) Assess the effect of maturation and *pepeta* processing on the nutrient content and *in-vitro* digestibility of starch and protein of rice grains and *pepeta* products.

c) Investigate the impact of roasting (i.e., dry-heat processing) at different conditions on the nutritional composition and *in-vitro* starch and protein digestibility of immature rice-based products.

d) Assess the effect of maturity and different processing practices on the visual quality properties and volatile profiles of rice and immature rice-based products.

1.6 Thesis outline

Fig. 1.2 presents a schematic overview of the thesis outline, including the specific research questions to address the identified objectives. This general introduction provides information on the importance of cereals commonly consumed in SSA, emphasising the immature cereals, their nutritional relevance and the research issues that this thesis addresses. **Chapter 2** focuses on gaining insight into the *pepeta* processing knowledge and assessing variations in the processing conditions and parameters across the study area in Tanzania. **Chapter 3** further establishes the nutritional and digestion properties of *pepeta* products and immature rice used for *pepeta* production. The knowledge from both chapters serves as a base for chapters 4 and 5. **Chapter 4** investigates the impact of maturity and roasting conditions (soaking and temperatures) on the nutritional composition and *in-vitro* starch and protein digestibility of immature rice-based products. These processing conditions were identified as important parameters that improve the specific nutritional, functional and sensory properties of traditionally processed *pepeta* products. Subsequently, **chapter 5** assesses the effect of maturity and different processing practices on the visual quality and volatile profiles of rice and immature rice-based products. Finally, **chapter 6** presents the general discussion of the main findings, their implications, as well as opportunities, challenges and future developments of the utilisation of immature cereals grains in SSA.

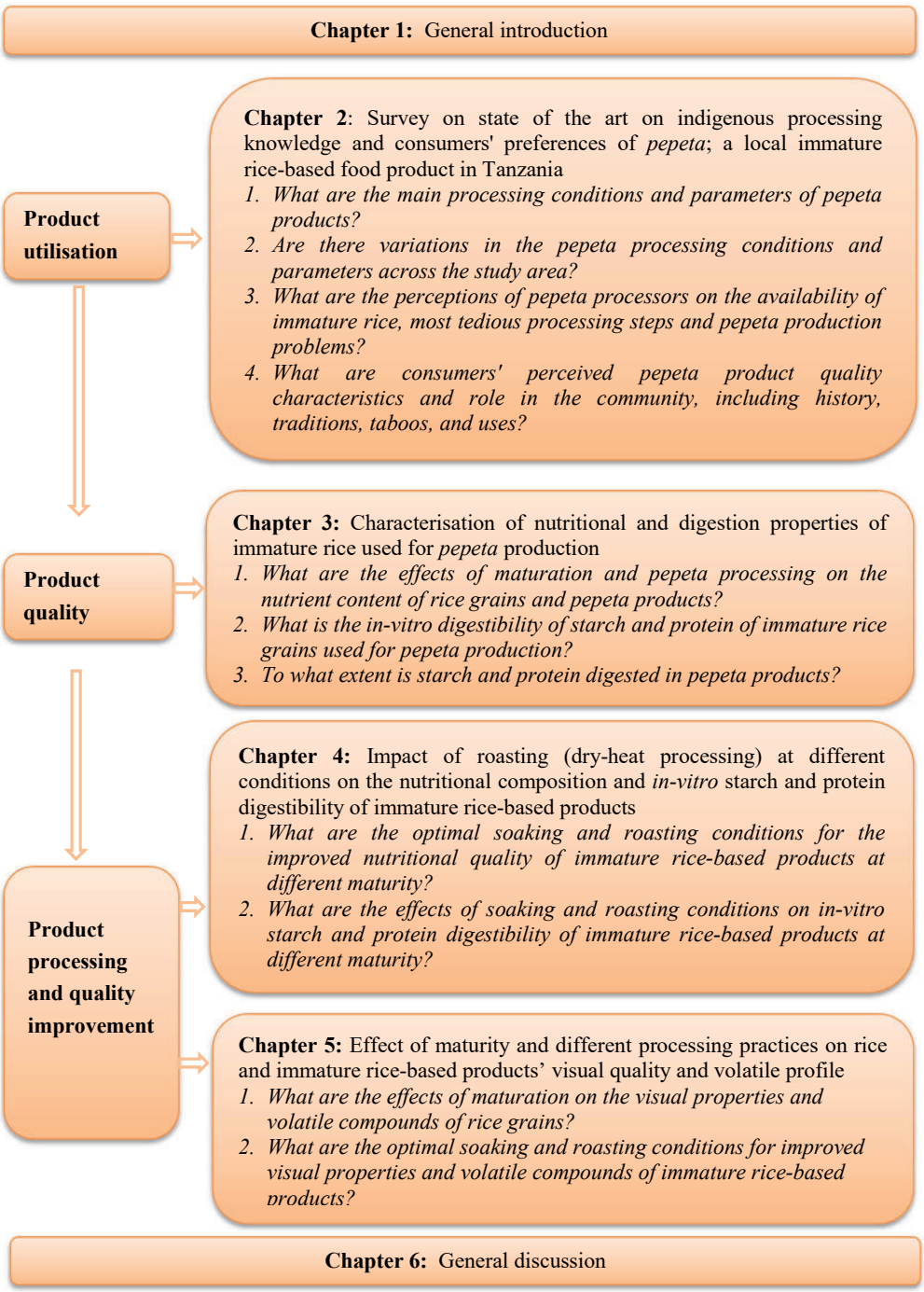

Fig. 1.2: Schematic overview of the thesis outline.

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2

CHAPTER 2.

Utilisation of *Pepeta*, a locally processed immature rice-based food product, to promote food security in Tanzania

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Abstract

Consumption of *pepeta*, a traditionally processed immature rice product, is common in Tanzania, where it contributes to food security as an early rice food i.e. when there is no other food available at the household while the crops in the field not yet fully ripe. Many production steps are needed to guarantee a consistent, good quality *pepeta* product, and this hinders its utilization in other rice-growing sub-Saharan regions. This study aims to gain insight into the *pepeta* processing knowledge and final product, and assess variations in the processing conditions and parameters across the study area. A survey among 257 Tanzanian processors and consumers revealed that the *pepeta* product is widely known, rated second (73.5% respondents) as rice-based food after *wali* (cooked white rice, (100%)) and linked to traditions of the communities in the study area. Harvest of immature rice grain, roasting, pounding, cleaning, and packing are the main process steps of *pepeta* production. Method of rice harvest, rice suitability for *pepeta* production after optimum harvest, dryness of grains and number of pounding as indicator to terminate roasting and pounding process respectively, and packaging materials used varied significantly across respondents in the study area. Reported criteria considered by respondents for product acceptability did not vary significantly across study area. The criteria include colour (76.5%), general appearance (60.8%), texture (64.7%) and taste (52.9%). Immature rice paddy and *pepeta* were sold at a higher price than mature rice paddy and white rice, respectively, which implies that options to facilitate *pepeta* processing through, for instance, standardization of processing conditions and parameters could lead to increased income.

Keywords: *Pepeta*, traditional processing, *Oryza sativa*, immature rice, cereal snack, food security

2.1 Introduction

Rice (*Oryza sativa* L.) is an important staple crop for global food security. Its global utilization is estimated at 503.9 million tonnes (milled basis), of which 80.5% in food uses accounting to a per capita food consumption of 53.9 kg.[1] In the sub-Saharan region, including Tanzania, rice farming is a key subsistence activity and serves a dual purpose as a major source of households' income and food security.[2] In this cropping system, rice is mainly grown on small farms of 0.5–3 ha per household, covering up to 75% of the rice production area.[3,4] The sub-Saharan region witnesses an increase in rice consumption at a higher pace than ever before due to increased urbanization and an income rise. On average, the annual per capita consumption of milled rice in the region is estimated at 31.0 kg in 2018, which is about 30% more than ten years before.[1]

Rice is mainly consumed as milled kernels, i.e. after removing the outer hard layer (husk) to produce brown rice or after further polishing by removing the germ and the inner soft layer (bran) to produce white rice.[5] Nutritionally, the mature dry rice grain contains 80% starch, 12% water, 7.5% protein and 0.5% ash, while providing up to 46 and 43% of dietary energy and dietary protein in the sub-Saharan region, respectively.[3,6] The rice germ and bran are valuable sources of iron and zinc, dietary fibre, vitamin B (i.e. riboflavin, thiamine and niacin), and vitamin E.[3] The exclusive consumption of white milled rice has caused vitamin and mineral deficiencies, despite the good nutritional potential of whole rice. This is attributed to losses of B vitamins and minerals, which are concentrated in the husk, germ and bran, which are removed during milling.[7]

Studies to improve the nutritional quality of milled rice generally focus on optimising product and processing aspects. These include rice flour processing and development of related products such as fermented, baked, extruded and fried products.[8] Hydrothermal rice processing technology, i.e., parboiling significantly improve the nutritional quality of rice by

enhancing the diffusion of some minerals and water-soluble vitamins into the endosperm.[9,10] Most studies concern mature rice, with little attention on the use of immature rice grains. Use of immature cereal grains has potential nutritional benefits, since nutrient contents tend to decrease as grain matures. Several studies reported higher amounts of nutritive components such us protein, reducing sugar, calcium, potassium, iron, β-carotene, vitamin C, vitamin B_2, B_3 and B_6, vitamin E and γ-oryzanol in immature grains when compared to mature grains.[11–13] Physical and economic access to nutritious food is among main components of food security.[14] Sales of crops to meet household cash obligations have been linked to the improvement of sustainable food and livelihood security.[2,15] Apart from premium selling, use of immature grains can directly increase smallholder farmers' income through reduced farm management costs due to early harvest and exclusion of postharvest operations like drying and storage. The gained cash income can either be used by households to increase yields (hence physical food accessibility) through improved crop management practices or obtain food from the local market (economic food accessibility).[2]

Currently, there is a growing interest at valorising potentially nutritious though neglected immature cereal-based food products.[16–18] Traditional processing of immature rice to produce *pepeta* is common in Tanzania and has existed in isolation from rest of the sub-Saharan region. *Pepeta* is widely consumed by communities because its natural flavour, which resembles the buttered popcorn aroma. As snack food, *pepeta* has potential due to increased consumer's demand for nutritious, healthy, ready-to-eat processed food with satisfying taste and ease of portability because of rising urbanization and increased employment of women in industrial and public sectors worldwide.[19,20] However, very little information (except nutrition composition[21]) is available in the literature related to *pepeta* processing knowledge and the final product. Therefore, the present study was undertaken to gain insight into the *pepeta* processing knowledge and assess variations in the processing conditions and parameters

across the study area. Perceived *pepeta* product quality characteristics, its role in the community including history, traditions, taboos and uses, and *pepeta* problems and trade supply chain in the study area were investigated as well. The documented information serves as a basis for research into possible ways to optimise specific processing conditions to improve nutritional quality, and/or other fields along *pepeta* value chain to enhance its competitiveness.

2.2 Methodology

2.2.1 Study design

Both quantitative and qualitative approaches were used in this study. A face-to-face interview through questionnaires together with focus group discussion and on-field observation via demonstrations were the main data collection tools used. The questionnaire was based on a conceptual model that represent simplified *pepeta* value chain and its link to food and livelihood security.

2.2.2 Questionnaire design

2.2.2.1 Conceptual model used to design the questionnaire

Fig. 2.1 show a conceptual model used to design the questionnaire (S1 Questionnaire) and checklist (S2 Checklist). The model reflects the key actors (rice farmers, processors and consumers) along *pepeta* value chain and its linkage to food and livelihood security. Immature rice grains are sole ingredients for processing of *pepeta* products, the availability and accessibility can substantially affect *pepeta* processing and consumption. Rice is preferred for human consumption and plays a significant role in Tanzanians' culture, traditions, and religion.[22] It is a major source of income, food and employment in rural areas, providing about 95% of the national food requirements, accounts for more than 70%

livelihoods of the Tanzanian population.[23] The influence of processing conditions on product quality properties and acceptability are fundamental in processed food products like *pepeta*,[24] affecting food product utilization. Therefore, indigenous knowledge on immature rice grains harvesting and processing, information on *pepeta* product quality and acceptability, and existing problems are included in the model as well. Food product characteristics such as appearance, taste, texture, colour, aroma, and socio-economic characteristics of consumer like gender, age, education and income can influence food choices and preference.[25]

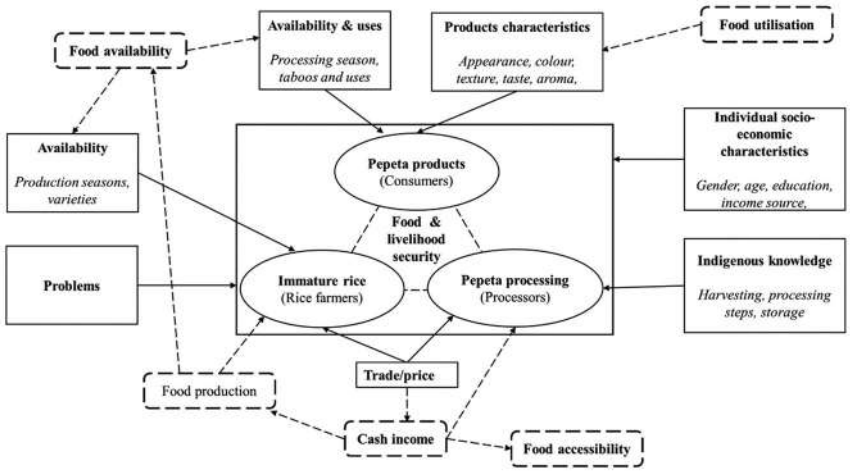

Fig.2.1: Conceptual framework for the design of the questionnaire used to collect information along *pepeta* value chain. ——— indicates possible factors that can affect *pepeta* value chain, and — — — · indicate elements of *pepeta* processing interact with food and livelihood security.

2.2.2.2 Questionnaire

The questionnaire was developed based on the conceptual model (Fig. 2.1) to collect information on the following aspects: (1) General information and awareness of *pepeta*, (2) *Pepeta* processing knowledge, (3) Product quality criteria and utilization, and (4) Product processing, marketing and storage constraints. It was divided into sections containing specific

questions to extract information from different actors along *pepeta* processing value chain (S1 Questionnaire), as follows:

- *General information:* Gender, age, education, income source, awareness about *pepeta* and type of actor in the value chain, i.e., processor, consumer and/or trader.
- *At consumer level:* Consumer information on consumption frequency of *pepeta*. Place of purchase (e.g., informal market, farmer-gate, and/or supermarket), prices relative to product quality (freshness and seasonality). Properties considered during purchase (e.g. colour, appearance, texture, taste and aroma).
- *At processor level: Pepeta* processing techniques and product quality. Sources of raw materials (immature paddy), parameters considered when purchasing raw material for *pepeta* processing (i.e., varieties and maturity level) and prices. Places of sales of *pepeta*, consumer perception of product quality (e.g., colour, appearance, texture, taste and aroma), constraints and most tedious process steps.
- *At trade level:* Places of sales of *pepeta*, consumer perception of product quality (e.g. colour, appearance, texture, taste and aroma).

2.2.3 Study area

The study was conducted in the Eastern and Southern Highland parts of Tanzania, focusing on five district administrative boundaries: Kilombero (08°07'S, 36°40'E), Ulanga (09°00'S, 36°40'E), Mvomero (06°18'S, 37°27'E), Bagamoyo (06°26'S, 38°54'E) and Iringa rural (07°46'S, 35°42'E) (Fig. 2.2). The study area comprises several valleys that are considered among the most fertile areas in the country, suitable for irrigation agriculture, including rice crop cultivation. The valleys include Great Ruaha River (208 000 ha) and the Little Ruaha floodplain (4800 ha) in Iringa rural district council, the Kilombero valley floodplain (329 600 ha) in Kilombero and Ulanga district councils, the Wami floodplain (169 000 ha) in Mvomero

district council, and the Ruvu floodplain (117 000 ha) in Bagamoyo district council.[26] The district administrative areas were chosen based on the relevance of rice as one of key crops in the farming system and its dual purpose as a major source of households' income and food security. According to the perception of local people (community leaders) and Village Agriculture and Extension Officers (VAEOs), the area has also been serving as a centre of *pepeta* processing knowledge for many decades.

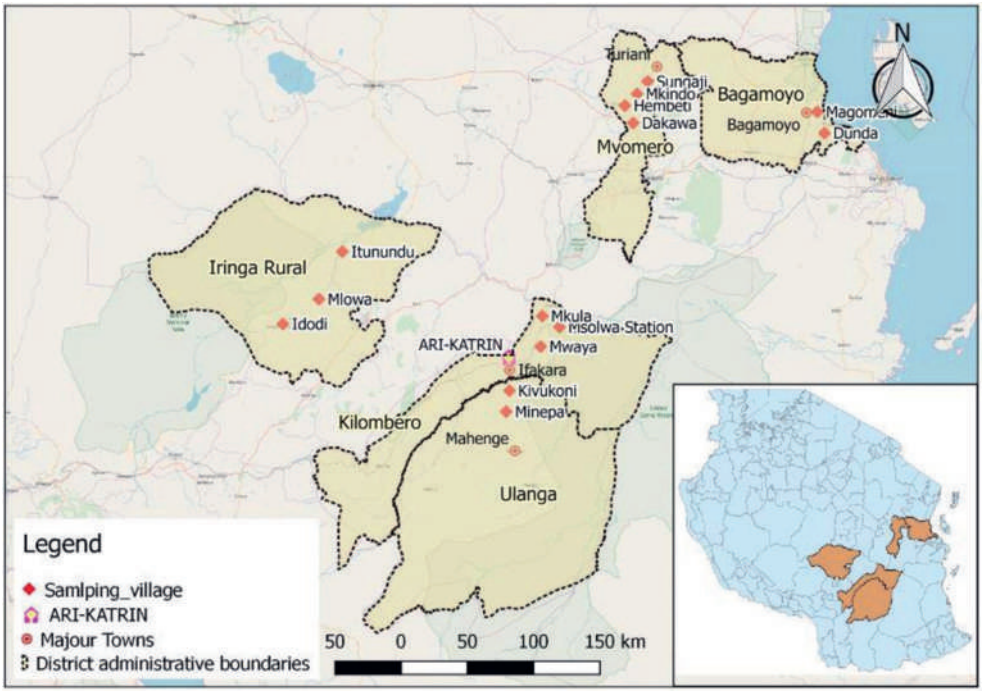

Fig. 2.2: Study area surveyed for *pepeta* product and its indigenous processing knowledge in Tanzania. Coordinates for district administrative boundaries are indicated as follows: Kilombero (08°07'S, 36°40'E), Ulanga (09°00'S, 36°40'E), Mvomero (06°18'S, 37°27'E), Bagamoyo (06°26'S, 38°54'E) and Iringa rural (07°46'S, 35°42'E). ARI-KATRIN = Agricultural Research Institute KATRIN. Source: Survey data, September 2017 – February 2018. The map background and shapefiles were taken from OpenStreetMap Foundation (OSMF, United Kingdom) and National Bureau of Statistics (NBS, Tanzania) with permission under a CC BY license, respectively.

2.2.4 Sampling of respondents/informants

Prior to data collection, a random observation was done on 117 food venders selling food at local markets to estimate the proportion "p" of venders selling rice-based food products. The research team assessed whether a vender was selling at least one common rice-based food product found in the community. The obtained proportion was then used to estimate the total number "n" of respondents to be interviewed in the study, using the following formula:[27]

$$n = \frac{U^2_{1-\alpha/2} \times p(1-p)}{d^2}$$

where n is the total sample size; p is the proportion of targeted informants; $U_{1-\alpha/2}$ = value of the Normal random variable for a probability value of 0.975 (or α=0.05), $U_{1-\alpha/2} \approx 1.96$; d = margin error of the estimation fixed at 5% (0.05).

The district population size was used to compute the number of participants to be interviewed for each district council (Table 2.1) according to:[28]

$$ni = \frac{Ni}{N} * n$$

where n_i is the number of participants in the district council; N_i is the total number of the population in the district council; and N is the total number of people living in the five selected district councils. The used population data were based on Tanzania National Bureau of Statistics report.[29]

Table 2.1: Population distribution overview in the study area

District council	Population size*	Proportion of the population	Number of informants
Kilombero	339,092	0.27	68
Ulanga	169,853	0.14	38
Mvomero	351,075	0.28	72
Bagamoyo	108,811	0.09	24
Iringa rural	268,840	0.22	55
Total	1,237,671	1	257

*According to Tanzania National Bureau of Statistics (NBS)[29]

2.2.5 Field data collection

Data collection was from September 2017 to February 2018, using a structured questionnaire, by focus group discussions and observations. The questionnaire was first tested and adjusted before being administered to the 257 respondents selected from five district administrative boundaries (Table 2.2). For face-to-face interviews, respondents from each household in the selected villages were randomly selected from the list of rice farmers and/or *pepeta* processors who were willing to participate provided by VAEOs. The interviews were held in *Swahili*, the national language of Tanzania, which was well understood by all respondents.

Table 2.2: Demographic profile of respondents (as percentages) in the surveyed administrative districts for *pepeta* product and its indigenous processing knowledge in Tanzania

	Districts					Overall % (n=257)
	Kilombero (n=68)	Ulanga (n=38)	Mvomero (n=72)	Bagamoyo (n=24)	Iringa rural (n=55)	
Gender*						
Males	26.5	15.8	40.3	70.8	67.3	41.6
Females	73.5	84.2	59.7	29.2	32.7	58.4
Age group (years)*						
18 – 29	20.6	23.7	19.4	8.3	20.0	19.5
30 – 44	47.1	39.5	33.3	8.3	40.0	37.0
45 – 59	27.9	31.6	34.7	50.0	29.1	32.7
60^{+}	4.4	5.3	12.6	33.4	10.9	10.9
Level of education*						
No education	13.2	15.8	9.7	8.3	0.0	9.3
Primary level	79.4	78.9	73.6	62.5	78.2	75.9
Others [a]	7.4	5.3	16.7	29.2	21.8	14.8
Main source of livelihood [b]						
Agricultural activities*	52.9	26.3	66.7	54.2	70.9	56.8
Rice cultivation only*	48.5	73.7	33.3	45.8	29.1	43.6
Pepeta processing*	50.0	52.6	0.0	0.0	0.0	21.0
Others*[c]	1.5	7.9	41.7	45.8	49.1	28.0

[a] Combined secondary level, vocational training and tertiary/collage options since the number of each case was small, [b] more than one answer possible, [c] combined casual labour, remittances, petty trade, mechanics and civil servant options since the number of each case was small, *Significance difference among districts at $p < 0.05$. Source: Survey data, September 2017 – February 2018.

Focus group discussions were employed to comprehend the collected information from individual face-to-face interviews, as people normally tend to mention the most important things when asked to freely recall under a given short time.[27] Such discussions consisted of 12 - 15 participants, purposively selected following criteria such as age (youth, people of reproductive age and old), gender balance, and different actors (farmers/consumers, processors and traders) along the *pepeta* processing value chain (Table 2.3). The focus group discussions last for 2 – 3 hours for each conversation and were guided by a checklist. A total of 5 focus group discussions were conducted; 3 in Kilombero district and 2 in Ulanga district, depending on the population sample size. The observation data were collected during harvesting and handling of rice and *pepeta* processing to verify data collected from interviews. Physical properties data i.e. moisture content, weight, temperature and duration of various processing steps of *pepeta* production were measured using a digital grain moisture meter (SATAKE, MOISTEX SS7), weighing scale (Endel™, EWS-H-PLUS), digital thermometer (Fluke, model 52 II), and digital timer (Fisherbrand™), respectively.

Table 2.3: Overview of focus group discussion

Participants category	Number of participants	
	Kilombero district	Ulanga district
Gender		
Male	6	7
Female	6	8
Total (per FGD*)	12	15
Age		
Youth (< 30 years)	2	2
Adults (30 – 49 years)	4	5
Elders (> 49 years)	6	8
Total (per FGD)	12	15
Pepeta actors		
Rice farmers/consumers	6	8
Pepeta processors/traders	6	7
Total (per FGD)	12	15
Number of FGD conducted	3	2

*Focus group discussion. Source: Survey data, September 2017 – February 2018.

The study was approved by and conducted in collaboration with Tanzania Agricultural Research Institute (TARI) – Ifakara center, following all relevant regulations. The individual pictured in Fig. 2.5 and 2.6 has provided written informed consent (as outlined in PLOS consent form) to publish their image alongside the manuscript.

2.2.6 Data processing and analysis

Data from individual household interviews were subjected to descriptive statistics (percentages or frequencies) using IBM SPSS statistics (version 23, USA) and Microsoft Excel 2016. A chi-square test of independence at 0.05 level of significance was performed to determine if there was significant different between *pepeta* processing practices of Kilombero and Ulanga respondents and ascertain the relationship between *pepeta* knowledge (awareness) and respondents' demographic data (gender and age). The chi-square test was performed independently for each individual option in the multiple response questions. Data for process efficiency, and moisture, weight and heat losses, the independent t-test ($p < 0.05$) was computed to evaluate any significant different between Kilombero and Ulanga districts. Qualitative data gathered through focus group discussion and observation were content analysed. The collected GPS data were used to prepare a map using QGIS (version 3.4.3).

2.3 Results and discussion

2.3.1 Product history, taboo and uses

According to the focus group discussions, *pepeta* means *"flattened grains"* and its processing knowledge dates back since introduction of rice in the community. This knowledge has been passed on from generation to generation. *Pepeta* and its processing knowledge form an integral part of social rites, rituals and festivals of the *Ndamba, Mbunga, Ngindo, Pogoro, Kwere* and *Doe* ethnic tribes found in the study areas as mentioned by respondents. According

to the interviewees, no girl would qualify for marriage if she would not know how to process *pepeta*. Therefore, *pepeta* processing was one of the trainings given to young girls during *unyago* rituals, a practice to celebrate the coming of age of girls or during weddings. According to *kwere* and *doe* tribes, a grounded *pepeta* product, known as *bwimbwi*, was used as a participation fee in *jando*, a circumcision rite for boys. *Unyago* and *jando* rituals involved instructing youth about sex and conjugal life.[30] It was further believed that a marriage became happy if a spouse (wife) prepared a large amount of *pepeta* to proudly serve her husband throughout a year or until the next rice harvesting season. Preparing *pepeta* is a way of showing affection and therefore *pepeta* was also given to special guests like in-laws as a symbol of respect and care. Reserving traditional snacks for special, esteemed individuals like men and in-laws is a common practice among Tanzania communities.[31]

Pepeta was also recognized as a symbol for the start of a new harvest season for *Ndamba, Mbunga, Ngindo* and *Pogoro* tribes. No household was allowed to start the new harvest before *pepeta* was prepared and sent to the *chief*, a community leader, for making a ritual sacrifice asking protection against natural calamities, wild animals and birds.

In the study area, respondents explained their reason for using immature rice to prepare *pepeta:* as a means of securing food when no other food was available at the household while the crops in the field not yet fully ripe. Therefore, mothers were forced to prepare *pepeta* to feed their children while waiting for the main dish. These findings illustrate the tremendous importance of *pepeta* in the community living in the surveyed areas: “a means of communication, affirming and reinforcing social relations, of expressing one’s personal or group identity and of connecting to the living or ancestral peer group”.[32]

2.3.2 Product popularity

Though the popularity of *pepeta* varied significantly (x^2= 109.193, df = 4, p = 0.000) across respondents in the study areas, most of the respondents (73.5%) knew the product and rated the product second after *wali* (cooked white rice) (Table 2.4), the main form of consuming rice in Tanzania.[23] *Pepeta* was mentioned as one of the common rice-based food products by 100.0% of respondents in Kilombero and Ulanga districts, and by 79.2 and 70.8% in Bagamoyo and Mvomero rural districts, respectively. Contrarily, only 23.6% of respondents from Iringa rural districts knew *pepeta.* This difference could be due to observation that *pepeta* was no longer processed (hence unavailable) in Iringa rural district, whereas it is regularly processed for both sales and household consumption in Kilombero and Ulanga districts, and occasionally processed in small quantity for household consumption in Mvomero and Bagamoyo districts. *Pepeta* processing, as traditional knowledge, is attributed to several factors including gender and generation.[32] To confirm this concept, the association between *pepeta* knowledge (awareness) and respondents' demographic data (gender and age) among districts (Mvomero, Bagamoyo and Iringa rural) was tested. The analysis in Table 2.4 show gender differed significantly (x^2= 8.817, df = 2, p = 0.012) across respondents in the districts, more males knew *pepeta* product in Bagamoyo and Iringa rural districts, and the vice versa is true for Mvomero district. However, no significant differences were found in the overall respondents between males (53.0%) and females (47.0%) for *pepeta* knowledge (x^2= 3.417, df = 1, p = 0.065), indicating location is the main influencing factor for the observed differences across districts in the study area. To evaluate the impact of age on *pepeta* knowledge, respondents were categorized into three groups: below 30 years (regarded as youth with little or no knowledge of *pepeta*), between 30 and 44 years (regarded as adults with moderate *pepeta* knowledge), and 45 years and above (considered as elders with much experience on *pepeta*). There was a significant difference in the overall respondents regarding

pepeta knowledge among age groups (x^2=9.696, df = 2, p = 0.021), with 61.5% of those familiar with *pepeta* (55.0% of the respondents) being 45 years and above, while 12.0% were below 30 years (Table 2.4). Also, age groups differ significantly (x^2= 6.265, df = 4, p = 0.018) across the respondents in the study area. The data suggest that *pepeta* processing, like other traditional knowledge, is at risk of extinction due to adaptation to surroundings and culture changes from one generation to another.[32] In this study no data about *pepeta* processing and/or consumption was collected at Mvomero, Bagamoyo and Iringa rural districts as respondents in these districts could not recall the last time they processed and/or ate *pepeta*. They obtained *pepeta* from local markets or as a gift from Kilombero and Ulanga folk.

Table 2.4: Frequencies (as percentage) of respondents on awareness of rice-based products and *pepeta* knowledge in the study area

	Districts					Overall
	Kilombero	Ulanga	Mvomero	Bagamoyo	Iringa rural	(%)
Rice-based product popularity [a]	n=68	n=38	n=72	n=24	n=55	n=257
*Pepeta**	100.0	100.0	79.2	70.8	23.6	73.5
Wali (cooked white rice)[#]	100.0	100.0	100.0	100.0	100.0	100.0
Wali (cooked brown rice)*	23.5	0.0	18.1	0.0	1.8	11.7
Mchopeko (parboiled rice)*	48.5	31.6	15.3	0.0	0.0	21.8
Vitumbua (rice dough)*	66.2	71.1	70.8	45.8	87.3	70.8
Mkate (Bread)*	63.2	57.9	52.8	75.0	14.5	50.2
Ungalishe (composite flour)*	45.6	15.8	30.6	37.5	30.9	33.1
Ugali (stiff porridge)*	5.9	13.2	16.7	12.5	30.9	16.0
Visheti (puffed rice)	8.8	2.6	5.6	4.2	0.0	4.7
Togwa (non-alcohol drink)*	19.1	34.2	0.0	0.0	0.0	10.1
Pombe (alcohol drink)*	20.6	52.6	5.6	0.0	10.9	17.1
Pepeta knowledge as affected by						
Gender*			n=57	n=17	n=13	n=87
Male			35.3	68.4	69.2	53.0
Female			64.7	31.6	30.8	47.0
Age*[$]						
< 30 years (youth)			15.7	5.3	7.7	12.1
30 – 44 years (adult)			29.4	10.5	38.5	26.5
> 44 years (elder)			54.9	84.2	53.8	61.4

[a] More than one answer possible, [#]no statistical analysis computed, *Significance difference ($p < 0.05$) among districts, [$]Significance difference ($p < 0.05$) in the overall respondents.
Source: Survey data, September 2017 – February 2018.

2.3.3 Processing season and availability

Pepeta is processed in large amounts twice a year, following the rice production calendar, which controls the availability of immature rice grains. The main production season is from April to July, i.e., the major rainy season when immature rice is sourced from both rainfed and irrigated fields, and the second season is from October to December when irrigated fields are the only source of rice. *Pepeta* processing as a source of household income was only mentioned by 50.0% (Kilombero) and 52.6% (Ulanga) of the respondents, the informants in the survey (Table 2.2), and was exclusively an activity of women in the visited areas. These findings are in line with previous research,[33,34] which underpins the important role women play in preserving and transferring traditional food processing knowledge from generation to generation in sub-Saharan region.

2.3.4 *Pepeta* processing

Pepeta resembles flaked rice, but the use of freshly harvested immature rice grains make the product and production process distinct from many traditional and commercial flaked rice products in Asian and other rice–consuming countries.[35] The main processes involved in the production of *pepeta* are harvesting of immature rice grains, cold soaking, roasting, pounding, cleaning and packing (Fig. 2.3). Though the production is mainly at household level, variations exist for *pepeta* production destined for sale compared to that intended for home consumption, mainly regarding processing quantity. For commercial production, 60 – 180 kg of *pepeta* per interviewed processor and less than 15 kg of *pepeta* per household for home consumption is processed during each cropping season. During *pepeta* processing, rice grains undergo several physical changes, including drying, a colour change from greenish yellow to bright yellow, dehusking and flattening (Fig. 2.3a-d). Table 2.5 shows the moisture content, duration, weight and temperature for various processing steps of *pepeta*.

Table 2.5: Moisture content, duration, weight and temperature at various processing steps of *pepeta* product.

Process step	Sample	Duration	Weight (kg)	Moisture content (%)	Temperature (^{0}C)
Harvesting	Immature paddy (15 – 32 DAH)	2 – 12 h	18 – 36 (1 – 2 buckets)	31 - 36	Ambient temperature (21 – 31*)
Soaking	Immature paddy	Overnight (12 – 16 h)	18 – 36 (1 – 2 buckets)	--	Ambient temperature
Roasting	Immature paddy	3 – 8 min	0.25 – 0.41	9 – 15	Frame temperature (181 – 270) Vessel temperature (117 – 180) Paddy temperature (80 – 129)
Pounding	Roasted paddy	1 – 3 min	0.17 – 0.34	9 – 15	Ambient temperature
Cleaning	Pounded paddy		0.16 – 0.31		Ambient temperature
Packing/ storage	*Pepeta*		0.1 – 0.2	9 – 15	Ambient temperature

*Mkoma and Mjemah.[36] Source: Survey data, September 2017 – February 2018.

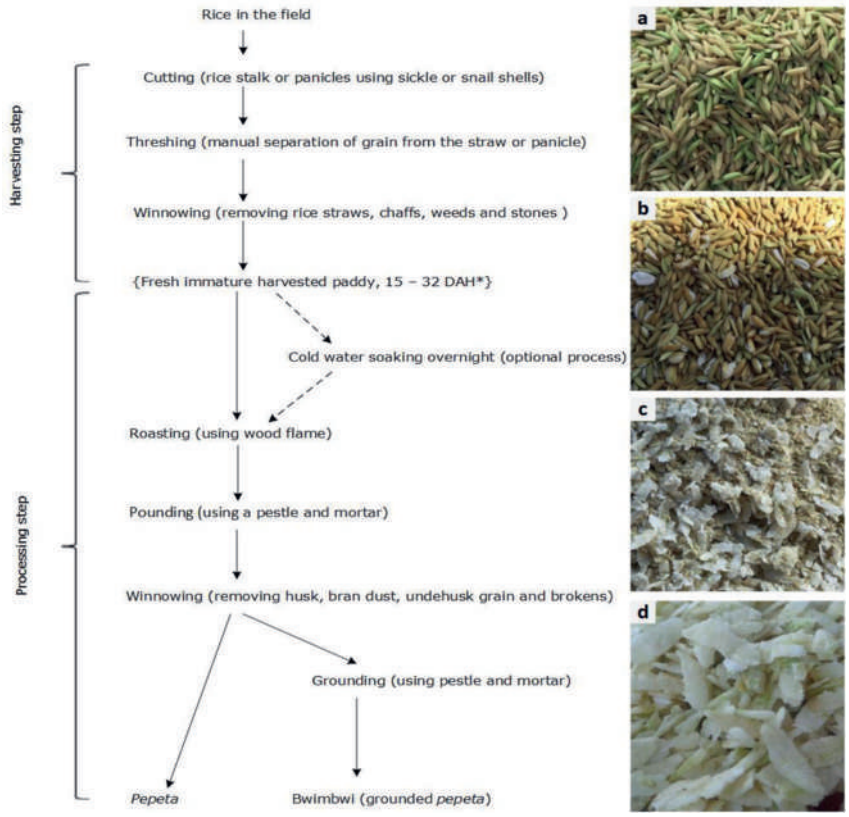

Fig. 2.3: *Pepeta* processing flow diagram. DAH – days after 50% heading, **a** – fresh immature harvested paddy grains, **b** – roasted paddy grains, **c** – pounded paddy grains, and **d** – cleaned *pepeta* product

Selection of rice cultivar and optimum harvest time are critical steps for the quality and flavour characteristics of *pepeta*. Generally, *pepeta* is prepared from different aromatic landraces of rice (Table 2.6). Each informant cited at least four cultivars known to them and/or found in their locality. The most frequently cited cultivar was TXD306 mentioned by 100% and 95% of respondents in Kilombero and Ulanga districts, respectively. Participants were also asked to specify the most preferred cultivar. TXD306 (87.5% of respondents), Kalimata (7.1%) and Mbawambili (5.4%) were most preferred (Fig. 2.4) and differ significantly (x^2= 7.554, df = 2, p = 0.023) between Kilombero and Ulanga respondents. Respondents considered TXD306, which is a semi-aromatic hybrid variety, to have quality traits and availability advantages over highly aromatic landraces with a long growth cycle (i.e., Kalimata and Mbawambili). TXD306 can be cultivated twice annually, guarantees the availability of immature rice grains and hence *pepeta* processing. In addition, the awns of the Mbawambili variety tend to burn during roasting, introducing black spots and thereby affecting the general appearance of the *pepeta* product.

Table 2.6: Inventory of rice varieties commonly used for *pepeta* production in the surveyed area.

Local name (Swahili)	Category	Aroma	Citation in location (%)		Overall % (n=55)
			Kilombero (n=35)	Ulanga (n=20)	
SARO5/TXD306	Hybrid	Semi-aromatic	100.0	95.0	98.2
Kalimata*	Landrace	Aromatic	0.0	90.0	32.7
Mbawambili*	Landrace	Aromatic	74.3	45.0	63.6
Nondo*	Landrace	Aromatic	45.7	0.0	29.1
Ngome*	Landrace	Aromatic	0.0	45.0	16.4
Wahipesa*	Hybrid	Non-aromatic	11.4	40.0	21.8
Supa-India	Landrace	Aromatic	25.7	35.0	29.1
Lawama*	Hybrid	Non-aromatic	34.3	10.0	25.5
Zambia	Landrace	Aromatic	28.6	25.0	27.3
Komboka*	Hybrid	Non-aromatic	22.9	0.0	14.5
Nyengo*	Landrace	Aromatic	17.1	0.0	10.9
Afa-Mwanza	Landrace	Aromatic	8.6	0.0	5.5
Kalimawangu	Landrace	Aromatic	5.7	0.0	3.6
Kisegese	Landrace	Aromatic	2.9	5.0	3.6
Most preferred variety*					
TXD306			94.3	75.0	87.5
Kalimata			0.0	20.0	7.1
Mbawambili			5.7	5.0	5.4

* Significance difference between Kilombero and Ulanga districts ($p < 0.05$)
Source: Survey data, September 2017 – February 2018.

Fig. 2.4: Most preferred rice verities used to process *pepeta* mentioned by respondents in the study area; a) TXD306/SARO 5, b) Mbawambili, and c) Kalamata.
Source: Survey data, September 2017 – February 2018.

Harvesting of immature rice grains

Harvesting immature rice destined for *pepeta* processing involves cutting, threshing and cleaning. Rice in the field is harvested by cutting rice panicles (60.0%) using a snail shell or cutting rice stalks (40.0%) using a sickle (Table 2.7, Fig. 2.5a and 2.5b), and differed significantly (x^2= 16.042, df = 1, p = 0.000) between respondents of Kilombero and Ulanga districts. Harvesting by cutting rice panicles involves sorting of appropriate rice grains, leaving rice in the field that is considered unfit (too immature or too mature) for *pepeta* processing by processors. In contrast, the entire field is harvested when using sickles. Changes of leaf and panicle colour (92.7% of respondents) as rice matures, and biting through grain (25.5%) are common maturity indicators for optimum maturity of rice in the community, harvested two weeks (50.9% of respondents) or three weeks (32.7%) after 50% flowering of rice in the field. The colour of the grain seed coat changes from green to a distinct colour in accordance with rice cultivar at the onset of ripening, and grain endosperm hardens as it develops.[37,38] Though no significant different in the indicators for optimum rice maturity, the suitability of rice in the field after optimum maturity varied significantly (x^2= 17.595, df = 2, p = 0.000) between respondents of Kilombero and Ulanga districts. According to respondents, rice in the field remained suitable for one (63.6% of respondents) to two weeks (34.5%) after attaining the optimum maturity level for *pepeta* processing.

Fig. 2.5: Harvesting of immature paddy rice grains during pepeta production using traditional equipment and methods; a) snail shell, b) sickle placed on harvest rice stalks, c) traditional hand threshing of harvested rice panicles, and d) traditional cleaning (winnowing) of threshed paddy.
Source: Survey data, September 2017 – February 2018.

Threshing, the process that involves separating rice kernels from panicles but not removing the husk, is done immediately after cutting by hand (Fig. 2.5c) or by beating with a stick on the paddy to maintain freshness of the harvested rice kernels. After threshing, rice grains are cleaned by a combination of winnowing and hand sorting (Fig. 2.5d). Unfilled kernels (92.7% of informants), rice straws and chaffs (81.8%), stones (36.4%) and weed seeds (27.3%) were major unwanted materials removed during cleaning (Table 2.7). The unwanted materials removed during cleaning did not differ significantly between Kilombero and Ulanga respondents, except for weed seeds (x^2= 11.786, df = 1, p = 0.001). The moisture content of fresh harvested rice kernels ranged from 30 – 36% (Table 2.5).

Table 2.7: Overview harvesting of immature rice grains and its preparation before *pepeta* processing

	Citation in location (%)		Overall %
	Kilombero (n=35)	Ulanga (n=20)	(n=55)
Method of rice harvest*			
Manual by cutting rice panicles	80.0	25.0	60.0
Manual by cutting rice stalks	20.0	75.0	40.0
Indicators for rice maturity [a]			
Changes of leaf and panicle colour	91.4	95.0	92.7
Grain biting	20.0	35.0	25.5
Days after seeding/transplanting	25.7	10.0	20.0
Days after 50% heading	25.7	25.0	25.5
Optimum harvest for *pepeta*			
Don't know	14.3	20.0	16.4
2-weeks after 50% flowering	57.1	40.0	50.9
3-weeks after 50% flowering	28.6	40.0	32.7
Rice suitability after optimum maturity*			
1-week	82.9	30.0	63.6
2-weeks	14.3	70.0	34.5
3-weeks	2.9	0.0	1.8
Materials removed on harvested grains [a]			
Unfilled kernels	88.6	100.0	92.7
Rice straws and chaffs	82.9	80.0	81.8
Stones/sands	37.1	35.0	36.4
Weed seeds*	42.9	0.0	27.3
Pre-treatment before roasting			
Paddy soaking overnight	68.6	65.0	67.3
Roasting of freshy harvested paddy	31.4	35.0	32.7

[a] More than one answer possible, *Significance difference between Kilombero and Ulanga districts ($p < 0.05$).
Source: Survey data, September 2017 – February 2018.

Soaking

We observed cold water paddy soaking as an optional process, mainly in commercial production. It is done when harvesting until late in the evening to prolong freshness of harvested paddy until the next day. Table 2.7 indicates 67.3% of the respondents soaked freshly harvested immature paddy overnight, typically into 10 – 20 litre plastic containers. Paddy soaking is also said to soften and standardize the moisture content of harvested paddy kernels. Indeed, rice in the field matures heterogeneously, and large variations up to 46% in individual kernel moisture content at harvest have been reported.[39,40] Generally, freshly harvested immature paddy is preferred, as it gives a more white-greenish colour. The soaked paddy is water drained at ambient temperature for about 2 – 4 hours before roasting.

Roasting

In the study areas, fresh or soaked paddy is roasted in dry aluminium (Fig. 2.6a) or earthenware (Fig. 2.6b) pots, with continuous stirring using a big wooden spoon. We noticed no roasting medium such as sand or fine silt,[35] used in many related flaked rice products. About 0.25 to 0.41 kg of paddy was roasted at a time for about 3 – 8 min. The roasting vessel was warmed up to 117 – 180°C and paddy reached a temperature from 80 – 129°C (Table 2.5). Table 2.8 show many of the respondents mentioned 1 – 5 min (49.1%) and 6 – 10 min (29.1%) as roasting duration. However, 56.4% of the informants indicated variations in the duration of roasting, depending on the amount of paddy roasted at a time, hotness of the firewood flame and efficiency of stirring.

Pepeta processors depend on knowledge, experience and observations to determine when to terminate the roasting process. Dryness of the grains (90.9% of respondents), puffing of grains (56.4%) and colour changes of grains (52.7%) were common indicators to determine the end of the roasting process (Table 2.8, Fig. 2.3b). There was no significance difference on

various roasting parameters between Kilombero and Ulanga respondents, except for dryness of the grains (x^2= 4.526, df = 1, p = 0.033) a factor used as indicator for termination of roasting process. According to processors, dryness of the grain could be assessed by the ease of stirring due to a decrease in adhesion as grain dried during roasting, and/or easiness of dehulling when roasted grain is rubbed between hands. These findings differ from,[35] who reported the initiation of a popping sound (puffing of grains) as the main indicator used for the termination of roasting in preparation of rice flakes.

Fig. 2.6: Major process steps of *pepeta* processing; a) hand roasting of paddy using aluminium pot, b) hand roasting of paddy using earthenware pot, c) traditional pounding using pestle and mortar, d) traditional cleaning. Source: Survey data, September 2017 – February 2018.

Table 2.8: Overview of main *pepeta* processing steps in the study area

	Citation in location (%)		Overall %
	Kilombero (n=35)	Ulanga (n=20)	(n=55)
Roasting duration			
Don't know	5.7	25.0	12.7
1 to 5 minutes	45.7	55.0	49.1
6 to 10 minutes	37.1	15.0	29.1
11 to 15 minutes	11.4	5.0	9.1
Variation in roasting duration	62.9	45.0	56.4
Indicators for terminating roasting [a]			
Dryness of the grains*	97.1	80.0	90.9
Puffing of grains	62.9	45.0	56.4
Colour change of grains	60.0	40.0	52.7
Pounding duration			
Don't know	8.6	25.0	14.5
1 to 5 minutes	62.9	60.0	61.8
6 to 10 minutes	28.6	10.0	21.8
11 to 15 minutes	0.0	5.0	1.8
Variation in pounding duration	68.6	60.0	65.5
Indicators for terminating pounding [a]			
Absence of undehusked paddy grains	91.4	95.0	92.7
Flatness of the pounded grains	68.6	75.0	70.9
Number of pounding*	25.7	0.0	16.4
By-products of the pounding process [a]			
Broken grains	74.3	75.0	74.5
Husk/brans	82.9	95.0	87.3
Undehusked grains	80.0	60.0	72.7
Packaging materials used [a]			
Polyethylene bags	66.7	55.6	62.7
Plastic containers*	21.2	50.0	31.4
Paper bags*	24.2	0.0	15.7
Aluminium/earthenware pots	6.1	5.6	5.9
Most tedious processing step [a]			
Harvesting (cutting)	25.7	5.0	18.2
Threshing	20.0	30.0	23.6
Roasting	11.4	10.0	10.9
Pounding	74.3	70.0	72.7

[a] More than one answer possible, *Significance difference between Kilombero and Ulanga districts ($p < 0.05$).
Source: Survey data, September 2017 – February 2018.

Pounding

Pounding is the labour intensive and critical operation of *pepeta* processing, traditionally done by hand pounding the roasted paddy using a pestle and mortar (Fig. 2.6c). Through pounding, the roasted paddy gets dehusked and flattened concurrently. Hand pounding is common

practice when preparing traditional flaked rice from mature rice at household level in India.[35] Many respondents (65.5%) mentioned variations in the duration of pounding, where 61.8% and 21.8% reported a pounding duration of 1 – 5 min and 6 – 10 min, respectively (Table 2.8). However, during various *pepeta* processing demonstrations, about 0.17 – 0.34 kg of roasted paddy was pounded for 1 – 3 min by two or three people at a time (Table 2.5). We observed that pounding speed and hotness of the roasted paddy were important parameters. For effective processing, the pounding should immediately start and end while the roasted paddies are still hot. Even a slight delay from pounding may affect the thickness of the flattened grains and general appearance of the end product. Absence of undehusked paddy grains (92.7%) and flatness of the pounded grains (70.9%) were considered by respondents as major indicators for termination of the pounding process (Table 2.8, Fig. 2.3c). No significant difference observed between Kilombero and Ulanga respondents in pounding parameters, except for number of pounding (x^2= 6.149, df = 1, p = 0.013) used as a factor to end the pounding process, mentioned by respondents (25.7%) in Kilombero district only.

Cleaning and storage

Fig. 2.6d shows the cleaning process using the traditional winnowing method. The pounded grains are cleaned to remove by-products of the pounding process, namely broken grains (74.5% of respondents), husk/brans (87.3%), and undehusked grains (72.7%) (Table 2.8). In this study we found that the cleaning was done in two stages: first cleaning immediately after each pounding to remove brans, and a second cleaning at the end of *pepeta* production to remove remaining brans, broken and undehusked grains (Fig. 2.3d). According to processors, any delay to remove brans immediately after pounding, affect whiteness and hence general appearance of the *pepeta* product. After cleaning, *pepeta* is stored in polyethylene bags (62.7% of respondents) and plastic containers (31.4%) of different sizes. Aluminium pots,

earthenware pots and paper bags are seldom used. Although there was no significant difference in cleaning parameters, the packaging material used i.e. plastic containers (x^2= 4.483, df = 1, p = 0.034) and paper bags (x^2= 5.175, df = 1, p = 0.023) differed significantly between Kilombero and Ulanga respondents, with Kilombero being more sustainable-oriented. Paper bags which were only mentioned by respondents in Kilombero (24.2%), are considered as eco-friendly sustainable packaging material due to its biodegradability properties i.e. decompose by biological activity such as through bacteria or fungi into natural metabolic by-products.[41]

2.3.5 Process efficiency and losses

Several terms are used by experts to evaluate the efficiency of processing methods. In this study we use *pepeta* recovery percentage to evaluate the process efficiency of *pepeta* processing. Based on the rice milling recovery concept,[42] we define *pepeta* recovery (i.e. process efficiency) as the percentage of *pepeta* yield based on the initial paddy weight before roasting. The survey found 40% recovery when immature paddy was processed into *pepeta* (Fig. 2.7), a lower value than the 50 – 60%[43] when mature dried paddy is milled. The apparent low yield of *pepeta* attributed to 35% weight loss due to remove of unwanted materials (husk, bran dust, broken and undehusked paddy grains) during cleaning, 20% weight loss during roasting, and 6% weight loss due to grain scattering during pounding. High moisture loss (65%) was the main factor contributing to weight loss during roasting. However, processing efficiency, loss at roasting and cleaning as well as heat loss from flame to paddy differed significantly between Kilombero and Ulanga respondents, with Ulanga being more efficiency compared to Kilombero.

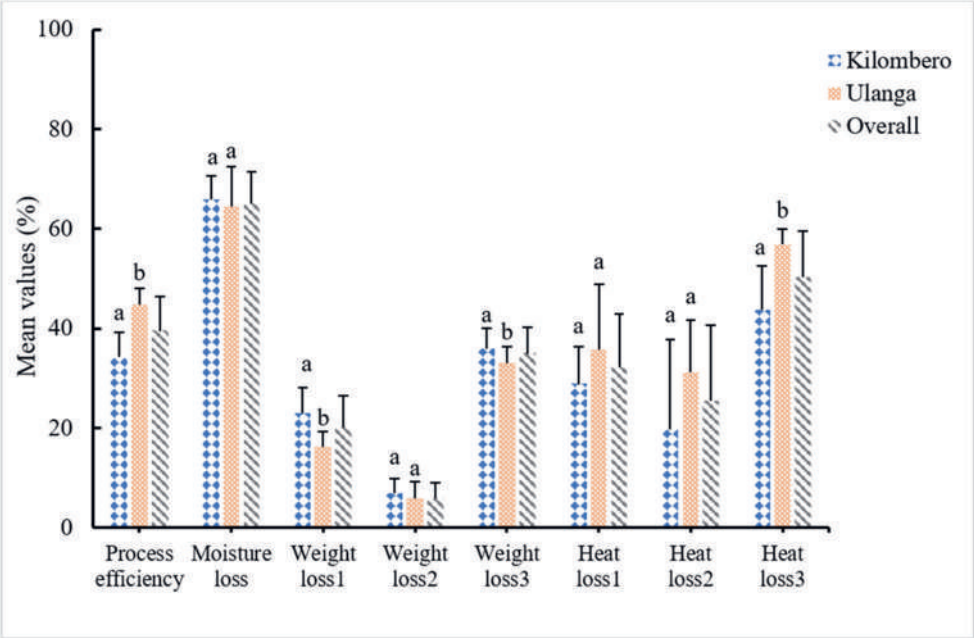

Fig. 2.7: Mean values for process efficiency, and moisture, weight and heat losses which occur at various steps of *pepeta* processing; weight loss1 – loss at roasting, weight loss2 – loss at pounding, weight loss3 – loss at cleaning, Heat loss 1 – frame to vessel loss, Heat loss 2 – vessel to paddy loss, Heat loss 3 – Frame to paddy loss. Error bar in the chart represent standard deviation of mean value percentages. Bar with different letter are significant different at $p < 0.05$ for each parameter.
Source: Survey data, September 2017 – February 2018.

2.3.6 *Pepeta* trade

In the surveyed area, *pepeta* trade is largely carried out in the Morogoro region, with by far the largest proportion of the commercial production and distribution chain dominated by women. The trade starts in Kilombero and Ulanga districts, which are the centres for *pepeta* production in the region. In these districts, processors bought immature paddy directly from the field, which was sold at a price almost three times that of dried mature paddy. This could be a way to offset losses due to the fact that when harvesting immature rice the yield is lower than for mature rice.[44] According to respondents, prices varied from one place to another, and depending on seasonal availability: lower during bumper harvest and higher during scarce supply. *Pepeta* was sold mainly along roadside selling centres (100.0% of respondents),

seldomly at local markets (18.2%) and train stations (10.9%) (Table 2.9). Although there was no significant difference in roadside selling centres, *pepeta* selling at train stations differed significantly (x^2= 3.848, df = 1, p = 0.049) between Kilombero (17.1%) and Ulanga (0.0%) respondents. It is important to note that Ulanga district is not accessible by train as Kilombero district. The study found three major *pepeta* roadside selling centres, namely *Ruaha getini* (07.66°S 036.97°E) and *Mang'ula kona* (07.85°S 036.89°E) in Kilombero district, and *Kivukoni getini* (08.20°S 036.69°E) in Ulanga district. The processors who are also traders, have formed their groups, which regulate the number of processors selling the product in a daily rotation routine. This is because there are more processors than the capacity of the vending centre. However, processors complained about a lack of reliable markets opportunities, indicating the challenge of *pepeta* distribution chain and the potential to improve the trading network.

Table 2.9: Overview of *pepeta* trade in the study area

	Citation in location (%)		Overall %
	Kilombero (n=35)	Ulanga (n=20)	(n=55)
Pepeta uses			
As snack	97.1	100.0	98.2
As breakfast	2.9	0.0	1.8
Reason for processing *pepeta*			
Vending only	54.3	45.0	50.9
Vending and household consumption	45.7	55.0	49.1
Pepeta selling location [a]			
Roadside[#]	100.0	100.0	100.0
Local market	22.9	10.0	18.2
Train station*	17.1	0.0	10.9
Middle persons/mobile call out	5.7	0.0	3.6

[a] More than one answer possible, [#]No statistical analysis computed, *Significance difference between Kilombero and Ulanga districts ($p < 0.05$). Source: Survey data, September 2017 – February 2018.

The price of *pepeta* did not change during the processing season and was twice that of milled white rice. This is illustrative of the importance of adding value to agricultural produce for household income generation and hence livelihood and food security improvement.[2,45] This study found that *pepeta* was also sold to the neighbouring municipalities such us Morogoro

and Dar es Salaam by middle-persons (3.6% of respondents). Processors mentioned to produce *pepeta* for either selling (50.9%) or both selling and household consumption (49.1%). Though the contribution to household income has not yet been documented, most processors cited that it covers by far the greatest proportion of their daily needs such as food, clothes and school fees.

2.3.7 Product quality criteria and acceptability

Fig. 2.8 indicates factors considered by respondents in the study area when buying and consuming *pepeta*. According to respondents, major factors considered were colour (76.5% of respondents), general appearance (60.8%), texture (64.7%) and taste (52.9%). In addition, aroma, flatness and number of broken grains were also considered. Location had no effect on quality criteria considered by respondents between Kilombero and Ulanga district. Colour was mentioned as a highly critical factor in the focus group discussions as well. According to the interviewees, a white-greenish colour (Fig. 2.3d) is most preferred as it indicates that the product is made from immature rice grains. The informants cited general appearance to include presence or absence of black spots, husks, bran, undehusked grain, dust, broken, and flatness of pounded grains. We observed that the general appearance and colour of *pepeta* product act as eye-catching traits, highly differentiate *pepeta* products among processors and play a key role in consumer decisions to taste the product before buying. This is possible because *pepeta* is marketed in unpacked form, whereby consumers are invited to taste the product before buying.

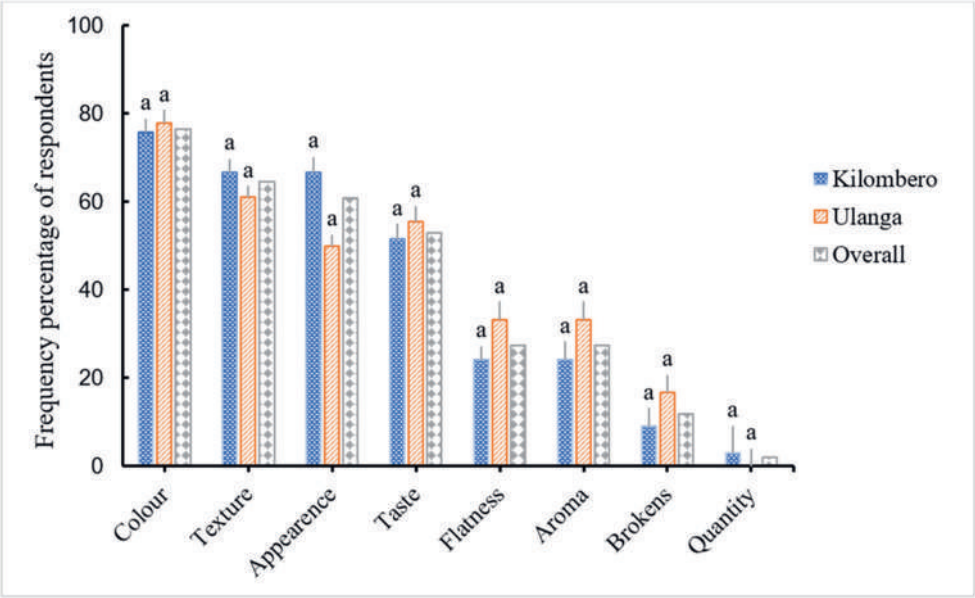

Fig. 2.8: Factors considered by consumers when buying *pepeta* product in the study area. Kilombero – Kilombero district, Ulanga – Ulanga district. Bar with different letter are significant different at $p < 0.05$ for each parameter. Source: Survey data, September 2017 – February 2018.

After tasting, flavour and texture played decisive roles in consumers' buying decisions. According to interviewees, a good *pepeta* texture is not too crunchy, moderately soft but not too sticky and thin. Thick and hard *pepeta* was reported to cause jaw aching even when consumed only for a short time. Generally, the use of immature rice grains gives a butter-like popcorn flavour to *pepeta*, which is highly appreciated by many consumers (98.0%) in the surveyed areas. Indeed, the maturity level of cereal grain has been associated to the taste of *Firiks* (or *Frekeh*), a traditional product prepared from immature wheat.[16,46] We found that the most common way of consuming *pepeta* is as a snack (98.2% of respondents), whereas 1.8% used *pepeta* as breakfast. In this study, snack refers to consumption as a leisure activity rather than as a meal, with no specific time.

2.3.8 *Pepeta* production problems

Fig. 2.9 presents a general overview of *pepeta* processing and quality problems identified by respondents in the study area. *Pepeta* processors used colour changes of rice grains and hardening of endosperm as criteria to identify the optimum rice maturity level for *pepeta* processing. This method is very subjective, resulting in variations in maturity levels of the harvested rice grains, and hence colour and flavour of *pepeta* end products among processors.

Harvesting by cutting and threshing immature rice grains is done under wet conditions, a period when water in the rice fields is still needed for crop growth. Generally, it is difficult to control the water level within individual rice fields due to poor infrastructure. This makes the use of local harvesting tools and methods inevitable as mechanised tools cannot be used in wet conditions, making the process laborious and time-consuming. In some occasions, processors soak immature paddy in cold water to extend the freshness of harvested paddy until the next day when harvesting ends late in the evening. However, a focus group interviewee said that soaking imparted undesirable colour changes and off-flavour to the end product, similar complaints as reported by Sulochana *et al*.[35]

Dryness, puffing and colour changes of rice grains are important factors used by processors to determine the end of the roasting process, a subjective method based on past experience and visual observations. In addition, these factors highly depend on the amount of paddy being roasted, temperature and stirring speed. Though the amount of paddy is easily controlled by roasting a known amount, maintaining a constant temperature and stirring speed is difficult due to the use of firewood and manual stirring. Properties such as wood density, moisture content, flammability, flame brightness, and flaming period affect flame temperature.[47] These are not considered much by processors as the type of firewood used in the study area is based on availability.

Processing step	Processing problems	Quality problems
Harvest →	Variable maturity levels Labour intensive Seasonal availability of immature rice grains Slow harvesting rate	Variable colour and flavour
Soaking →	N/A	Impair colour and flavour
Roasting →	Variable roasting time and temperature Variable roasting termination point Labour intensive Limited availability of firewood	Variable moisture/softness, colour and flavour
Pounding →	Labour intensive Limited availability of energy	Variable flatness/thickness
Cleaning →	Less efficiency/	Impair whiteness and general appearance
Packaging/ storage →	Inefficient storage facilities Poor hygienic condition	Quality loss due to moisture access into the product Insect, dust and mould contamination

Fig. 2.9: *Pepeta* processing and quality problems identified by respondent in study area
Source: Survey data, September 2017 – February 2018.

Usually, roasting is done in open air, a situation which further aggravates the problem of uncontrolled heat distribution during roasting due to air velocity. An uncontrolled heat flow and inefficient stirring result in over-roasted paddy due to high heat or under-roasted paddy due to false puffing of rice grain, respectively. This causes a poor quality of the end product, with defects concerning moisture content, softness, colour and flavour (Fig. 2.9). To prevent such issues, processors have to continuously stir while regulating the amount of chopped wood to maintain a proper heat flow during roasting, a tiresome process according to processors.

About 72.7% of the respondents mentioned pounding as the most tedious process step, at least two times more frequent than the remaining processing steps: threshing (23.6%), cutting (18.2%) and roasting (10.9%) (Table 2.8). Application of labour serving technology like roaster and rice flaking machine[48] will substantially relief the workload. To achieve the

desired flatness, processors have to manually pound roasted paddy at a high speed to finish the process quickly while the paddies are still hot. This is because cold pounded rice grains do not flatten; instead normal milled white rice kernels are obtained. Processors complained about physical discomfort, including hand, back and chest pain.

Respondents mentioned an impairment of whiteness and general appearance of the end product when cleaning is delayed after pounding. Other constraints include quality degradation due to moisture uptake by the product, and contamination by insects, dust and mould as a result of poor storage facilities and hygienic conditions. *Pepeta* as a dried product, tends to absorb water from the surroundings. To maintain product quality, *pepeta* was stored in plastic containers with tight-closing lids and kept in cool and dry conditions. In addition, respondents in the focus group discussion mentioned to re-sundry *pepeta* to extend its shelf life when stored for a long period, especially during the rainy season. The results concur with Ngadze *et al*.[33] who reported re-sundrying of traditionally prepared dry monkey orange (*Strychnos* spp.) products to extend shelf life. Wet and high humidity storage conditions facilitate clump formation, mould growth, discolouration, a bad smell and bitter taste; factors that were much considered by consumers when rejecting a *pepeta* consignment.

2.3.9 General utilization of immature cereal-based products

Cereals are the major staple food for many people. Wheat, maize and rice comprise at least 75% of the world's grain production.[49] Consumption of immature maize as a roasted product or boiled whole kernel, and/or traditional processed products is common in Africa.[50,51] *Mohlefe* or *malitsibana* is a common green maize bread in Lesotho, prepared by wet-milling maize kernels to a thick dough, shaped into cob-like forms, covered with maize leaves and steamed until completely cooked.[51] Similar to *pepeta*, the preparation of *mohlefe* is done during the harvest season when households are waiting for crops to mature in the field.

Degree of maturity and varietal differences significantly impact the quality properties of *mohlefe*. Preferred attributes are a whitish or yellowish colour, depending on the maize variety used, and an intense aroma of immature green maize kernels.[51] Contrary to *pepeta*, salt is added during preparation of *mohlefe*, and this food is mostly consumed as breakfast.

Firik (also known as *frikeh* or *frekeh* or *freekah*) is a common immature whole wheat-based food consumed in the Middle East and North African countries.[16,52] Processing knowledge and quality attributes of *firik* have been extensively studied.[16,46,53–55] To prepare *firik*, immature wheat ears are scorched or roasted on open fire, sundried, threshed, after which the kernels are separated from the hulls and cracked.[46] Similar to *pepeta*, harvesting time, wheat cultivar and processing conditions determine the quality attributes of *firik*. The best harvest time for *firik* ranges from the late-milk to mid-dough stages, which gives a better taste than for the ones processed at the full ripe stage. Hard durum wheat (*Triticum durum*) is preferred for *firik* production. Scorching gives the *firik* its unique, appetizing smocked flavor.[16] Generally accepted high quality *firik* is plump, firm when fresh, slightly burnt, green when dried, containing few remains of the pleas, lemmas, glumes, and free from stones and debris.[52] *Firik* is widely used as an ingredient in the preparation of some specific meals, and especially consumed with meat, tomatoes, stuffed squash, eggplant, grape leaves, and chicken broth.[16,46]

Novel processing of immature grains to increase their applications in food industry has been documented. Previous study[56] assessed the application of fluidized-bed coating technology in improving the health benefits i.e. antioxidant activities of Khao Mao cereal product (a traditional puffed pounded-unripe rice in Thailand). Yilmaz *et al.*[18] investigated the potential use of infrared radiation to unfold the limitation use of immature rice grains due to high rancidity rate compared to mature rice grains. Infrared stabilized immature rice grain flour has been used in preparation of extruded rice products,[57] gluten free bread,[17] and Tarhana, a cereal based fermented food.[58] Çetin-Babaoğlu *et al.*[59] and Pepe *et al.*[60] evaluated the application of

immature wheat flour in straight-dough and sour-dough (fermentation) processes for production of wheat bread. In addition, fermentation technology has been widely used to improve the nutritional quality of other immature cereal-based products like green spelt wheat tempe,[61] and green maize (*melie*) and green sorghum (*senkhoana*) breads.[51]

2.4 Conclusions and recommendations

This study presents the current use, potential and challenges of *pepeta* and its traditional processing knowledge on promoting physical and economic accessibility to early season food, which are main aspects of food security, at household level in Tanzania: "Food security is achieved when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life".[14] Selling immature rice and *pepeta* provides economic benefits for local farmers and processors since these products are commonly sold at prices that are about three times higher than for mature paddy and white rice. The premium price earned contributes to the livelihood of the household.

However, the current traditional processing method has disadvantages: the process is slow, laborious and not suited for upscaling as this cannot guarantee a consistently good quality *pepeta* product. There are no standards for rice harvesting, and processing conditions and parameters. These problems affect its full utilization across sub-Saharan Africa, further aggravate food insecurity in the region. In order to develop a nutritious high sensorial quality *pepeta* product with better shelf life and ease of portability, research on the factors which influence its quality including nutritional, physico-chemical, rheological and aroma properties is of paramount importance. Research to optimise the major process parameters of *pepeta* processing, such as maturity level and moisture content of immature rice grains, and roasting conditions (i.e., temperature and time), for high nutritional and sensorial quality *pepeta*

product should not be neglected by researchers. Ultimately, assessing proper preservation, packaging, storage conditions and shelf life stability, and possibility for value addition of *pepeta* product can create new markets in the cities and towns in the country and outside Tanzania. As these food technological problems and solutions are products specific, the social culture, food culture and socio-economic impact of *pepeta* improvement must be considered by the researchers.

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3

CHAPTER 3.

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

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Abstract

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.

Key words: nutritional properties, *in-vitro* digestion, *pepeta*, immature rice, rice-based food product.

3.1 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.[1,2] 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.[3] However, several researches reported higher amounts of protein, reducing sugars, calcium, potassium, iron, β-carotene, vitamin C, and vitamin B_2, B_3 and B_6 in immature cereal grains,[4,5] 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.[6] 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.[7,8]

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[9] 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.[9,10] Similar studies on immature wheat have reported high contents of total dietary fibre, fructo-oligosaccharides and phytochemicals, such as phenolics and flavonoids.[11,12]

Wholegrain and polished rice are utilized in a very wide range of foods,[13] 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. 3.1). The effect of cooking on the physical and nutritional behaviour of rice kernels is well-documented: swelling 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.[14,15] 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.[16] This technique is widely used in processing of flaked, puffed and expanded rice-based snacks.[17] It increases the digestibility of starch in processed products compared to raw products,[16–18] and perhaps the nutrient content as well due to an increased total ash in rice- and wheat-based roasted products.[19,20] 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.

3.2 Materials and methods

3.2.1 Materials

3.2.1.1 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.*[10]: dough grain stage (DGS, 15 – 21 DAH) – the milky liquid in the endosperm begins to solidify into sticky white matter and the size of the endosperm continues to expand; mature grain stage (MGS, 22 – 28 DAH) – the endosperm continues to expand in size, the grain is hard and the colour of seed coat becomes distinct in accordance with rice varieties; fully ripe stage (FRS, 29 – 35 DAH) – the optimum harvest stage where the grain is fully mature, the endosperm expands to the largest size, the grain is hard and the colour of seed coat strongly represents the colour of the corresponding rice varieties; and over ripe stage (ORS, 36 – 43 DAH) – beyond optimum harvest stage. In this study, rice grains were considered immature at DGS and MGS, since fully mature grains are those above 28 DAH.[10] 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.

3.2.1.2 Reagents

Pepsin from porcine gastric mucosa (P6887, 3200–4500 U/mg), porcine bile extract (B8631), amyloglucosidase from *Aspergillus niger* (10113, 129.3 U/mg), trypsin from porcine pancreas (T7409, 1000–2000 U/mg), α-chymotrypsin from bovine pancreas (C4129, ≥40 U/mg), α-amylase from porcine pancreas (A4268, 700–1400 U/mg), sodium dodecyl sulfate (SDS), o-phthaldialdehyde (OPA), DL-dithiothreitol (DTT), L-serine, thiamine hydrochloride (B_1), nicotinic acid (B_3, ≥ 99.5 % HPLC), and nicotinamide (B_3') 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.

3.2.2 Sample preparation

3.2.2.1 *Pepeta* processing

A part of the harvested immature paddy rice grains was traditionally processed into *pepeta* (Fig. 3.1). 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.

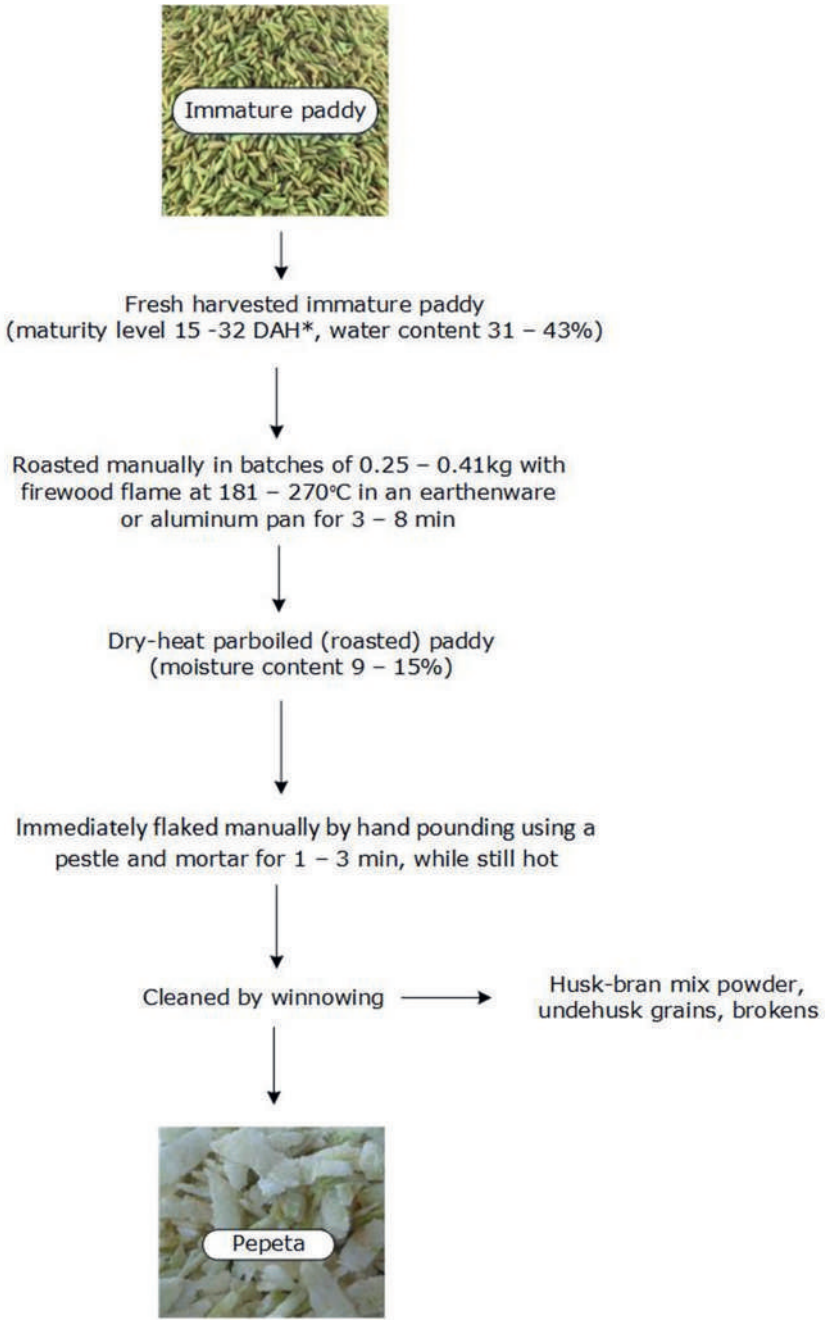

Fig. 3.1: *Pepeta* processing flow chart. *Days after 50% heading/flowering

3.2.2.2 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^3/h). 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.

3.2.2.3 Grinding

Pepeta, raw milled white rice and cooked rice samples were cryo-milled (Freezer mill 6875D, Spex Sample Perp) 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.

3.2.3 Proximate analysis

3.2.3.1 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.[21]

3.2.3.2 Total ash content

Flour samples (2 g) were combusted in a furnace at 550 °C overnight and the ash content was quantified.[21]

3.2.3.3 Total lipid

Lipid content was determined according to standard methods[21] 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.

3.2.3.4 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 covert the nitrogen content in the sample to protein.[22]

3.2.4 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 α-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 correspoding SDF and IDF values. Total dietary fibre (TDF) was calculated as the sum of SDF and IDF.

3.2.5 Surface lipid content

Surface lipid content (SLC) was determined to quantify the degree of milling (DOM) according to Matsler and Siebenmorgen,[23] following same procedure as in section 3.2.3.3. In this analysis, whole kernels of *pepeta* and milled rice samples were used.

3.2.6 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.

3.2.7 Determination of selected vitamin B_1 and B_3

Determination of thiamine (B_1), nicotinic acid (B_3) and nicotinamide ($B_{3'}$) was by a modified procedure of Chen *et al*.[24] 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 3000 g for 10 min at room

temperature. The supernatant was filtered by a 0.2 μm membrane filter and 20 μL extract was used for HPLC analysis. The mobile phase was 25 mM KH_2PO_4 (pH3): $CNCH_3$ in a ratio of 97:3 at a flow rate of 1.0 mL/min. The HPLC system was operated with a Prevail C18 column (5 μm, 4.6×250 mm) and a UV detector set at 245 and 270 nm at room temperature.

3.2.8 Starch characterisation analysis

3.2.8.1 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.

3.2.8.2 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).

3.2.8.3 Starch isolation

Starch was isolated from rice grains according to modified wet-milling method of Syahariza *et al*.[25] 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.

3.2.8.4 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.

3.2.8.5 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[26] and presented as mean particle size, Dx10 (mean particle size of the smallest 10 %), Dx50 (mean particle size of the lower 50 %), and Dx90 (mean particle size below which 90 % of the sample is found).

3.2.8.6 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 µL 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. Onset (To), peak (Tp) and conclusion (Tc) temperatures, and enthalpy change (ΔH) were analysed using Pyris™ software (Version 11, PerkinElmer, Inc. Waltham, USA).

3.2.9 *In-vitro* digestion

In-vitro digestion of starch and protein hydrolysis was performed based on the harmonized INFOGEST protocol[27] 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 α-amylase, simulated gastric fluids (SGF) and freshly prepared pepsin (2000 U/ml), 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), and a pancreatic enzyme solution consisting of α-amylase (200 U/ml), trypsin (100 U/ml) and chymotrypsin (25 U/ml). 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 3000 g 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.

3.2.9.1 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) 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_∞, 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.

3.2.9.2 Determination of protein hydrolysis

The concentration of free amino groups (NH_2) in TCA samples was determined using the ortho-phthalaldehyde (OPA) method.[28] In order to quantify the total content of NH_2 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:

$$DH(\%) = \frac{NH_{2(DS)} - NH_{2(t=0)}}{NH_{2(Total)} - NH_{2(t=0)}} \; x\,100$$

where:

NH_2 (DS) = free amino groups from digested sample

NH_2 (t=0) = free amino groups from samples at time 0 of digestion

NH_2 (Total) = maximum amount of NH_2 present in the sample

3.2.10 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.

3.2.11 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 ± standard deviation of at least two replicates.

3.3 Results and discussion

3.3.1 Proximate analysis

Table 3.1 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/100 g in TXD306 and 0.80 (DGS) – 0.99 (ORS) g/100 g in Lawama, showing lower amounts than in previous research[29] 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 3.2.5), as rice bran contains about 20% lipid.[30] The protein content was 6.54 (DGS) – 9.74 (MGS) g/100 g in TXD306 and 6.19 (DGS) – 8.33 (MGS) g/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.[31] 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 Ji *et al*.[5] 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/100 g, DGS) and Lawama (0.96 g/100 g, DGS) than in fully matured grains in FRS (0.41 g/100 g) and ORS (0.41 g/100 g) respectively. Similar results have been reported for other rice varieties during their development.[5]

Table 3.1: Nutritional content of TXD306 and Lawama rice varieties as a function of maturity level and processing conditions.

Nutritional compound	Sample	TXD306 DGS (15- 21 DAH)	TXD306 MGS (22-28 DAH)	TXD306 FRS (29-35 DAH)	TXD306 ORS (36-43DAH)	Lawama DGS (15-21 DAH)	Lawama MGS (22-28 DAH)	Lawama FRS (29-35 DAH)	Lawama ORS (36-43 DAH)
Proximate									
Surface lipid content (g/100g, db)	Rice	0.76 ± 0.09^{aB}	0.75 ± 0.07^{aA}	0.26 ± 0.03^{b}	0.68 ± 0.15^{a}	0.44 ± 0.06^{aA}	0.41 ± 0.03^{aB}	0.58 ± 0.06^{ab}	0.66 ± 0.01^{b}
	Pepeta	1.66 ± 0.06^{aA}	1.32 ± 0.05^{bA}			1.10 ± 0.10^{aA}	1.37 ± 0.03^{aA}		
Total lipids (g/100g, db)	Rice	1.17 ± 0.02^{aB}	1.18 ± 0.04^{aB}	0.44 ± 0.02^{b}	0.85 ± 0.27^{ab}	0.80 ± 0.18^{aA}	0.89 ± 0.20^{aA}	0.92 ± 0.14^{a}	0.99 ± 0.03^{a}
	Pepeta	2.14 ± 0.12^{aA}	1.55 ± 0.00^{bA}			1.68 ± 0.04^{aA}	1.65 ± 0.09^{aA}		
Total protein (g/100g, db)	Rice	6.54 ± 0.09^{aA}	9.74 ± 0.21^{bA}	6.63 ± 0.73^{a}	9.53 ± 0.14^{b}	6.19 ± 0.08^{aA}	8.33 ± 0.29^{bA}	6.30 ± 0.18^{a}	7.12 ± 0.02^{c}
	Pepeta	7.33 ± 0.51^{bA}	9.66 ± 0.16^{aA}			6.58 ± 0.03^{aA}	8.36 ± 0.17^{bA}		
Total ash (g/100g, db)	Rice	1.03 ± 0.01^{aA}	0.76 ± 0.01^{bA}	0.41 ± 0.01^{c}	0.45 ± 0.01^{d}	0.96 ± 0.03^{aA}	0.59 ± 0.04^{bB}	0.96 ± 0.02^{a}	0.41 ± 0.01^{c}
	Pepeta	1.10 ± 0.03^{aA}	0.86 ± 0.03^{bA}			1.03 ± 0.04^{aA}	1.09 ± 0.03^{aA}		
Vitamins									
Thiamine (mg/100g, db)	Rice	1.88 ± 0.17^{aA}	0.46 ± 0.01^{bB}	0.47 ± 0.05^{b}	0.43 ± 0.01^{b}	0.51 ± 0.00^{aA}	0.65 ± 0.00^{bA}	0.60 ± 0.03^{b}	0.56 ± 0.02^{a}
	Pepeta	1.48 ± 0.03^{bA}	2.49 ± 0.03^{aA}			0.52 ± 0.02^{aA}	0.55 ± 0.06^{aA}		
Nicotinic acid (mg/100g, db)	Rice	49.7 ± 3.92^{aA}	45.2 ± 0.37^{aA}	16.1 ± 0.16^{b}	18.5 ± 3.47^{b}	34.2 ± 7.30^{abA}	46.04 ± 4.45^{aA}	28.06 ± 4.09^{ab}	21.3 ± 4.04^{b}
	Pepeta	32.6 ± 4.81^{aA}	21.7 ± 0.43^{aB}			24.8 ± 0.48^{aA}	31.0 ± 8.51^{aA}		
Nicotinamide (mg/100g, db)	Rice	2.79 ± 0.09^{aA}	1.22 ± 0.06^{bB}	1.21 ± 0.00^{b}	1.48 ± 0.22^{b}	1.95 ± 0.17^{aA}	2.32 ± 0.75^{aA}	2.06 ± 0.37^{a}	1.67 ± 0.24^{a}
	Pepeta	3.13 ± 0.35^{aA}	2.45 ± 0.19^{aA}			3.43 ± 0.85^{aA}	2.87 ± 0.61^{aA}		
Mineral									
Iron (mg/kg, db)	Rice	0.59 ± 0.44^{aA}	0.64 ± 0.17^{aA}	0.31 ± 0.06^{a}	1.27 ± 0.37^{a}	0.58 ± 0.03^{bA}	2.05 ± 0.02^{aA}	0.89 ± 0.45^{b}	1.06 ± 0.23^{ab}
	Pepeta	0.82 ± 0.14^{bA}	1.66 ± 0.05^{aA}			1.34 ± 0.44^{aA}	1.85 ± 0.17^{aA}		
Zinc (mg/kg, db)	Rice	0.75 ± 0.00^{cB}	0.93 ± 0.01^{cB}	1.73 ± 0.07^{b}	2.11 ± 0.13^{a}	0.62 ± 0.05^{dB}	1.53 ± 0.08^{bA}	0.96 ± 0.05^{c}	2.14 ± 0.00^{a}
	Pepeta	1.88 ± 0.00^{aA}	1.77 ± 0.01^{bA}			1.65 ± 0.05^{aA}	1.78 ± 0.01^{aA}		

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. 3.2 (Panels a – 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.[9] 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 3.1 and Fig. 3.2 (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 significant increased total lipid content in TXD306 and total ash content in Lawama at MGS. These results are likely because of the bran residue.[30] 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 varieties, possibly due to associated trend with SLC.

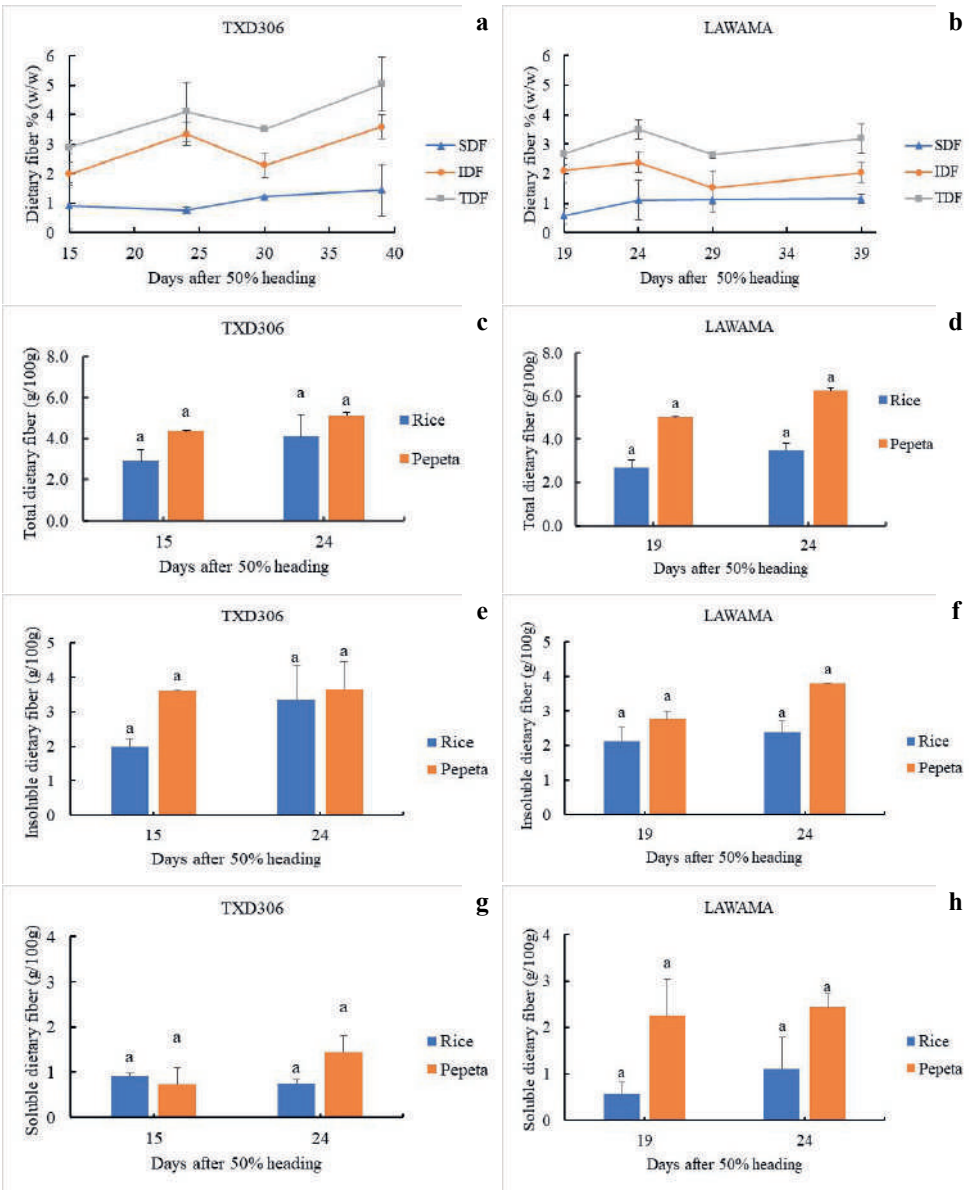

Fig. 3.2: Dietary fibre contents of two rice varieties (TXD306 and Lawama) as affected by maturity level (a, b) and effect of processing into *pepeta* (c, d, e, f, g, h). TDF – total dietary fibre, IDF – insoluble dietary fibre, SDF – soluble dietary fibre, Rice – raw rice flour, *Pepeta* – locally prepared rice flake. Labels with different letters within maturity level indicate a statistically significant difference in fibre content ($p \leq 0.05$).

3.3.2 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. 3.3). Staining of the cell walls (light blue) in the micrographs of raw rice (Fig. 3.3 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.[32,33] CLSM micrographs of *pepeta* (Fig. 3.3 Panels d – 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[34,35] 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. 3.3 Panel 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 3.2.2.2).

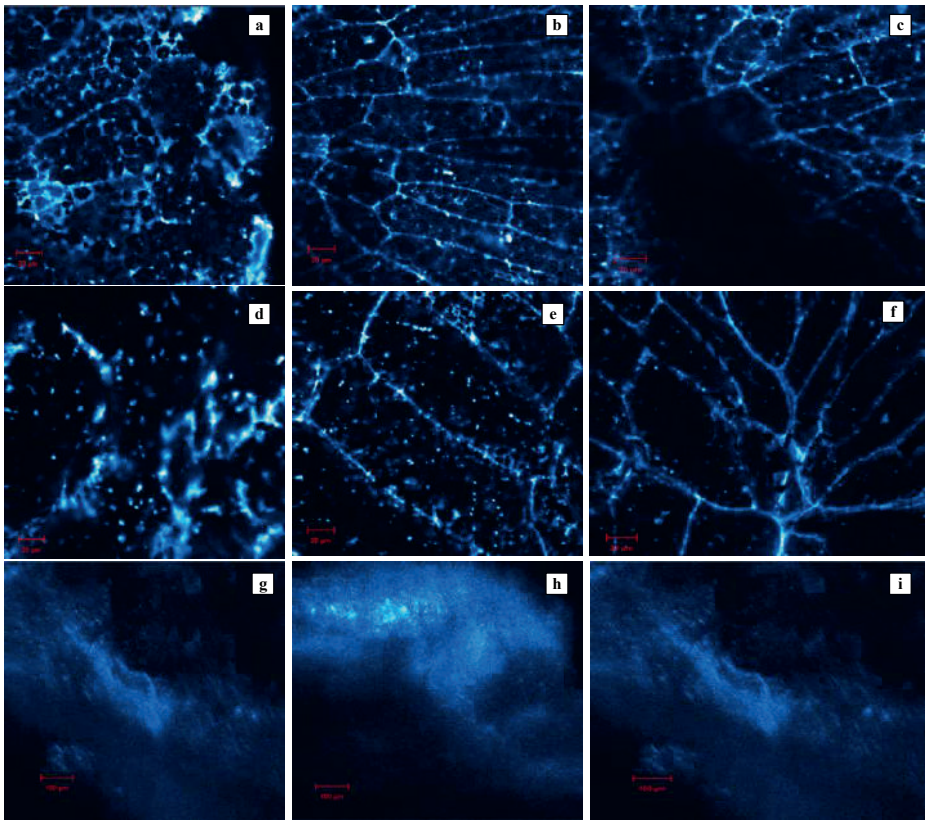

Fig. 3.3: Confocal images of raw rice (a – c), *Pepeta* (d – f) and cooked rice (g – i) in different area of the cross section of the kernel; outer kernel layer (a, d, g), central starchy endosperm (b, e, h), and inner region of kernel (c, f, i). Cell walls were stained in light blue. Micrographs a – f were taken using 40x objective lens while g – i 20x.

3.3.3 Vitamins and minerals

Table 3.1 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/100 g in TXD306 and 21.3 (ORS) – 46.06 (MGS) mg/100 g in Lawama) was abundant, followed by nicotinamide (1.21 (FRS) – 2.79 (DGS) mg/100 g in TXD306 and 1.67 (ORS) – 2.32 (MGS) g/100 g in Lawama). The amount of nicotinic acid and nicotinamide significant decreased during grain development; TXD306 at FRS and Lawama at ORS contained the lowers amounts. Significantly higher thiamine contents, 1.88 mg/100 g for

TXD306 and 0.65 mg/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[5] on two Korean rice varieties during maturation. Other studies[5,36,37] 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.[38–40]

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 3.1). In both varieties, *pepeta* contained higher levels of nicotinamide (2.45 (MGS) – 3.13 (DGS) mg/100 g in TXD306 and 2.87 (MGS) – 3.43 (DGS) mg/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/100 g for TXD306 and 0.58 – 2.05 mg/100 g for Lawama during rice maturation (Table 3.1), 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/100 g for TXD306 and Lawama, respectively. These results differ from previous work,[5] possibly due to the use of different rice varieties and cultivation conditions.[41,42]

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

significant increased during *pepeta* processing at DGS (1.88 mg/100 g) and MGS (1.77 mg/100 g) in TXD306, and at DGS (1.65 mg/100 g) in Lawama. These results are attributed to the high amount of bran and germ remains in *pepeta* (section 3.3.1). Maturity level significantly affect iron and zinc composition of *pepeta* in TXD306, high values found at MGS and DGS respectively. However, no significant difference was observed in Lawama, possibly due to observed concomitant SLC trend.

3.3.4 Starch characterisation

Total starch of rice grain ranged from 79.0 (ORS) to 82.8 (DGS) g/100 g in TXD306 and from 80.9 (FRS) to 84.0 (DGS) g/100 g in Lawama (Table 3.2), similar to previous research.[43] 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.[4,44] 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 3.3.1). Reportedly, an increase in protein content in cereal grains negatively correlates with amylose accumulation, a component of starch.[45,46] In addition, a rice proteomic study[7,8] 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 3.3.1).

Amylose content ranged from 17.1 (FRS) to 20.2 (ORS) g/100 g in TXD306 and 15.2 (ORS) – 20.1 (DGS) g/100g in Lawama, similar to previous work,[47] 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 differences

were not significant. This is in line with previous study,[48] suggested the accumulation of amylose in rice during the mid-stage of grain filling. Rice grains are classified according to amylose content.[49] 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.[50] For a realistic comparison between the RS content of *pepeta* and rice, cooked rice grains were used as rice is normally eaten cooked,[51] and the results were expressed per 100 g total starch (Table 3.2). The RS in cooked rice ranged from 0.28 to 0.54 g/100g in TXD306 and 0.05 to 0.40 g/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.[52] Cooking (wet heat) and *pepeta* (dry heat) processing methods show no significant difference in RS content for both varieties. Dutta *et al*.[16] and Sagum *et al*.[53] 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 exhibits very low levels of RS when cooked and processed into *pepeta*.[16,52,53]

In both varieties, particle size distribution analysis of rice starch granules showed no significant effect for mean particle size, Dx10, Dx50 and Dx90 as rice grains matured (Table 3.2), indicating that rice starch granules are fully developed at DGS. The mean particle size ranged from 5.24 (MGS) to 5.54 (FRS) µm in TXD306 and 5.16 (MGS) to 5.23 (ORS) µm in Lawama, which was similar as reported for six other rice varieties.[54]

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. 3.2). Significant effects were found between DGS and MGS on Tp, Tp and Tp of rice flour and rice starch samples. However, there was no significant

Table 3.2: *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	TXD306 DGS (15-21 DAH)	TXD306 MGS (22-28 DAH)	TXD306 FRS (29-35 DAH)	TXD306 ORS (36-43 DAH)	Lawama DGS (15-21 DAH)	Lawama MGS (22-28 DAH)	Lawama FRS (29-35 DAH)	Lawama ORS (36-43 DAH)
Starch characterization									
Total starch (g/100g, db)	Rice	82.8 ± 2.30^{aA}	81.4 ± 2.85^{aA}	81.2 ± 2.84^{a}	79.0 ± 0.14^{a}	84.0 ± 2.20^{aA}	81.5 ± 5.57^{aA}	80.9 ± 2.22^{a}	81.4 ± 2.51^{a}
	Pepeta	78.2 ± 2.31^{aA}	77.1 ± 1.18^{aA}			78.7 ± 1.20^{aA}	78.2 ± 2.31^{aA}		
Resistant starch (g/100g starch db)*	Rice	0.54 ± 0.09^{aA}	0.41 ± 0.01^{abA}	0.28 ± 0.00^{b}	0.33 ± 0.05^{ab}	0.35 ± 0.05^{abA}	0.40 ± 0.13^{aA}	0.05 ± 0.01^{b}	0.24 ± 0.09^{ab}
	Pepeta	0.35 ± 0.13^{aA}	0.34 ± 0.21^{aA}			0.33 ± 0.11^{aA}	0.21 ± 0.07^{aA}		
Amylose (g/100g, db)	Rice	19.2 ± 1.35^{a}	18.6 ± 3.16^{a}	17.1 ± 2.43^{a}	20.2 ± 0.03^{a}	20.1 ± 0.81^{a}	18.2 ± 2.74^{a}	19.1 ± 2.97^{a}	15.2 ± 2.03^{a}
Particle size distribution									
Mean Particle size (µm)	Rice	5.35 ± 0.20^{a}	5.24 ± 0.29^{a}	5.54 ± 0.06^{a}	5.35 ± 0.07^{a}	5.23 ± 0.19^{a}	5.16 ± 0.05^{a}	5.21 ± 0.22^{a}	5.23 ± 0.04^{a}
Dx10 (µm)	Rice	3.68 ± 0.05^{a}	3.49 ± 0.09^{a}	3.73 ± 0.08^{a}	3.55 ± 0.02^{a}	3.62 ± 0.08^{a}	3.42 ± 0.14^{a}	3.55 ± 0.12^{a}	3.49 ± 0.09^{a}
Dx50 (µm)	Rice	5.17 ± 0.18^{a}	5.02 ± 0.18^{a}	5.34 ± 0.03^{a}	5.12 ± 0.05^{a}	5.05 ± 0.18^{a}	4.94 ± 0.08^{a}	5.03 ± 0.02^{a}	5.04 ± 0.05^{a}
Dx90 (µm)	Rice	7.21 ± 0.37^{a}	7.20 ± 0.75^{a}	7.56 ± 0.24^{a}	7.38 ± 0.18^{a}	7.02 ± 0.33^{a}	7.10 ± 0.06^{a}	7.06 ± 0.35^{a}	7.26 ± 0.01^{a}
Starch hydrolysis									
C_∞ (g/100g, db)*	Cooked rice	98.45± 2.35^{aA}	91.28± 8.15^{aA}	95.85 ± 1.39^{a}	99.07 ± 00^{a}	88.47± 1.90^{aA}	85.11± 1.92^{aA}	94.48 ± 5.11^{a}	93.80 ± 0.51^{a}
	Ground *pepeta*$^{\#}$	82.99 ± 2.13^{aB}	76.69 ± 3.97^{aA}			85.04 ± 2.30^{aB}	83.81 ± 6.79^{aA}		
	Coarse *pepeta*$^{\$}$	81.81 ± 3.73^{aB}	79.32 ± 0.91^{aA}			79.78 ± 1.57^{aB}	76.05 ± 0.81^{aA}		
k (min^{-1})	Cooked rice	0.32 ± 0.02^{aA}	0.33 ± 0.06^{aA}	0.29 ± 0.00^{a}	0.25 ± 0.02^{a}	0.35 ± 0.02^{aA}	0.32 ± 0.00^{aA}	0.24 ± 0.02^{a}	0.26 ± 0.16^{a}
	Ground *pepeta*	0.29 ± 0.02^{aA}	0.31 ± 0.02^{aA}			0.34 ± 0.05^{aA}	0.27 ± 0.00^{aB}		
	Coarse *pepeta*	0.20 ± 0.00^{aB}	0.18 ± 0.05^{aA}			0.25 ± 0.04^{aA}	0.20 ± 0.01^{aC}		
Protein hydrolysis									
Gastric digestion (g/100g, db)	Cooked rice	19.17 ± 6.16^{aA}	05.98 ± 0.98^{aA}	13.41 ± 5.53^{a}	09.42 ± 0.80^{a}	13.77 ± 4.74^{aA}	08.34 ± 2.36^{aB}	12.04 ± 7.60^{a}	14.75 ± 8.57^{a}
	Ground *pepeta*	31.02 ± 8.85^{aA}	26.92 ± 18.4^{aA}			12.94 ± 2.67^{aA}	20.94 ± 0.69^{aA}		
	Coarse *pepeta*	08.60 ± 3.96^{aA}	08.74 ± 3.25^{aA}			21.07 ± 13.4^{aA}	11.86 ±4.18^{aAB}		
Intestinal digestion (g/100g, db)	Cooked rice	71.66 ± 14.2^{aA}	46.87 ± 4.54^{aA}	68.92 ± 11.1^{a}	61.44 ± 0.94^{a}	73.10 ± 8.50^{aA}	54.36 ± 5.88^{aB}	70.47 ± 0.39^{a}	63.97 ± 19.3^{a}
	Ground *pepeta*	84.06 ± 3.60^{aA}	74.50 ± 9.74^{aA}			79.47 ± 15.6^{aA}	94.48 ± 4.50^{aA}		
	Coarse *pepeta*	75.77 ± 8.66^{aA}	50.77 ± 16.1^{aA}			77.51 ± 7.32^{aA}	63.68 ± 3.74^{aB}		
Gelatinization behaviour									
To (^{o}C),	Rice	68.53 ± 0.37^{aA}	68.14 ± 0.25^{aA}			67.17 ± 0.18^{aA}	65.74 ± 0.17^{bA}		
	Rice starch	64.90 ± 0.26^{aA}	63.99 ± 0.12^{bB}			64.07 ± 0.17^{aB}	61.99 ± 1.12^{aA}		
	Pepeta	nd	nd			nd	nd		
Tp (^{o}C)	Rice	75.75 ± 0.18^{aA}	75.27 ± 0.22^{aA}			74.19 ± 0.03^{aA}	72.47 ± 0.17^{bA}		
	Rice starch	71.32 ± 0.29^{aB}	70.71 ± 0.41^{aB}			70.32 ± 0.04^{aB}	67.95 ± 0.99^{aA}		
	Pepeta	nd	nd			nd	nd		
Tc(^{o}C)	Rice	85.36 ± 0.30^{aA}	83.06 ± 0.18^{bA}			84.21 ± 0.01^{aA}	81.41 ± 0.22^{bA}		
	Rice starch	80.05 ± 0.69^{aA}	78.93 ± 0.36^{aB}			79.26 ± 0.06^{aB}	75.57 ± 0.94^{bA}		
	Pepeta	nd	nd			nd	nd		
ΔH (J/g)	Rice	11.21 ± 1.15^{aA}	11.04 ± 0.26^{aA}			11.67 ± 0.15^{aA}	11.21 ± 0.06^{aB}		
	Rice starch	14.80 ± 1.27^{aA}	15.82 ± 1.22^{aA}			15.33 ± 0.58^{aA}	15.12 ± 0.27^{aA}		
	Pepeta	nd	nd			nd	nd		

*Expressed in g/100 g of total starch, $^{\#}$Finely ground *pepeta*, $^{\$}$Coarsely ground *pepeta,* 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, Dx10 – average particle size of smallest 10% , Dx50 – average particle size of the smallest 50%, Dx90 – average particle size of the smallest 90%, C_∞ - equilibrium hydrolysis, k- rate constant, To – onset temperature, Tp – peak temperature, Tc – conclusion temperature, ΔH – enthalpy change. Data expressed as mean ± standard deviation.

effect between maturity levels for the ΔH associated with gelatinization. The To, Tp, and Tc 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.[55,56] In addition, we observed significant differences in To, Tp and Tc in both varieties, and ΔH of Lawama variety for rice flour as compared with rice starch samples. The To, Tp and Tc 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*.[57] who reported an increase in susceptibility 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.

3.3.5 *In-vitro* digestibility of starch and proteins

3.3.5.1 Starch hydrolysis

Fig. 3.4 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 α-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.[58,59] 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.[57,60]

According to Fig. 3.4 Panel a – b, the ORS in TXD306 and MGS in Lawama had slightly lower digestibility under simulated conditions. The C_∞ and k, estimated by fitting the experimental data to the first order kinetic equation (section 3.2.9), showed an inconsistent trend for maturation for both varieties (Table 3.2). The C_∞ (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) min^{-1} in TXD306 and 0.24 (FRS) – 0.32 (DGS) min^{-1} in Lawama) values for cooked rice are in line with previous work.[31,57,61] However, no significant effect on C_∞ 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,[16–18] 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.[56] However, none of these factors seems to play a role when starch is fully gelatinized (section 3.2.2.2) as in our study.

Digestograms (Fig. 3.4 Panels c – 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_∞ at DGS in both varieties and k of Lawama variety at MGS) between cooked rice and *pepeta*. Slightly lower C_∞ and k values were observed in *pepeta* as compared to cooked rice (Table 3.2). Chitra *et al*.[17] 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. 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 3.2.2.2 and 3.3.4). 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 3.3.2). 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.[62,63] 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*,[64] and fine ground *pepeta* (FGP, < 0.425 mm) were compared for their *in-vitro* starch digestibility. Fig. 3.4 (panels e – 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.[65] Though no significant decrease in C_∞ 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 3.3.2), has an effect on *pepeta* starch digestibility, as repeatedly reported for other cereals.[66–68] The slightly lower starch digestibility in *pepeta* is therefore possibly related to the higher level of structural integrity as compared to cooked rice.

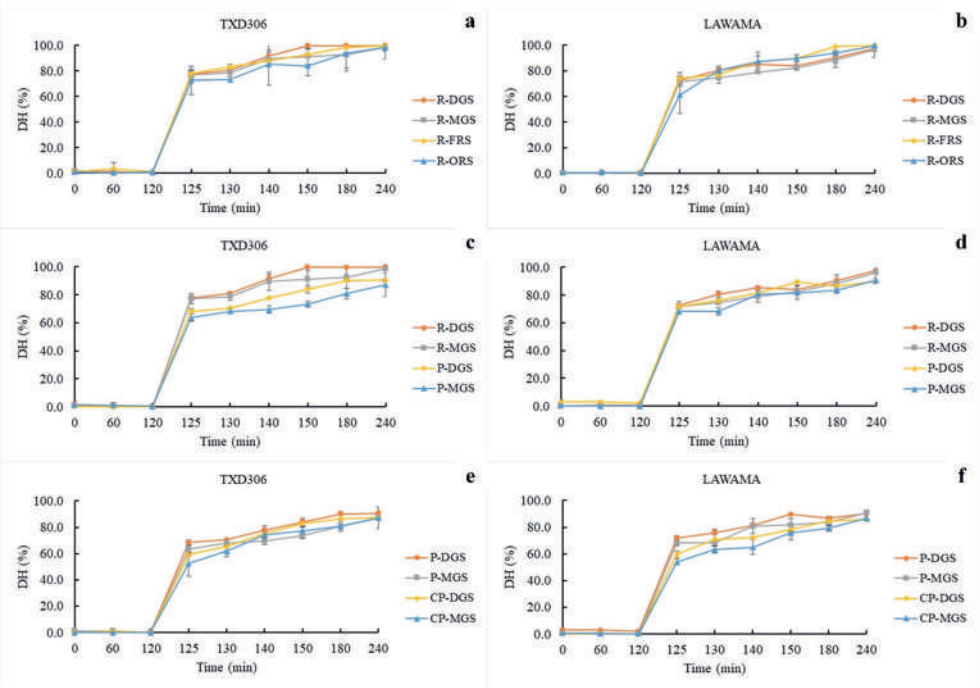

Fig. 3.4: *In-vitro* hydrolysis of starch in two rice varieties, TXD306 and Lawama during different stages of development (a and b) and as a function of processing method (c and d) and particles sizes (e and f). R-DGS – cooked rice at dough grain stage (15 – 21 days after 50% heading (DAH)), R-MGS – 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 (≤ 0.425mm) prepared at dough grain stage, P-MGS – *pepeta* powder (≤ 0.425mm) prepared at mature grain stage, CP-DGS – *pepeta* coarse (2 – 1mm) prepared at dough grain stage, an CP-MGS – *pepeta* coarse (2 – 1mm) prepared at mature grain stage.

3.3.5.2 Protein hydrolysis

Fig. 3.5 Panels a – 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 3.2). The fact that immature grains contain less digestible protein than fully mature grains may be responsible. Previous studies[7,8] 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

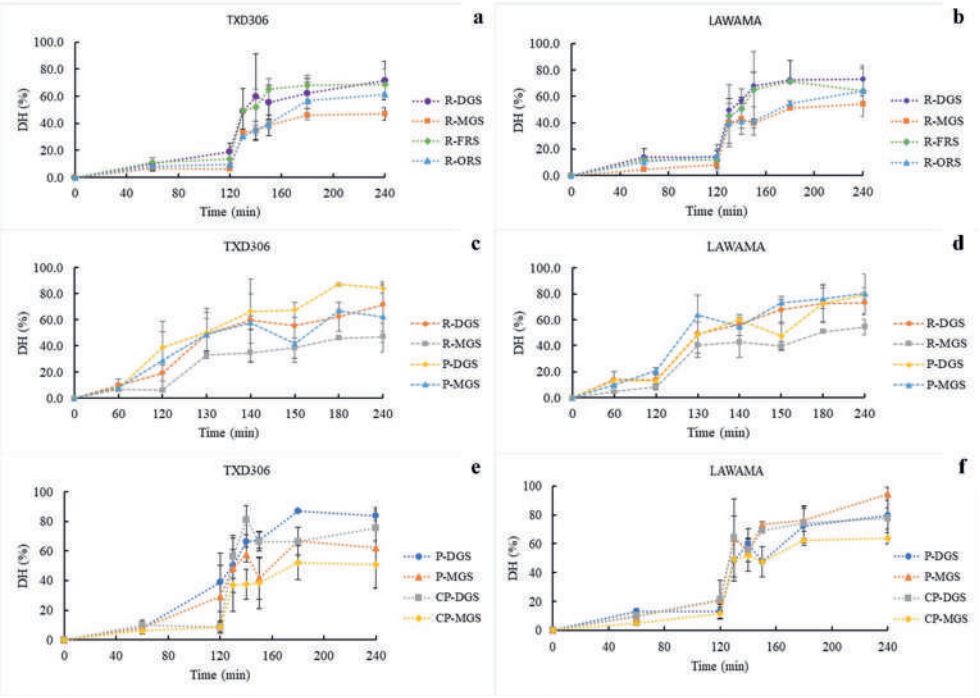

Fig. 3.5: Digestograms of protein enzymatic hydrolysis *in-vitro* for TXD306 and Lawama rice varieties during different stages of development (a and b), and as a function of processing method (c and d) and particles sizes (e and f). R-DGS – cooked rice at dough grain stage (15 – 21 days after 50% heading (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 (≤ 0.425mm) prepared at dough grain stage, P-MGS – *pepeta* powder (≤ 0.425mm) prepared at mature grain stage, CP-DGS – *pepeta* coarse (2 – 1mm) prepared at dough grain stage, an CP-MGS – *pepeta* coarse (2 – 1mm) prepared at mature grain stage.

was observed for fine *pepeta* samples (Fig. 3.5 Panels c – 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 3.2). 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[53,69,70] 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.[70,71] 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. 3.5 Panels e – 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 3.3.2). The results are consistent with previous studies on the effect of particle size on *in-vitro* digestibility of legume and cereal flours.[28,65] 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.

3.4 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.

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Conflict of interest The authors declare that they have no conflict of interest.

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4

CHAPTER 4.

Dry-heat processing at different conditions impact the nutritional composition and *in-vitro* starch and protein digestibility of immature rice-based products

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Abstract

Immature grain represents a precious nutritional source in many rural Africa areas. To optimize processing of immature rice into *pepeta* (a traditional rice-flakes produced from immature rice grains), immature rice (TXD306 variety) harvested at 18 and 26 days after 50 % heading were processed in the laboratory under different soaking (0 and 12 h) and roasting temperature (80, 100 and 120 °C) regimes. Riboflavin, nicotinic acid, nicotinamide and iron concentration increased with severity of roasting temperature, while thiamine has an opposite trend. Heating promoted the transformation of insoluble into soluble dietary fiber, increased lipid digestibility decreasing protein one, which showed the highest value when rice was roasted at 100 °C. Soaking before roasting significantly increased moisture and iron content while slightly increased riboflavin, nicotinic acid and nicotinamide when compared to unsoaked products. Among roasted products, starch digestibility increased with roasting temperature. Microstructure analysis indicated a complete loss of cell wall integrity in cooked rice, determining a complete starch and protein digestion while this is delayed in raw rice and roasted products. We concluded that roasting at 100 °C is the optimum temperature to produce *pepeta* of the highest protein digestibility and low starch digestibility. Soaking before roasting at 120 °C is best when retaining micronutrients is considered.

Keywords: dry-heat processing, nutritional composition, *in-vitro* digestion, *pepeta*, immature rice-based products.

4.1 Introduction

Consumption of immature cereal-based products is common in communities where cereals are staple food. Firik (also known as frikeh or frekeh or freekah) is a scorched/roasted immature whole wheat-based food consumed in Arabic countries in the Middle East and Northern Africa.[1–4] Breads prepared from green maize and sorghum kernels are widely consumed in sub-Saharan Africa.[5] In Tanzania, *pepeta*, a locally prepared rice flakes from immature grains, is common among rice consuming communities.[6,7] Currently, consumption of immature cereal-based products is gaining popularity worldwide due to their nutritional and health potential benefits compared to fully mature cereal grains. Nutritional components such as protein, reducing sugars, calcium, potassium, iron, β-carotene, vitamin C, and vitamin B_2, B_3 and B_6[7–9] and functional compounds like dietary fiber, fructo-oligosaccharides and phytochemicals (phenolics and flavonoids)[1,3,10,11] decrease as cereal grain mature. These variations are the physiological consequences of cellular and physiological changes during grain development.[12]

However, the processing of some immature cereal-based products like *pepeta* is still done only at the household level. The production process is slow, labor intensive, with no established standards for harvesting, processing conditions and parameters.[6] This makes it impossible to guarantee a consistently good quality *pepeta* product and hinders its upscaling to the industrial production level.

Despite the valuable content of micronutrients *pepeta*, like that of cooked milled white rice, has a very high and fast starch digestibility properties.[6] Fast and high starch digestibility has been linked to high glycemic index (GI), associated with high occurrence of type II diabetes.[13–15] On the contrary, consumption of rice-based products with high digestible proteins can help improve the nutritional status (i.e. protein-energy malnutrition) of populations, as rice is the staple food and widely available and affordable for most of the

population.[16] In this framework, the main goal is to design cereal food products with high protein and low starch digestibility. In order to improve the nutritional quality and functional properties of the immature cereal-based product, insight into the changes in nutritional composition and digestibility properties of immature rice grains processed at different conditions is inevitable. This study focused on *pepeta* and simulated its production in the laboratory using different soaking and temperature regimes to optimize its processing conditions. The effect of soaking and roasting treatments on nutritional content and *in-vitro* digestion of starch and protein and their interaction was evaluated. The difference in nutritional content and *in-vitro* digestion of starch and protein between maturity levels were assessed as well to validates our previous findings as paddy grains were harvested at specific maturity date. Contrast to this study, paddy grains in the previous findings[7] were harvested according to processors knowledge, then their maturity levels classified into categories which could have produced artefacts due to minimum and maximum extremes of individual maturity levels withing category.

4.2 Materials and methods

4.2.1 Materials

4.2.1.1 Rice

Immature rice grains (at 31 – 43 % moisture content) of TXD306 variety suit for *pepeta* production were collected from a rice farmer at Ulanga district in Tanzania and used as research materials in this study. The grains were harvested at 18 and 26 days after 50% heading (DAH), based on our previous study that reported 15 – 28 DAH as the optimum maturity for *pepeta* production.[6] The heading date (at 50% heading) was determined when 50 percent of the panicles in the rice field were at least partially visible. For each maturity level,

5 kg of harvested wet rice grains (in portions of 500 g) were vacuum-sealed in plastic bags (Princess®, S-492967-001, China) and stored at -20 ºC until further use.

4.2.1.2 Reagent

Pepsin from porcine gastric mucosa (P6887, 3200–4500 U/mg), porcine bile extract (B8631), amyloglucosidase from *Aspergillus niger* (10113, 129.3 U/mg), trypsin from porcine pancreas (T7409, 1000–2000 U/mg), α-chymotrypsin from bovine pancreas (C4129, ≥40 U/mg), α-amylase from porcine pancreas (A4268, 700–1400 U/mg), sodium dodecyl sulfate (SDS), o-phthaldialdehyde (OPA), DL-dithiothreitol (DTT), L-serine, thiamine hydrochloride (B_1), nicotinic acid (B_3, ≥ 99.5 % HPLC), and nicotinamide ($B_{3'}$) 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 fiber analyses were acquired from Megazyme Inc. (Wicklow, Ireland). Other chemicals used in this study were of analytical grade.

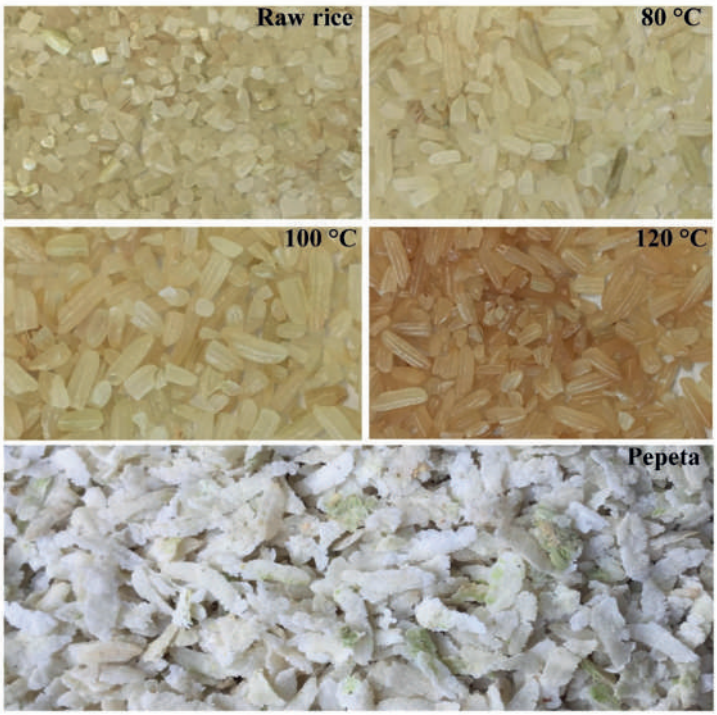
4.2.2 Preparation of immature rice-based products

Immature harvested rice grains were roasted in the laboratory, mimicking *pepeta* processing technology, using various soaking and temperature regimes, as shown in Fig. 4.1 A and B. Before roasting, one-half of the frozen paddy grains samples were kept at room temperature (20 – 25 °C) for 12 hours until their temperature equilibrated to that of the surroundings, whereas the second half was soaked in cold water at room temperature overnight (12 hours) and left to drain-off water for 6 hours. Replicate samples (100 g) of soaked and unsoaked paddy grains were each roasted at 80, 100 and 120 ºC for 8 minutes using hot air fluidized roaster (Toper, Optical Coffee Roaster, Turkey). For each temperature, three independent

samples were roasted. Once roasted, the samples were cooled down to room temperature before milling into rice grains using a milling machine (F. Walter - H. Wintersteiger K.G. Maschinen-Geratebau, Ried Innkreis, Austria) for 4 minutes. The machine employs single-stage milling technology

whereby dehulling and polishing of paddy grain is done concurrently. Tiny broken kernels, tips and fine powder were removed using a 2 mm sieve, while brans, rice straws, and other light chaffs were removed by blower system (Herding Filertechnik, HSL 1500-14-16/18 SB, German) set at 180 daPa. The milling machine was cleaned using compressed air (Kaeser Kompressoren, SM 12 SIGMA, Netherlands) set at 7.2 – 7.8 bar before milling another sample. Samples were then stored at -20 °C for further laboratory analyses.

A



B

Treatment codes, roasting and dehusking conditions for the rice samples in this study

Treatment code	Soaking time (h)	Roasting temperature (°C)	Roasting time (min)	Dehusking time (min)
80-NS	0	80	8	4
80-S	12	80	8	4
100-NS	0	100	8	4
100-S	12	100	8	4
120-NS	0	120	8	4
120-S	12	120	8	4
Raw rice*	Immature dried paddy			2*
Pepeta	Locally prepared pepeta from fresh harvested immature paddy			

*Raw rice samples were less hard compared to roasted rice samples, thereby dehusking beyond 2 minutes cause substantial sample losses in form of flour dust mixed with bran. NS – not soaked, S – soaked in cold water for 12 h before roasting, 80, 100 and 120 are corresponding roasting temperatures (°C).

Fig. 4.1: General appearance of raw and processed rice samples at different roasting temperatures (A), and tabulation description (B) of laboratory experiment design employed in this study.

Three control samples were also assessed: (i) raw rice – a portion (500 g) of immature harvested rice variety TXD306 that had been dried to 13 – 11 % using a hot air dryer (TG 200, Retsch GmbH, Haan, Germany) set at 50 °C and milled into white rice for 2 minutes, not roasted; (ii) *Pepeta* – a locally prepared *pepeta* from corresponding fresh harvested rice variety TXD306 that has been manually roasted on an open wood fire (181 – 270 °C, paddy

temperature 80 – 129 °C) for 3 – 8 min, immediately followed by hand pounding using a pestle and mortar for 1 – 3 min, as described in our previous study[6,7]; and (iii) cooked rice – a portion (10 g) of milled raw rice sample used for determination of starch properties, microstructure and *in-vitro* digestion analyses only, that had been well cooked in excess boiling water (ratio 1:25 w/v, respectively) until fully gelatinized. Cooked rice kernels were pressed between two glass slides to check for optimum gelatinization, i.e., when no clear white core was observed anymore. The processing treatments were assigned codes (Fig. 4.1 B), indicating roasting temperature (°C) and soaking time (h) for the laboratory simulated *pepeta* processing treatments.

4.2.3 Grinding

A cryo-mill (Freezer mill 6875D, Spex Sample Perp) was used to mill raw milled white rice, *Pepeta* and roasted rice products set at 2 cycles, 2 min cooling, 5 min grinding, and 15 cycles per second (cps). The retained flour particles (425 – 250 µm) were used in microstructure and *in-vitro* digestion analyses, whereas the particles that passed through 250 µm laboratory sieve were used for other analyses. For cooked samples, cooked rice grains were squeezed through a 425 µm laboratory sieve and used for starch properties, microstructure and *in-vitro* digestion analyses. Samples were immediately stored at -20 °C till further uses.

4.2.4 Proximate analysis

Dry matter content was determined by oven drying 2 g of samples overnight set at 105 °C and the weight difference calculated according to an AOAC method.[17] To quantify ash content, 2 g of samples were incinerated at 550 °C overnight following AOAC methods.[17] Soxhlet-petroleum ether extraction system was used to extract fat from 5 g of the sample according to an AOAC method.[17] The extracted fat was then expressed as the mass percentage of the

original sample. Dumas combustion method was used to estimate the nitrogen content. About 15 – 20 mg of sample was analyzed (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. To convert nitrogen content to protein, a specific conversion factor (Jones 'factor for rice products) of 5.95 was used.[18] Total carbohydrate content was calculated by the subtraction method, i.e., the fraction retained after deduction of other proximate compositions on a dry matter basis.

4.2.5 Degree of Milling

The degree of milling (DOM) was estimated by the surface lipid content (SLC) method according to Matsler and Siebenmorgen[19] using Soxhlet-petroleum ether extraction system as described in section 4.2.3. Whole kernels of *pepeta* and roasted products, and large broken grains (above 2/3 of the original size) of raw rice (due to lack of whole kernels), were used in this analysis.

4.2.6 Crude dietary fiber

The enzymatic gravimetric method using a megazymes kit (K-TDFR, Megazyme Int, Wicklow, Ireland) was employed to determine Soluble (SDF) and insoluble (IDF) dietary fiber. In summary, the mixture of the sample (1 g) and MES-TRIS buffer solution (40 mL) was incubated in series with α-amylase, protease, and amyloglucosidase enzymes before being filtering to obtain the IDF. For SDF quantification, hot ethanol (95 %, v/v) at 60 °C was used to precipitate the filtrate. SDF and IDF values were corrected by subtracting the corresponding ash and protein residuals. Total dietary fiber (TDF) was determined as the sum of SDF and IDF.

4.2.7 Analysis of Fe and Zn

ICP-AES (Inductively Coupled Plasma – Atomic Emission Spectrometry) using Thermo iCAP-6500 DV equipment (Thermo Fisher Scientific, Waltham, USA) was employed to estimate the content of Fe and Zn, as described in our previous study.[7] In summary, 300 mg of flour sample was digested with the concentrated nitric-hydrochloric acid mixture and hydrogen-peroxide in series[20] before analyzed on ICP-AES. The flour samples were dried at 70 °C overnight before the analysis.

4.2.8 Determination of vitamin B_1, B_2 and B_3

The determination of thiamine (B_1), riboflavin (B_2), nicotinic acid (B_3) and nicotinamide ($B_{3'}$) was done following the modified procedure of Chen *et al.*[21] To extracts B vitamins, mixtures of 0.5 g ground samples with 25 mL milli Q water in 50 mL grainer tubes were sonicated at 40 °C for 5 h before centrifuging at 3000 g for 10 min at room temperature. A 0.2 µm membrane filter was used to filter the supernatant, and 20 µL extract was used for HPLC analysis. For thiamine, nicotinic acid and nicotinamide quantification, the mobile phase was 25 mM KH_2PO_4 (pH 3): $CNCH_3$ in a ratio of 97:3. Riboflavin was analyzed using 5mM ammonium formate buffer/ acetonitrile buffer in a ratio of 70 to 30, respectively. The HPLC system was operated with a Prevail C18 column (5 µm, 4.6×250 mm), at a flow rate of 1.0 mL/min and a UV detector set at 245 and 270 nm at room temperature.

4.2.9 Starch characterization analysis

4.2.9.1 Total, resistant and digestible starch

Resistant starch (RS) and digestible starch (DS) were evaluated by an enzymatic gravimetric method using a commercial Megazyme kit (K-RSTAR 05/19, Megazyme Int., Wicklow, Ireland). Cooked rice products containing higher moisture (80-84 % wet basis) were treated as

wet samples. Total starch (TS) was calculated as the sum of resistant starch and non-resistant (digestible) starch.

4.2.9.2 Starch isolation and preparation of cooked starch

Isolation of starch was performed according to the modified wet-milling method described in Syahariza *et al*.[22] In brief, a rice slurry was prepared by milling for 10 minutes, a 50 g sample of soaked rice grains in sodium metabisulfite (150 mL, 0.45% w/v) for 72 hours at 4 °C using a commercial blender. To remove protein, the slurry was repeatedly and vigorously mixed with 450 mL NaCl (0.1 M) solution and 50 mL toluene was employed till the toluene layer was clear and free from protein. The starch was then recovered by filtrating through a filter paper (Whatman 595 ½, Whatman International Ltd., Kent, UK), rinsed with 96% ethanol and left at room temperature for 2 hours to allow complete evaporation of ethanol before storing at -20 °C for further analysis. To prepare cooked rice starch, 1 g from raw rice starch was dispersed in 4 g distilled water and incubated at 70 °C for 10 min with constant shaking (200 rpm). Samples were then placed in a boiling water bath for 30 min. To avoid retrogradation, the cooked starch pastes were immediately stored at -20 °C till further analysis.

4.2.9.3 Amylose/amylopectin ratio

The amylose/amylopectin megazyme kit (K-AMYL 06/18, Megazyme Int., Wicklow, Ireland) was used to quantify the amylose content of processed rice flour and starch samples. Indirect measurement of amylopectin content was done by subtracting the amylose percentage from the TS percentage of the samples.

4.2.10 Protein aggregations analysis

4.2.10.1 Protein solubility

A solvent dependent solubility test on 26 DAH samples was used to evaluate changes in protein interactions according to the modified protocol of Liu and Hsieh[23] and Van Der Borght.[24] Four different solvents were used to extract protein; solvent 1 (S1, 0.1 M phosphate buffer salt (PBS) at pH 7.5), solvent 2 (S2, 0.1 PBS/2% (w/v) sodium dodecyl sulphate (SDS) at pH 7.5), solvent 3 (S3, 0.1 PBS/2% (w/v) SDS/6 M urea at pH 7.5), and solvent 4 (S4, 0.1 PBS/2% (w/v) SDS/6 M urea/ 1% (w/v) dithiothreitol (DTT) at pH 7.5). Flour samples (< 250 mm sieve) were mixed with solvent in a ratio of 1:25 (w/v), respectively and shaken for 1 h at 150 rpm and 25 °C. Subsequently, the solutions were centrifuged (4816 g, 15 minutes, 25 °C) to recover the solubilized protein in the supernatants. Before extraction, each flour sample was freeze-dried, defatted with petroleum ether (soxhlet system) heated at 60 °C for 6 h, and recovered flour in the soxhlet thimble was air-dried at 25 °C under a fume hood. The protein content of the supernatants was determined using a Pierce BCA protein assay kit (Thermo Fisher Scientific, Massachusetts, USA). The total protein content of protein samples was measured by the Dumas combustion method using the analyzer (EA 1112 NC, Thermo fisher scientific Inc., Waltman, USA) (section 4.2.3). The protein solubility was calculated as the percentage of protein in the supernatant to the protein in samples.

4.2.10.2 Free-thiol content

Free thiol content was determined according to the method described by Chan and Wasserman[25] with modifications. In brief, flour samples (75 mg) were suspended in 1.0 mL reaction buffer composed of 8 M urea, 1% (w/v) SDS, 3 mM EDTA (ethylene-diaminetetraacetic acid) and 0.2 M Tris-HCl, pH 8.0. Samples were incubated at 21 °C for 1 h with intermittent shaking. Subsequently, samples were centrifuged at 10000 g for 10 minutes.

From the supernatant, 50 µL was taken and mixed with 950 µL of 0.1 mM DTNB working solution, prepared by dissolving 40 mg DTNB (5,5-dithiobis (2-nitrobenzoic acid)) in 10 mL DMSO (Dimethyl sulfoxide), followed by 100-fold dilution with 0.1 M Tris-HCl, pH 7.5. A blank was set by adding 50 µL 0.1 M Tris-HCL buffer to 950 µL DTNB working solution. Absorbance was read with a spectrophotometer at 412 nm, and free thiol content was calculated using the molar extinction coefficient of NTB (14150 M^{-1} cm^{-1}).

4.2.10.3 Total thiol content

The disulphide content was determined according to Thannhauser *et al.*[26] with slight modifications. 50 mg of flour sample was dissolved in 1 mL reaction buffer, then diluted with 0.1 M Borate buffer pH 9.0 to obtain the concentration range of 5-0.5 mg/mL flour in solution. The reaction buffer was prepared by mixing 20 parts of the stock solution (6.3 M Guanidine-HCl, 1 mM EDTA, 0.2M Tris-CL, pH 9.5) and 1 part of the 2 M Na_2SO_3 solution. Subsequently, samples were centrifuged at 10000 g for 10 minutes. Thiols were determined by adding 10 µL sample aliquot to 3 mL of 50 mM NTSB (2-nitro 5-thio sulfo benzoic acid) solution, synthesized by dissolving 29.8 mg DTNB in 3 mL 1M Na_2SO_3 pH 9-9.5 solution. The reaction mixture was then incubated at room temperature (21 °C) in the dark for 25 minutes. Absorption was read at 412 nm, and disulphide content was calculated using the Extinction coefficient 13.600 M^{-1} cm^{-1} per disulphide.[27] The same concentration range without added sample was made as a reference.

4.2.10.4 Surface hydrophobicity

Surface hydrophobicity (H_0) was determined following the method described by Wang *et al.*[28] using 8 mM 1-anilino-8-naphthalene sulfonate as the hydrophobic fluorescence probes with modification. Defatted flour samples were prepared at a protein concentration (on a dry matter

flour basis) of 5 mg/mL in a 0.01 M phosphate buffer (pH 7), stirred at room temperature for 1 h and centrifuged 5000 g for 30 mins. The supernatants were serially diluted with the same buffer to various protein concentrations ranging from 0.5 to 0.005 mg/mL. Subsequently, 25 µL of ANSA (8.0 mM in 0.1M phosphate buffer, pH 7) was added to 2.7 mL sample. The fluorescence intensity (FI) was measured by the LS50B luminescence spectrometer (Perkin Elmer, Massachusetts, USA) at 338 nm (excitation) and 496 nm (emission) wavelengths. The slope (linear regression fit) of the FI versus the protein concentration of the sample gave the protein surface hydrophobicity.

4.2.11 *In-vitro* digestion

A two-phase gastro-intestinal *in-vitro* starch and protein hydrolysis was employed following a modified consensus INFOGEST protocol[29] as described in our previous study.[6] Before analysis, all samples (except cooked rice) were mixed with milli-Q water in a ratio of 1:2 (w/v), respectively. In all samples, a pre-warmed simulated salivary fluid (SSF) without salivary α-amylase was added to a final ratio of 1:1 (w/v), respectively. Immediately, the mixture was combined with simulated gastric fluids (SGF) and freshly prepared pepsin to a final ratio of food to SGF of 1:1 (v/v) and enzyme activity of 2000 U/mL, respectively. The pH of the mixture was adjusted to 3 with 1 M HCl before the gastric digestion was simulated by incubating sample tubes at 37 °C for 120 min. Thereafter, the gastric chyme was mixed with warmed simulated intestinal fluids (SIF) in a final ratio of 1:1 (v/v) to simulate the intestinal phase, respectively. Fresh bile and pancreatin solution were added by considering the final concentration of 28.8 mg/mL and trypsin enzymatic activity of 100 U/mL, respectively. The chyme pH was adjusted to 7 with 1 M NaOH and incubated at 37 °C for 120 min to complete a 240 min *in-vitro* digestion.

The experiments were performed in an oven by placing sample tubes in a rotor (Multi Rs-60, Biosan, Riga, Latvia) set at 40 rpm. For each sample tubes, an aliquot sample (0.2 mL) were sampled at 0, 60, 120, 125, 130, 140, 150, 180 and 240 min for starch hydrolysis, and at 0, 60, 120, 150, 180 and 240 min for protein hydrolysis. Immediately, absolute ethanol and 20 % TCA were separately added to the aliquot samples in the eppendorf tubes to a ratio of 1:4 (in both starch and protein) to stop amylase and protease activity, respectively. The aliquots sample mixtures were left to cool at room temperature for 20 min before centrifuging at 3000 g for 10 min at 0 °C. Subsequently, the supernatants were obtained in the new eppendorf tubes and stored at −20 °C until further analysis.

4.2.11.1 Determination of starch hydrolysis

A mixture of 0.5 mL amyloglucosidase solution (27.17 U/mL) in 0.1 M sodium acetate buffer (pH 4.8) and 0.1 mL ethanolic supernatant obtained after the termination of amylase activity were incubated at 37 °C for 1 h. To quantify the amount of glucose, a Megazymes D-glucose assay kit (GOPOD FORMAT, K-GLUC, Megazyme Inc., Bray, Ireland) was employed. A factor of 0.9 was multiplied to convert the amount of glucose into starch, expressing the results in g of digested starch per 100 g of total starch on a dry basis. The experimental data were then fitted into a first-order equation to estimate the kinetics of starch hydrolysis:

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

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

4.2.11.2 Determination of protein hydrolysis

Prior quantification of free amino groups (NH_2), non-digested samples were hydrolyzed with 6 M HCl, incubated at 110 °C for 24 h. After this, the free amino groups in both digested

(TCA) samples and non-digested samples were estimated by ortho-phthalaldehyde (OPA) method.[30] The degree of hydrolysis (DH) was calculated based on the following equation:

$$DH(\%) = \frac{NH_{2(DS)} - NH_{2(t=0)}}{NH_{2(Total)} - NH_{2(t=0)}} \; x\,100$$

where:

NH_2 (DS) = free amino groups from digested sample

NH_2 (t=0) = free amino groups from samples at time 0 of digestion

NH_2 (Total) = maximum amount of NH_2 present in the sample

4.2.12 Confocal laser scanning microscopy

Zeiss 510 inverted microscope (Carl Zeiss microscopy, Oberkochen, Germany) was used to visualize the endosperm cell wall microstructure of raw and processed rice products destined for *in-vitro* starch and protein hydrolysis. Flour particles (425 – 250 µm) of raw rice and roasted rice products, and freshly prepared cooked rice particles (squeezed through 425 µm) were stained with 0.02 % calcofluor-white dye and left to incubate for 10 min. Samples were then excited at 405 nm, and images were taken using a 40x (N.A. 1.3 oil immersion) objective lens.

4.2.13 Statistical analysis

One-way ANOVA was performed on the data collected to determine the significance of the processing treatments and maturity level using SPSS (version 11.5, SPSS Inc., Chicago, USA). Data were treated independently for each processing treatment, but they were pooled out together irrespective of processing treatments to evaluate maturity level effect. Post hoc comparisons between independent variables were conducted using Tukey's HSD tests at $p < 0.05$. Two-way ANOVA was computed to assess interactions between soaking and roasting conditions of simulated *pepeta* processing technology, treating "roasting" as a fixed effect and

"soaking" as a random effect. Pearson correlation was used to investigate the relationship between protein digestibility and heat-induced protein interactions. Data are presented as means ± standard deviation of at least two replicates of an immature polished rice flour.

4.3 Results and discussions

4.3.1 Impact of processing on DOM and proximate composition of immature rice-based products

The DOM and proximate composition of immature rice-based products at different maturity stages, soaked or not and roasted at a different temperature, are shown in Table 4.1. As expected, moisture decreased with increased roasting temperature, where roasted products at 120 °C had the lowest values in both 18 and 26 DAH maturity. Soaking before roasting significantly increased the final moisture content of roasted products; due to the high initial moisture level of soaked grains.

Roasting with and without soaking significant decreased (except for 18 DAH) the lipid content of immature rice products, raw rice and *pepeta* showed higher values compared to roasted products in both maturity levels. This could be due to the observed low DOM, indicating a substantial amount of bran residual on the surface of raw rice and *pepeta* compared to other products (Table 4.1). In this study, DOM refers to the quantity of bran and polish removed from brown rice during milling operations.[19] Thus high amount of bran residual (less DOM) could be associated with increase in the total lipids content of raw and *pepeta* samples as rice brans contain 20 % lipids.[31] Roasting at 80 °C significantly enhanced the protein content of raw rice, but slightly decreased as the roasting temperature increased. This suggests that proteins are not yet concentrated on the surface of the endosperm in immature grains used in this study. Less DOM (higher amount of bran fraction/ surface lipid content) in heavily roasted grains diluted the endosperm, so less amount of N measured by

Dumas. Proteomic studies[32–34] revealed metabolic changes of rice grain during reserve accumulations; variations among grains at 7, 10 and 14 days after flowering (DAF) were much higher compared at 28 and 42 DAF.[35] Besides, rice proteins are solely produced in the starchy endosperm.[36] However, they accumulate on the outer surface of the endosperm as rice grains develop to optimum maturity.[37] Though soaking before roasting had no significant effect (except at 100 °C, 18 DAH) on protein content, slightly low values were observed in roasted products after soaking, indicating a more severe heat denaturation or aggregation in soaked products (section 4.3.4). Total carbohydrate slightly increases with the roasting temperatures. This could be associated with the decrease in protein content which is the second in abundant after starch in the polished rice, as carbohydrates content was determined by the subtraction method (section 4.2.3). Maturity level had a significant effect on lipid, protein and carbohydrate contents; high values were observed at 18 DAH compared to 26 DAH for protein content. The opposite was observed for lipids and carbohydrate contents. The decrease in protein content and increase in carbohydrate content could be related to the accumulation of starch during rice grain development.[8,38] An interaction between soaking and roasting temperature was significant in DOM, total lipid and total carbohydrate at 26 DAH.

Table 4.1: DOM and proximate analysis of TXD306 rice at different maturity levels and roasting conditions

Treatment	DOM (g/ 100 g, dwb)	Moisture (g/ 100 g)	Nutritional component (g/ 100 g, dwb)			
			Total lipid[#]	Total protein[#]	Total ash	Total carbohydrates[#]
18 DAH						
80-NS*	0.28 ± 0.04^{a}	19.11 ± 1.80^{d}	0.62 ± 0.02^{a}	9.01 ± 0.16^{c}	1.15 ± 0.41^{a}	89.22 ± 0.17^{a}
80-S	0.41 ± 0.03^{ab}	20.01 ± 0.28^{d}	0.58 ± 0.06^{a}	8.50 ± 0.52^{bc}	1.09 ± 0.09^{a}	89.84 ± 0.51^{ab}
100-NS	0.65 ± 0.03^{b}	15.09 ± 0.13^{c}	0.70 ± 0.27^{a}	8.71 ± 0.36^{c}	1.11 ± 0.10^{a}	89.49 ± 0.56^{ab}
100-S	0.64 ± 0.10^{b}	15.74 ± 0.29^{c}	0.65 ± 0.06^{a}	7.98 ± 0.04^{b}	1.12 ± 0.09^{a}	90.26 ± 0.04^{ab}
120-NS	0.32 ± 0.07^{a}	10.11 ± 0.36^{a}	0.53 ± 0.07^{a}	8.48 ± 0.03^{bc}	0.76 ± 0.65^{a}	90.23 ± 0.67^{ab}
120-S	0.46 ± 0.09^{ab}	12.32 ± 0.18^{b}	0.55 ± 0.02^{a}	7.86 ± 0.13^{ab}	1.08 ± 0.10^{a}	90.50 ± 0.22^{b}
Pepeta	1.23 ± 0.04^{d}	10.80 ± 0.46^{ab}	1.68 ± 0.04^{b}	7.44 ± 0.24^{a}	1.34 ± 0.16^{a}	89.54 ± 0.43^{ab}
Raw rice	0.99 ± 0.08^{c}	11.33 ± 0.26^{ab}	1.57 ± 0.08^{b}	7.90 ± 0.10^{b}	1.23 ± 0.15^{a}	89.30 ± 0.23^{a}
26 DAH						
80-NS	0.68 ± 0.01^{bc}	16.18 ± 0.10^{f}	1.48 ± 0.65^{d}	7.91 ± 0.33^{d}	1.08 ± 0.09^{a}	89.53 ± 0.92^{a}
80-S	0.70 ± 0.01^{bc}	17.20 ± 0.19^{g}	0.89 ± 0.09^{b}	7.78 ± 0.09^{cd}	1.04 ± 0.08^{a}	90.30 ± 0.12^{ab}
100-NS	0.48 ± 0.05^{ab}	13.64 ± 0.26^{d}	0.52 ± 0.05^{a}	7.85 ± 0.09^{cd}	1.03 ± 0.07^{a}	90.61 ± 0.06^{ab}
100-S	0.77 ± 0.03^{c}	14.33 ± 0.17^{e}	1.38 ± 0.29^{bd}	7.77 ± 0.23^{cd}	1.05 ± 0.13^{a}	89.79 ± 0.34^{a}
120-NS	0.40 ± 0.07^{a}	10.45 ± 0.19^{a}	1.04 ± 0.03^{bc}	7.10 ± 0.27^{ab}	1.25 ± 0.10^{a}	90.61 ± 0.18^{ab}
120-S	0.52 ± 0.06^{ab}	11.65 ± 0.23^{c}	0.58 ± 0.03^{a}	6.98 ± 0.06^{ab}	0.99 ± 0.06^{a}	91.46 ± 0.14^{b}
Pepeta	1.37 ± 0.06^{e}	10.08 ± 0.19^{a}	1.85 ± 0.12^{d}	7.16 ± 0.01^{b}	1.44 ± 0.51^{a}	89.56 ± 0.53^{a}
Raw rice	1.15 ± 0.19^{d}	11.15 ± 0.04^{b}	1.63 ± 0.07^{cd}	7.36 ± 0.11^{bc}	1.27 ± 0.19^{a}	89.75 ± 0.34^{a}

*80, 100 and 120 are roasting temperatures (°C), NS – not soaked, S – soaked in cold water for 12 h before roasting, DAH – days after 50% heading, dwb – dry weight basis, DOM – degree of milling by surface lipid content. For each maturity level, values in each column with different superscripted letters are statistically different ($p \leq 0.05$).

[#]Significance difference between maturity levels ($p \leq 0.05$). Data expressed as mean ± standard deviation of three replicates.

Consumption of dietary fiber is associated with reduced risk for cardiovascular disease, diabetes and colorectal cancer.[39,40] Table 4.2 reports the results of dietary fiber content for the two maturity levels. In all samples (raw rice, *pepeta* and roasted products), very low SDF content was observed compared to IDF in the corresponding samples, in line with previous paper.[11] Roasting significantly affects SDF content, with *pepeta* showing the highest values possibly due to the lower DOM. Though no significant changes were observed among roasted products, the SDF/TDF ratio increased with roasting temperatures (Table 4.2), indicating possible conversion of insoluble fiber to soluble fiber during heat processing.[41] Intake of SDF increases viscosity in the digestive tract whereby prolong gastric emptying, reduce rate of starch digestion and glucose absorption, and associated to increased satiety and reduced postprandial blood glucose level.[41–43] On the contrary, IDF and TDF varied significantly (except in 26 DAH at 100 °C) at 120 °C, decreasing as roasting temperature increases. These results agree with Naumann *et al.*[41], who reported TDF degradation in extruded lupin kernels as a function of temperature when processed at 25, 100 and 150 °C. In that study, high heat treatment (at 120 °C) may have caused glycosidic bonds cleavage, converting insoluble polysaccharides to smaller fractions,[39,44] which are too small to be detected as dietary fiber due to low molecular weight.[45] It is worth noting that the retained residuals upon filtration (40-60 μm) of samples mixture after a series of incubation (section 4.2.5) were considered a dietary fiber. The IDF consumption increase bulk volume and decrease transit time associated with the increased dietary fiber supply, result in increased satiety and decreased saturated fat intake.[41,42] Soaked roasted products showed slightly lower values of SDF and higher values of IDF and TDF (except at 80 °C for 18 DAH) than unsoaked products for both maturity levels. Some energy could be used to evaporate extra water in soaked paddy grains during roasting, associated with reduction in dietary fiber degradation and conversion of IDF to SDF. No significant difference in IDF and TDF content were observed between *pepeta* and raw rice,

possibly due to high bran residual observed in both samples as DOM (Table 4.1). Rice bran is rich in dietary fiber (27.6 – 33.3 %) in which about 90% of the content is IDF.[46] IDF and SDF/TDF ratio differed significantly between maturity levels, with higher values observed at 18 and 26 DAH, respectively, suggesting increase in conversation of IDF to SDF when immature rice grain developed from 18 to 26 DAH. This increase could be associated with the impact of roasting temperature on dietary fiber, being prominent at 26 DAH due to less initial moisture content of immature grains compared to 18 DAH. Except for SDF and TDF at 26 DAH, no interaction effect was found between soaking and roasting.

Table 4.2: Dietary fiber contents (expressed as Mean ± standard deviation) of TXD306 rice at different maturity levels as affected by different processing conditions.

Treatment	Dietary fiber (g /100 g)			SDF/TDF (%)*
	SDF	IDF*	TDF	
18 DAH				
80-NS	0.55 ± 0.01^{b}	5.12 ± 0.17^{c}	5.68 ± 0.19^{b}	9.743 ± 0.12^{ab}
80-S	0.59 ± 0.11^{b}	4.97 ± 0.45^{c}	5.56 ± 0.33^{b}	10.67 ± 2.68^{ab}
100-NS	0.60 ± 0.03^{b}	4.74 ± 0.64^{bc}	5.34 ± 0.60^{b}	11.38 ± 1.94^{ab}
100-S	0.59 ± 0.06^{b}	5.01 ± 0.75^{c}	5.61 ± 0.69^{b}	10.75 ± 2.38^{ab}
120-NS	0.66 ± 0.13^{b}	2.41 ± 0.78^{a}	3.08 ± 0.65^{a}	22.49 ± 4.90^{b}
120-S	0.63 ± 0.08^{b}	2.68 ± 0.70^{a}	3.31 ± 0.78^{a}	19.22 ± 2.17^{ab}
Pepeta	1.02 ± 0.09c	4.52 ± 0.01^{abc}	5.54 ± 0.10^{b}	18.48 ± 1.24^{ab}
Raw	0.39 ± 0.03a	4.66 ± 0.31^{bc}	5.05 ± 0.28^{ab}	7.826 ± 1.08^{a}
26 DAH				
80-NS	0.70 ± 0.07^{b}	3.97 ± 0.02^{bc}	4.67 ± 0.05^{bcd}	14.97 ± 1.39^{bc}
80-S	0.67 ± 0.01^{b}	4.48 ± 0.46^{c}	5.14 ± 0.48^{d}	13.01 ± 0.95^{ab}
100-NS	0.72 ± 0.02^{bc}	2.62 ± 0.07^{a}	3.47 ± 0.11^{ab}	21.68 ± 0.04^{d}
100-S	0.71 ± 0.01^{bc}	3.66 ± 0.12^{abc}	4.37 ± 0.10^{abcd}	16.29 ± 0.54^{bc}
120-NS	0.86 ± 0.06^{bc}	2.61 ± 0.05^{a}	3.34 ± 0.09^{a}	24.75 ± 0.91^{d}
120-S	0.86 ± 0.09^{bc}	2.81 ± 0.19^{ab}	3.67 ± 0.28^{abc}	23.40 ± 0.74^{d}
Pepeta	0.94 ± 0.10^{c}	3.80 ± 0.34^{bc}	4.75 ± 0.24^{cd}	19.94 ± 3.12^{cd}
Raw	0.44 ± 0.02^{a}	4.48 ± 0.57^{c}	4.93 ± 0.59^{d}	9.049 ± 0.67^{a}

DAH – days after 50 % heading, SDF – soluble dietary fiber, IDF – insoluble dietary fiber, TDF – total dietary fiber. 80, 100 and 120 indicate rice roasted at the corresponding temperatures (°C), NS – not soaked, S – water-soaked rice at room temperature for 12 h prior roasting, *Pepeta* – locally prepared rice flakes, Raw – unprocessed rice. For each maturity level, labels with different letters indicate a statistically significant difference in fiber content ($p \leq 0.05$) for each dietary fiber parameter. *Significance difference between maturity levels ($p \leq 0.05$).

4.3.2 Effect of heat processing on selected vitamins and minerals

Fig. 4.2 (Panels a – h) present the content in selected vitamin Bs of processed rice products at 18 and 26 DAH maturity. Rice processing significantly affected the final concentration of thiamine, riboflavin, nicotinic acid and nicotinamide content; *pepeta* showed the highest values except for nicotinic acid at 18 DAH (Fig. 4.2 Panel e) and nicotinamide at 26 DAH (Fig. 4.2 Panel h). This is likely due to high bran residuals remaining on the surface of *pepeta* grains after pounding (Table 4.1) as vitamins concentrate in brans.[47,48] Vitamin Bs content varied significantly among roasted samples as well. Though no significant change was observed in 18 DAH, the thiamine content in both maturity levels (Fig. 4.2 Panels a – b) decreased as roasting temperature increase for both roasting with and without soaking, probably due to thermal breakdown. The results are contrary to a previous study[49] which reported thiamine content to increase with the severity of heat treatment (during soaking and steaming). This discrepancy could be due to different employed parboiling regimes; warm soaking and steaming (wet-heating treatment) in their study vs cold soaking and roasting (dry-heat treatment) in our study. Fig. 4.2 (Panels c – h) further indicates that riboflavin, nicotinic acid and nicotinamide contents increased with roasting temperature. Samples roasted after soaking showed slightly high value compared to those roasted without soaking. Since the content in these vitamins does not correlate to DOM, it is possible that some inward diffusion of nutrients from bran layer to endosperm had occurred during soaking[50,51] and roasting. Upon roasting of immature (both soaked and unsoaked) paddy grains, the starch granule in the endosperm expanded and swelled by absorbing the available free water molecule as it gelatinizes.[50] The onset of starch gelatinization created a moisture gradient between the bran layer and starchy endosperm caused inward diffusion of free water molecules and water-soluble vitamins from the bran layer into starchy endosperm to facilitate further the

gelatinization process.[52,53] It is important to note that the initial moisture content of fresh immature rice grain, below 28 DAH (day after 50% heading), was sufficient to fully

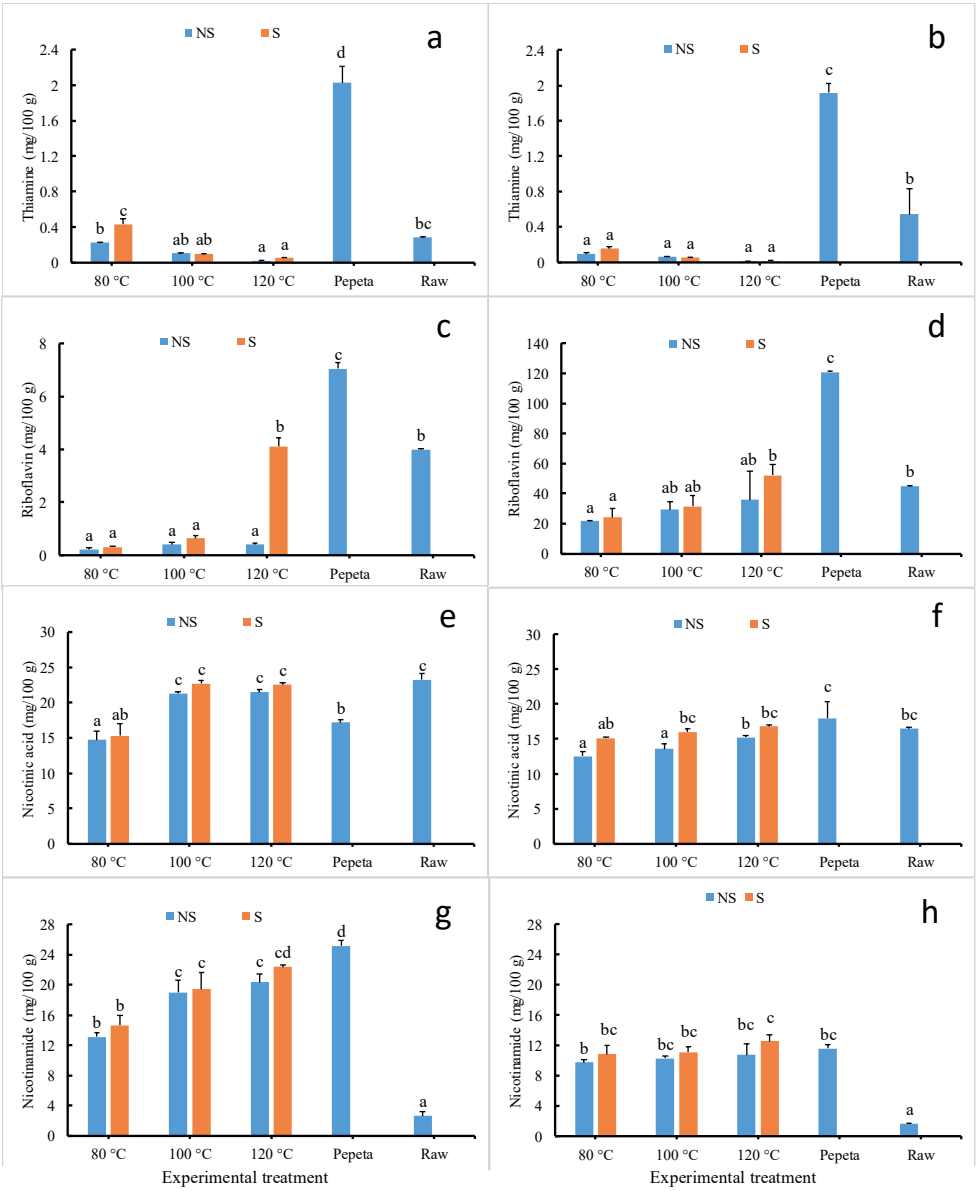


Fig. 4.2: Effect of processing condition on vitamin Bs content of TXD306 rice (in dry weight basis) at 18 DAH (days after 50% heading) (a, c, e, g) and 26 DAH (b, d, f, h) maturity levels. 80, 100 and 120 °C indicate rice roasted at the corresponding temperatures, *Pepeta* – locally prepared rice flakes, Raw – unprocessed rice, NS – not soaked, S – water-soaked rice at room temperature for 12 h prior roasting. Labels with different letters indicate a statistically significant difference in vitamin Bs content ($p \leq 0.05$). Each bar chart represents mean and standard deviation of two replicates.

gelatinize starch during *pepeta* processing.[7] Maturity level had a significant effect on riboflavin, nicotinic acid and nicotinamide contents; high values were observed at 26 DAH compared to 18 DAH for riboflavin content, whereas the opposite was observed for nicotinic acid and nicotinamide content. A study by Ji *et al.*[8] reported decreases in vitamins B contents, including riboflavin and/or their conjugates, during maturation of two Korean rice varieties, possibly due to their biochemical function as cofactors and precursors in regulating plant metabolism.[54–56] No interaction effect was found between soaking and roasting in all assessed vitamin Bs content.

Fig. 4.3 indicates significant changes in iron (Fig. 4.3 Panels a – b) and zinc (Fig. 4.3 Panels c – d) when rice processed under different conditions; the lowest iron content values were observed in raw rice. Among roasted products, the iron content significantly increased as roasting temperature increase; soaked products showed substantial-high values compared to roasted unsoaked products. The increase of minerals after roasting could be due to inward diffusion into endosperm during the soaking process[51] and fixation of micronutrients on the surface of the rice grain endosperm during the gelatinization process[57] for unsoaked rice products. On the contrary, roasting slightly reduced zinc content in roasted products, soaking before roasting showed an inconsistent trend. The results agree with the previous study,[58,59] which reported a contrasting heat treatment effect on iron and zinc; iron is highly affected compared to zinc upon heat treatment. This could be attributed to differential interactions between iron and zinc with the food matrix,[60–63] which has produced different inwards diffusion so that after removing different amount of bran (dehulling and polishing) it resulted in different amount of minerals accumulated in the endosperm. Though the maturity level had no significant effect, an interaction effect was found between soaking and roasting for iron and zinc at 18 DAH.

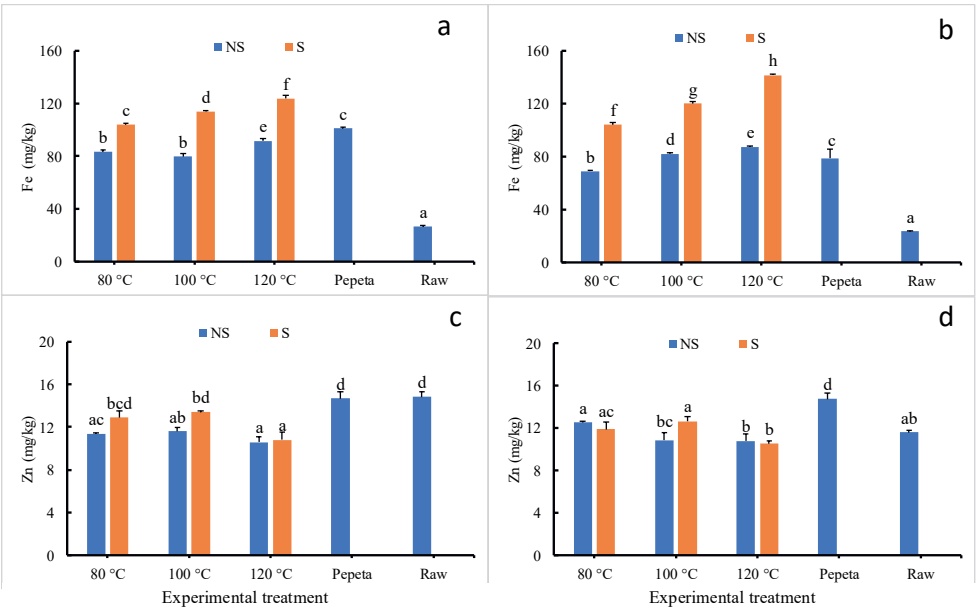


Fig. 4.3: Fe and Zn contents (in dry weight basis) of TXD306 rice at 18 DAH (days after 50% heading) (a, c) and 26 DAH (b, d) maturity levels as function of different processing conditions. 80, 100 and 120 °C indicate rice roasted at the corresponding temperatures, *Pepeta* – locally prepared rice flakes, Raw – unprocessed rice, NS – not soaked, S – water-soaked rice at room temperature for 12 h prior roasting. Labels with different letters indicate a statistically significant difference in Fe and Zn content ($p \leq 0.05$). Each bar chart represents mean and standard deviation of two replicates

4.3.3 Starch characteristics of processed rice products

Table 4.3 shows the starch properties of processed rice products at different maturity and under different processing conditions. The starch content, including the amount of RS, is mainly unaffected by roasting temperature and soaking, and comparable to the levels measured in locally prepared *pepeta*. Health benefit of RS intake as part of dietary fiber, include its contribution to gastrointestinal health, as a source of beneficial microbial fermentation in the large intestine, further associated with reduction in the glycaemic response.[64] However, the samples produced in the laboratory have a lower RS than raw rice, which suggests a very limited retrogradation when stored for 4 weeks before analysis. Rice cooking slightly decreased the amylose content compared to raw rice possibly due to amylose leaching into water during cooking.[65,66] It should be noted that rice was cooked in excess

boiling water until fully gelatinized (section 4.2.2). Contrary to cooking (wet heat), roasting (dry heat) enhanced the amylose content; roasted products showed higher amylose content than raw rice. The results agree with a previous study,[28] which reported higher apparent amylose content in heat-moisture treated starch than native starch in the rice. Though no significant effect observed among roasted samples (except for 18 DAH at 120 °C), the amylose content increased with roasting temperature in both flour and starch samples. These results can be explained by increased susceptibility towards hydrolysis by enzymes (fungal α-amylase and amyloglucosidase) on heat-treated samples due to more gelatinized starch, increasing the quantification of amylose by the enzymatic method used. Alternatively, it has been proposed that the level of apparent amylose can increase after heat moisture treatment because of the formation of the amylose-amylopectin complex upon heating.[28] This would limit the precipitation of amylopectin by concanavalin A, therefore, increasing the apparent amount of amylose. However, slightly lower values (except at 120 °C, 18 DAH) were observed in roasted products with soaking compared to roasted products without soaking, possibly due to amylose leaching into soaking water during the soaking process.[65,66] It is worth noting that the leaching out of amylose from paddy grains into soaking water is less pronounced compare to that during cooking of polished rice due to husk and bran barrier layers[67] and the fact the amylose is more tightly associated to amylopectin in native starch granules. The starch properties did not vary significantly between maturity levels except for starch content (TS and SS), high values observed at 18 DAH compared to 26 DAH. Similar results were found in our previous study on nutritional characterization of immature TXD306 and Lawama rice grains, reported decrease of starch content as rice grains developed from dough grain stage (DGS, 15 – 21 DAH) to mature grain stage (MGS, 22 – 28 DAH).[7] No interaction effect was found between soaking and roasting except RS for flour samples at 26 DAH.

Table 4.3: Starch properties of TXD306 variety at different maturity and roasting conditions

Treatment	Flour			
	Total starch[#] (g/ 100 g, dwb)	Resistant starch (g/ 100 g, dwb)	Soluble starch[#] (g/ 100 g, dwb)	Amylose (g/ 100 g, dwb)
18 DAH				
80-NS*	85.51 ± 0.52^{a}	0.63 ± 0.11^{a}	84.88 ± 0.41^{a}	12.70 ± 0.85^{ab}
80-S	83.46 ± 1.87^{a}	0.48 ± 0.02^{a}	82.98 ± 7.89^{a}	12.26 ± 2.17^{ab}
100-NS	79.24 ± 0.41^{a}	0.49 ± 0.01^{a}	78.76 ± 0.43^{a}	15.58 ± 0.99^{bc}
100-S	80.63 ± 0.51^{a}	0.52 ± 0.09^{a}	80.11 ± 0.41^{a}	14.23 ± 1.25^{abc}
120-NS	78.68 ± 1.71^{a}	0.35 ± 0.17^{a}	78.33 ± 1.88^{a}	16.78 ± 0.35^{bc}
120-S	79.09 ± 1.22^{a}	0.56 ± 0.01^{a}	78.53 ± 1.23^{a}	17.98 ± 0.20^{c}
Pepeta	80.10 ± 0.91^{a}	0.64 ± 0.03^{a}	79.46 ± 0.88^{a}	13.77 ± 1.91^{abc}
Raw rice	87.43 ± 4.26^{a}	2.65 ± 0.52^{b}	84.79 ± 4.78^{a}	12.08 ± 0.42^{ab}
Cooked rice	78.53 ± 3.09^{a}	0.56 ± 0.13^{a}	77.97 ± 3.22^{a}	10.52 ± 1.61^{a}
26 DAH				
80-NS	80.02 ± 0.76^{a}	0.68 ± 0.07^{b}	79.35 ± 0.83^{a}	12.61 ± 0.44^{ab}
80-S	80.95 ± 0.09^{a}	0.28 ± 0.09^{a}	80.67 ± 0.01^{a}	12.50 ± 0.79^{ab}
100-NS	77.90 ± 2.99^{a}	0.29 ± 0.01^{a}	77.61 ± 2.99^{a}	14.59 ± 0.47^{ab}
100-S	79.81 ± 0.62^{a}	0.27 ± 0.05^{a}	79.53 ± 0.57^{a}	14.18 ± 0.73^{ab}
120-NS	76.39 ± 3.98^{a}	0.24 ± 0.07^{a}	76.14 ± 3.91^{a}	16.32 ± 0.92^{b}
120-S	76.55 ± 0.50^{a}	0.23 ± 0.09^{a}	76.32 ± 0.40^{a}	16.09 ± 0.68^{b}
Pepeta	76.00 ± 0.01^{a}	0.49 ± 0.10^{ab}	75.51 ± 0.10^{a}	13.43 ± 2.36^{ab}
Raw rice	80.70 ± 5.69^{a}	2.93 ± 0.12^{c}	77.78 ± 5.57^{a}	12.41 ± 0.52^{ab}
Cooked rice	73.78 ± 3.92^{a}	0.52 ± 0.10^{ab}	73.26 ± 3.91^{a}	11.21 ± 1.23^{a}

*80, 100 and 120 are roasting temperatures (°C), NS – not soaked, S – soaked in cold water for 12 h before roasting, DAH – days after 50% heading, dwb – dry weight basis. Values in each column with different superscripted letters are statistically different ($p \leq 0.05$) for each maturity. [#]Significance difference between maturity levels ($p \leq 0.05$). Data expressed as mean ± standard deviation of three replicates.

4.3.4 Microstructure properties of processed rice products

To visualize the structure of the endosperm cells of the rice samples, confocal laser scanning microscopy (CLSM) was used (Fig. 4.4 Panels a – i). An intact cell wall structure (staining of the cell walls with light blue) was observed in raw rice flour samples (Fig. 4.4 Panel g), which was used as a reference sample for native structure in this study. This resembles previous findings in raw rice flour.[7] Fig. 4.4 (Panel h) shows partial disruption of the cell wall profile in *pepeta*, indicating possible mechanical damage due to the pounding and milling process. Cooked rice (Fig. 4.4 Panel i) showed complete loss of endosperm cell wall structure, which was used as a reference sample for maximum disruption. This was expected because rice

grains lose their structure when cooked in excess boiling water until fully gelatinized.[7] Intact cell walls were observed in roasted samples (Fig. 4.4 Panels a – f) even after mechanical milling of samples into flour particles (425 – 250 μm), with no noticeable differences in cell wall integrity among different roasting temperature, and between soaked and corresponding unsoaked samples. Change in endosperm cell walls profiles was observed in roasted samples compared to raw rice and related to increased cell volume with a decrease in elongation, possibly due to swelling and expansion of starch granules as it gelatinizes during roasting.[50] Soaking before roasting did not affect cell volume compared to roasted samples due to a slight moisture gradient between initial moisture content (fresh unsoaked immature paddy, 33-41%) and saturated moisture content (soaked immature paddy, 48-50 %) after cold water soaking, as the diffusion of water during soaking is governed by the moisture gradient between the surface of the grain and the center (starchy endosperm).[53,68] It is worth noting that when rice samples were cooked in excess boiling water, they reached a moisture content up to 82 %, resulted in rapturing of the cell wall as the consequences of the starch gelatinization process due to excessive water absorption.

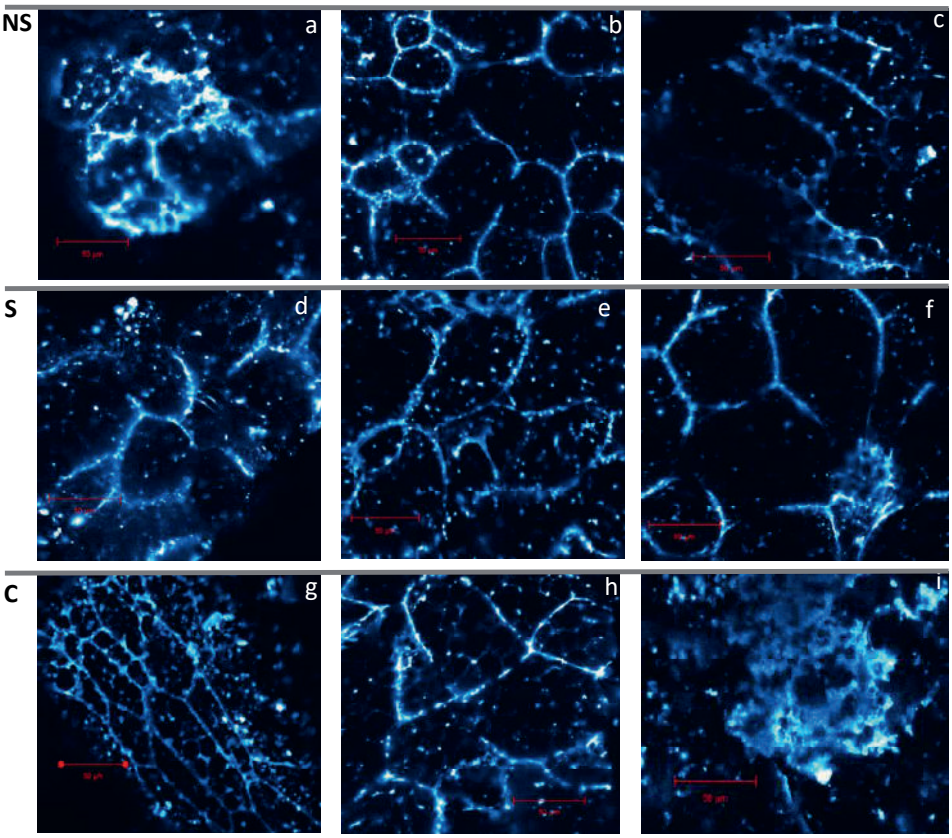


Fig. 4.4: Confocal images showing cell walls of rice flour particle (425 – 250 µm) for roasted rice at 80 °C (a, d), 100 °C (b, e), and 120 °C (c, f), raw rice (g), *Pepeta* (h) and cooked rice (i) squeezed through 425 µm sieve. NS – not soaked (a, b, c), S – water-soaked rice at room temperature for 12 h prior roasting (d, e, f), C – control samples (g, h, i). Cell walls were stained in light blue. Micrographs were taken using 40x objective lens. Scale bar is 50 µm.

4.3.5 Impact of rice processing on in-vitro starch and protein digestibility

4.3.5.1 Starch hydrolysis

Fig. 4.5 reports starch digestograms of the processed rice samples. The digestograms follow first-order kinetic equation (except raw rice), like those reported in previous studies.[7,28,69] All processed rice products exhibited a faster and higher extent of starch hydrolysis than raw rice (Fig. 4.5 Panels a, b, e). However, soaking showed inconsistent trends (Fig. 4.5 Panels c, d, f). The C_∞ and k values estimated by fitting the experimental data to the first-order equation justifies the observed trend of the digestograms (Table 4.4). The C_∞ and k values differ

significantly among rice products, raw rice showing the lowest values than processed rice products given the substantial amount of resistant starch type I and II.[70,71] The starch digestibility in roasted samples (except k value at 18 DAH) was lower than in cooked rice, possibly due to less damaged endosperm cell wall structure in roasted samples than cooked rice (section 4.3.4). Besides, aggregation of proteins (section 4.3.6), perhaps also favored by Maillard reaction, may have made starch granules more difficult to attack by amylase. Lower starch digestibility in dry heat parboiled rice compared to wet heat parboiled rice has been reported in a previous study,[72] indicate possible reduction of the glycemic index when consumed.

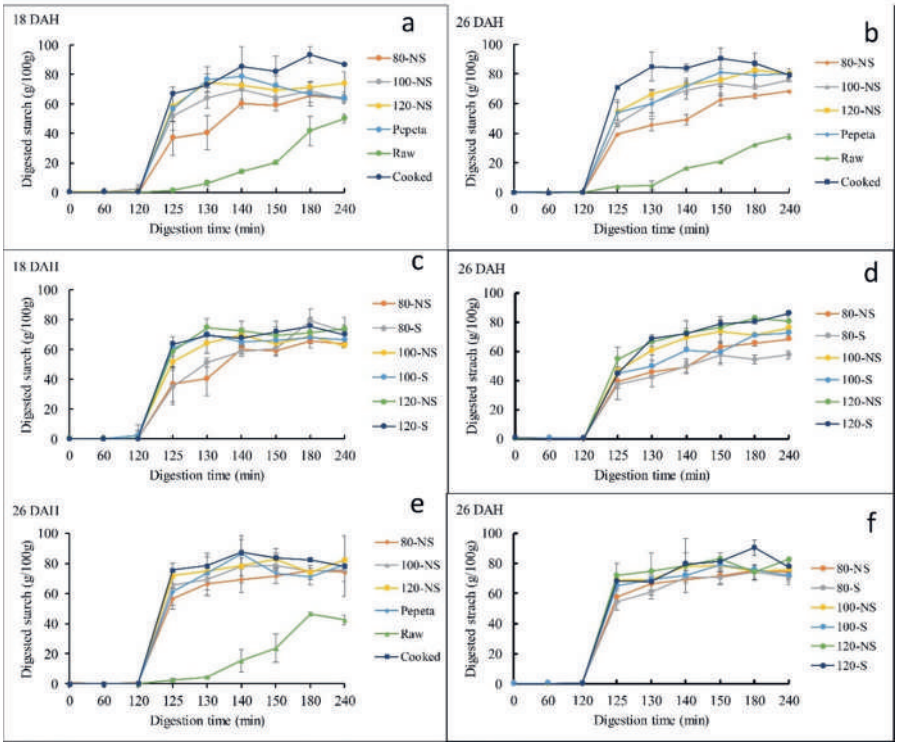


Fig. 4.5: Digestograms of starch enzymatic hydrolysis *in-vitro* for TXD306 flour (a – d) and starch (e, f) samples as a function of processing conditions (a, b, e) and soaking prior roasting effect (c, d, f). 80, 100 and 120 are roasting temperatures (°C), NS – not soaked, S – water-soaked rice at room temperature for 12 h prior roasting, *Pepeta* – locally prepared rice flakes, Raw – unprocessed rice, Cooked – cooked rice, DAH – days after 50% heading (maturity level). Error bar represent standard deviation for means of two replicates.

Roasting conditions significantly affected C_∞ and k among roasted products, increasing as the roasting temperature increased. The results could be due to a lower extent of gelatinization at lower roasting temperatures.[72,73] Though not significantly, soaking before roasting slight increased C_∞ and k values (except k value at 80 °C, 18 DAH) compared to roasting without soaking for 18 DAH, whereas no consistency trend was observed at 26 DAH for both flour and starch samples. Though the same trend in increasing starch digestibility as the roasting temperature increase was observed in isolated starch, the isolated starch samples had higher C_∞ and k (except k value of raw rice at 26 DAH) compared to flour samples. This result suggests a differential effect on the starch rather than on cell walls is one reason for the lower digestibility at a lower temperature. The rate constant (k) differed significantly between maturity levels, showing high values at 18 DAH compared to 26 DAH. This contrast to the pervious study, suggested both C_∞ and k values of cooked rice were not influenced by maturity level.[7] The discrepancy could be due to high extent of starch gelatinization in 18 DAH samples during roasting as a result of high initial moisture content of paddy grains compared to 26 DAH, associated with an increased enzymatic starch digestibility.[72,73] Except for C∞ for flour samples at 26 DAH, no interaction effect was observed between soaking and roasting.

Table 4.4: Estimated *in-vitro* digestion parameters for starch and protein of TXD306 rice variety at different maturity and processing conditions.

Treatment	Starch digestion: Flour		Starch digestion: Starch		Protein digestion[#]	
	C_∞ (g/ 100 g, dwb)	$k^{\$}$ (min^{-1})	C_∞ (g/ 100 g, dwb)	k (min^{-1})	Gastric (g/ 100 g, dwb)	Intestinal (g/ 100 g, dwb)
18 DAH						
80-NS*	63.25 ± 3.85^{ab}	0.16 ± 0.09^{ab}			2.51 ± 1.31^{a}	69.60 ± 6.75^{bc}
80-S	70.87 ± 2.91^{bc}	0.12 ± 0.05^{ab}			13.9 ± 1.79^{b}	64.97 ± 9.71^{bc}
100-NS	66.25 ± 5.85^{ab}	0.33 ± 0.10^{bc}			7.46 ± 5.10^{ab}	79.09 ± 3.61^{c}
100-S	67.17 ± 0.02^{ab}	0.48 ± 0.02^{c}			6.15 ± 0.39^{ab}	51.65 ± 6.91^{a}
120-NS	70.07 ± 0.78^{bc}	0.39 ± 0.09^{bc}			3.27 ± 0.31^{a}	69.08 ± 3.92^{b}
120-S	73.46 ± 0.08^{bc}	0.40 ± 0.01^{bc}			10.3 ± 1.19^{ab}	54.01 ± 2.20^{ab}
Pepeta	71.60 ± 7.41^{bc}	0.37 ± 0.11^{bc}			1.26 ± 0.06^{a}	58.76 ± 4.38^{abc}
Cooked rice	85.90 ± 7.44^{c}	0.28 ± 0.07^{abc}			3.73 ± 4.17^{a}	77.85 ± 3.91^{c}
Raw rice	51.27 ± 4.47^{a}	0.03 ± 0.01^{a}			1.99 ± 0.95^{a}	41.15 ± 1.17^{a}
26 DAH						
80-NS	63.38 ± 0.75^{bc}	0.14 ± 0.02^{ab}	72.23 ± 0.05^{b}	0.30 ± 0.04^{a}	14.7 ± 6.01^{a}	60.76 ± 7.50^{bc}
80-S	55.13 ± 1.39^{b}	0.20 ± 0.09^{bc}	70.85 ± 4.10^{ab}	0.26 ± 0.02^{a}	7.62 ± 4.22^{a}	48.80 ± 4.72^{ab}
100-NS	72.67 ± 3.23^{cd}	0.19 ± 0.01^{ab}	75.83 ± 2.63^{b}	0.38 ± 0.08^{a}	4.82 ± 4.62^{a}	75.27 ± 3.85^{cd}
100-S	67.75 ± 2.83^{c}	0.12 ± 0.02^{ab}	73.99 ± 1.27^{b}	0.41 ± 0.11^{a}	2.77 ± 0.37^{a}	60.63 ± 4.24^{bc}
120-S	77.84 ± 1.90^{cd}	0.23 ± 0.05^{bc}	78.78 ± 5.27^{b}	0.47 ± 0.14^{a}	4.11 ± 1.81^{a}	51.63 ± 4.52^{b}
120-S	80.72 ± 1.98^{d}	0.17 ± 0.04^{ab}	80.55 ± 3.56^{b}	0.32 ± 0.03^{a}	3.52 ± 0.38^{a}	46.91 ± 3.90^{ab}
Pepeta	78.06 ± 3.31^{d}	0.19 ± 0.05^{b}	77.25 ± 1.87^{b}	0.34 ± 0.13^{a}	4.49 ± 3.29^{a}	58.16 ± 6.04^{b}
Cooked rice	85.59 ± 4.31^{d}	0.36 ± 0.01^{c}	82.24 ± 8.86^{b}	0.82 ± 0.64^{a}	15.0 ± 3.74^{a}	89.95 ± 3.37^{d}
Raw rice	41.19 ± 3.01^{a}	0.03 ± 0.01^{a}	51.81 ± 7.81^{a}	0.02 ± 0.01^{b}	3.12 ± 3.57^{a}	31.56 ± 4.12^{a}

*80, 100 and 120 are roasting temperatures (°C), NS – not soaked, S – soaked in cold water for 12 h before roasting, [#]End of gastric and intestinal digestion, DAH – days after 50% heading, dwb – dry weight basis, C_∞ – equilibrium hydrolysis, k – rate constant, Values in each column bearing different superscripted letters are statistically different ($p \leq 0.05$) for each maturity. [$]Significance difference between maturity levels. Data expressed as mean ± standard deviation of two replicates.

4.3.5.2 Protein hydrolysis

Fig. 4.6 shows *in-vitro* hydrolysis of protein in the different rice products. Only a limited protein hydrolysis was achieved after gastric digestion, as previously reported for other plant-food products.[70,71] Rice processing significantly increased the protein hydrolysis; The lowest and the highest intestinal digestion values were observed in raw rice and cooked rice, respectively (Fig. 4.6 Panels a – b, Table 4.4). The low protein digestibility of raw rice is due to intact cellular structure (section 4.3.4), which hinder proteolytic enzymes to easily interact with substrates.[70,71]

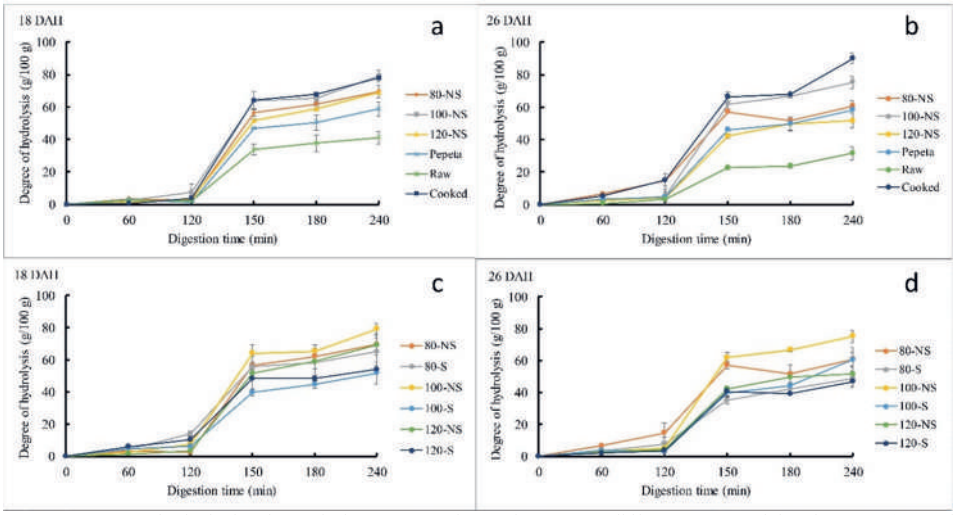


Fig. 4.6: *In-vitro* hydrolysis of protein in TXD306 rice variety at two different stages of development (18 and 26 DAH – days after 50% heading) as affected by different processing conditions (a, b) and soaking before roasting (c, d). 80, 100 and 120 are roasting temperatures (°C), NS – not soaked, S – water-soaked rice at room temperature for 12 h prior roasting, *Pepeta* – locally prepared rice flakes, Raw – unprocessed rice, Cooked – cooked rice. Error bar represent standard deviation for means of two replicates.

Dry roasting increases the digestibility of rice proteins, but protein digestibility in roasted samples is lower than that of cooked rice, contrary to what was reported in our previous work.[7] This inconsistency could be due to different particle size of the flour samples between the two studies: *pepeta* samples were pounded and sieved through a 425 µm sieve in the previous study.[7] In this study, roasted samples (including *pepeta*) were standardized between

425 – 250 µm while cooked rice grains were squeezed through 425 µm sieve (section 4.2.2). Besides, the lack of cell wall structure in cooked rice particles (Fig. 4.4) could be why the observed high protein digestibility compared to roasted samples. Fig. 4.6 and Table 4.4 also indicates the mild roasting (80 to 100 °C) increased protein digestion compared to raw rice due to loss of tertiary structure,[74] allowing exposure of peptide bonds to proteases. However, further increase in roasting temperature (at 120 °C) decreased the protein digestion, possibly due to highest formation of disulphide bonds (Fig. 4.7 Panel b). Furthermore, the intense brown color at 120 °C (Fig. 4.1 Panel A) suggests the formation of less digestible brown nitrogenous polymers, i.e., the melanoidins[75,76] through Maillard reaction. Though soaking before roasting had no significant effect on the digestion of protein (except at 100 °C, 18 DAH), lower values at the end of intestinal digestion were observed in soaked products (Fig. 4.6 Panels c, d) compared to unsoaked products, possibly due to slightly high disulphide bonds in soaked products (Fig. 4.7 Panel b). Maturity level had no significant effect on the digestibility of protein between 18 and 26 DAH. Except for gastric digestion of protein at 18 DAH, no interaction effect was observed between soaking and roasting.

4.3.6 Effect of heat treatment on rice protein aggregations

To gain a thorough understanding of the changes in the digestibility of rice protein upon roasting (section 4.3.5.2), the effect of heat treatment on the heat-induced protein interactions and the relationship among heat-induced interactions and protein digestibility (Table 4.5) were investigated. The heat-induced protein interactions were monitored by comparison of protein solubility in different solvents meant to break specific physical interactions so that the differences indicate the contribution of those interactions to the gain in protein solubility.[23] The analysis of free-thiol content and total thiol content (Fig. 4.7 Panel c), and surface hydrophobicity (Fig. 4.7 Panel d) were used to validate the protein solubility results.

As shown in Fig. 4.7 Panels a – b, hydrophobic interactions and hydrogen bonds were involved in maintaining the structure of rice protein because the solubility in raw rice sharply increased in S2 and S3 solvent. Simultaneously, disulphide cross-linking plays a less important role, confirming the relative insolubility of rice proteins.[24] The protein solubility in PBS (solvent S1) was significantly higher in raw rice and *pepeta* than roasted products and decreased with roasting temperature. Technically, the protein fractions soluble in the PBS buffer should be the albumin and globulin fractions, suggests that part of the albumin and globulin becomes insoluble after heat treatment,[77] whereas the mechanical pounding immediately after roasting

Table 4.5: The relationship between protein digestibility of rice subjected upon heat treatment and heat induced protein interactions at 26 DAH

Protein-protein interactions	Gastrointestinal protein digestion	
	Pearson correlation	*P*-value (2-tailed)
Native protein solubility	-0.571	**0.021**
Hydrophobic interactions solubility	0.066	0.809
Hydrogen bonds solubility	0.462	0.072
Disulfide bonds solubility	-0.206	0.326
Free thiols content	-0.421	0.105
Total thiols content	-0.461	0.072
Surface hydrophobicity	-0.620	**0.010**

P values marked in bold fonts are significantly different ($p < 0.05$)

during *pepeta* preparation could influence the solubility of albumin and globulin fractions. The sharpest increase in protein solubility was observed at 80 °C (55 – 58%) compared to raw rice (36%) sample in solvent S2, indicating aggregations of proteins by hydrophobic interactions. The increase in protein solubility in solvent S2 was lower at 100 (25 – 37%) and 120 °C (9 – 11%) compared to 80 °C. Moreover, surface hydrophobicity (Fig. 4.7 Panel d) increased significantly with roasting temperature, indicating the unfolding of proteins that

expose hydrophobic parts of protein molecules. This may have facilitated the formation of aggregates through hydrophobic interactions or S-S bonds in roasted samples.[77–79] However, the gain in protein solubility with solvent S2 was higher in samples roasted at lower temperatures suggesting that hydrophobic interactions were more critical in those samples. The protein solubility further increased in solvent S3 and S4, whereas a significant increase by 26% was observed in solvent S4 at 120 °C (Fig. 4.7 Panel b), suggesting disulphide bond formation. In addition to this observation, the decrease of total thiol content after roasting suggests that some of the -SH moieties become irreversibly engaged in covalent bonds, suggesting the formation of covalently cross-linked aggregated proteins. However, we did not observe any clear effect of roasting temperature nor any obvious relation with protein digestibility (Table 4.5). Though soaking had no effect (except total thiols content at 80 °C) on protein interactions, slightly high values at lower roasting temperatures (80 and 100 °C) in free thiols content, surface hydrophobicity and hydrophobic interactions were observed in soaked samples compared with corresponding unsoaked samples. By contrast, high values of disulphide bonds in soaked samples were observed in high temperatures (120 °C) as well. No interaction effect was observed between soaking and roasting except on free and total thiols contents. The solubility of *pepeta* protein was similar to that of roasted rice at 100 °C. This was expected as *pepeta* was roasted at a temperature ranging from 80 – 120 °C (section 4.2.2). In general, the results indicate disulphide bonds played a vital role in heat-induced protein interactions of rice protein. However, only the native protein conformation and surface hydrophobicity negatively correlated significantly with protein digestibility (Table 4.5).

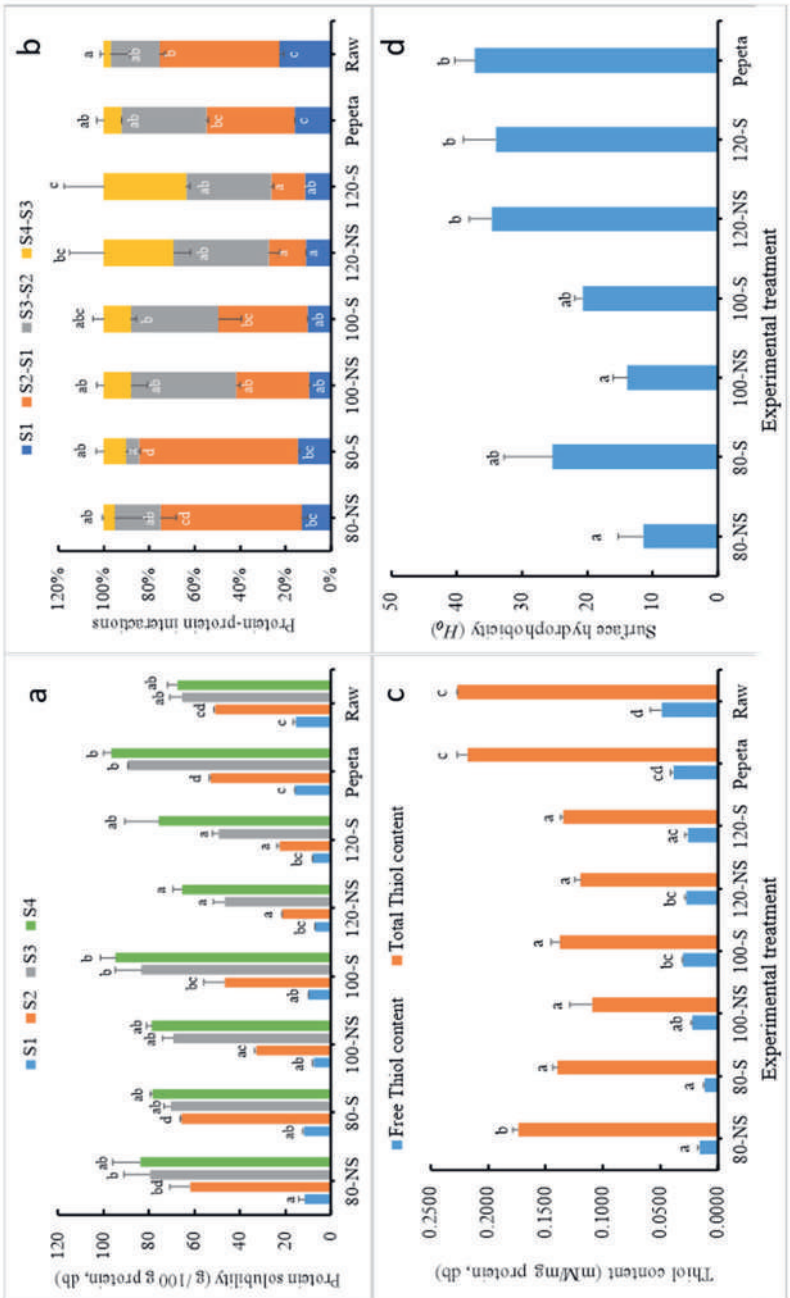


Fig. 4.7: Protein aggregations of rice protein subjected to different heat treatment conditions: (a) soluble protein recovery in 4 different solvents (S1: 0.1 M PBS (pH 7.5), S2: S1 + 2% SDS (pH 7.5), S3: S2 + 6 M urea (pH 7.5), S4: S3 + 1% dithiothreitol (pH 7.5)); (b) changes in protein-protein interaction forces (S2-S1= hydrophobic interactions, S3-S2 = hydrogen bonds, S4-S3 = disulfide bonds); (c) Thiol content; and (d) protein surface hydrophobicity. 80, 100 and 120 indicate rice roasted at the corresponding temperatures (°C), NS – water-soaked rice at room temperature for 12 h prior roasting, *Pepeta* – locally prepared rice flakes, Raw – unprocessed rice, db – dry basis. Labels with different letters indicate a statistically significant difference ($p \leq 0.05$) across treatment conditions for each parameter. Each bar chart represents mean and standard deviation of two replicates. Positive error bars in (b) represent disulfide bonds, while negative error bars represent hydrophobic interactions and hydrogen bonds accordingly.

4.4 Conclusion

This study explored the effect of processing conditions to optimize the nutritional quality and digestibility properties of immature cereal-based products such as *pepeta*, a processed immature rice flake typically consumed in Tanzania. The increase in riboflavin, nicotinamide and iron in both locally and laboratory simulated *pepeta* products provide evidence of its potential nutritional benefits. The dietary fiber transformation from insoluble to soluble fiber was linked with an increase in roasting temperature, showing *pepeta* processing technology can enhance fiber functionality of immature rice-based products. In the same vein, the increase of starch digestibility with the severity of the processing conditions among roasted products indicates the possibility of manipulating roasting temperature to produce immature rice-based products with a more favorable glycemic response than cooked rice. On the contrary, the optimum roasting temperature is required for maximum protein digestibility due to observed different patterns as processing conditions changes. According to the data from this study, roasting at 100 °C is the optimum temperature to produce *pepeta* of high-quality digestible protein and low starch digestibility, both of which have health benefits. The digestibility of starch and protein was not affected by soaking before roasting, but the vitamin and mineral profile improved when a soaking step was introduced. Soaking before roasting at 120 °C is best for retaining micronutrients such as iron, riboflavin and nicotinamide. Though maturity had inconsistence effect on some of the assessed nutritional quality, a 26 DAH is recommended over 18 DAH when a low starch digestibility due to low hydrolysis rate constant (k) is considered. Besides, the 26 DAH would minimize rice yield loss as unfilled grains due to harvesting of immature rice, but this need further evaluation. In general, the study indicates that current *pepeta* processing technology can ensure a product with good nutritional properties but that the process can be optimized to further improve its nutritional quality.

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Conflict of interest

The authors declare that they have no conflict of interest.

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5

CHAPTER 5.

Degree of maturity and dry-heat processing affect visual quality and volatile profile of roasted immature rice grains

This chapter has been submited to:
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Abstract

Foods from unripe cereals, such as flakes prepared in Tanzania from immature rice, are popular as they are available before the main harvest. However, the factors determining the sensory quality of the rice flakes are inadequately characterised, although this is critical for processors. In this study, grain size, colour, and volatile compounds of immature rice destined for so-called *pepeta* production were investigated at different maturity stages (i.e., dough-grain, mature-grain, fully-ripe, and over-ripe) and processing conditions (i.e., roasting at 80, 100 and 120 °C, with and without prior water-soaking for 12 h at room temperature). The highest brightness and lowest redness and yellowness values in *pepeta* resulted from the pounding process despite a decrease in brightness and an increase in red and yellow pigments upon roasting. In total, 53 volatile compounds were identified in Lawama and TXD306 variety; their concentrations decreased as grain matured except for Lawama at the mature-grain stage. Odour-active compounds such as 2-isopropyl-5-methyl-1-heptanol, butyl acetate, and butyl propionate were formed upon processing into *pepeta*, whereas abundant 2-pentyl-furan and 2-methoxy-4-vinyl phenol significantly increased with roasting temperature. No characteristic volatile pattern was observed to differentiate *pepeta* from other processed rice products. Soaking before roasting had no significant effect on grain size, colour, or volatile compounds, implying that this common practice of overnight storage had no detrimental effect on the end product. Degree of maturity and roasting temperature both impact the colour and volatile compounds, and subsequent sensory quality of the pepeta product, but overnight soaking does not.

Keywords: immature rice; *pepeta*; grain dimensions; volatile compounds; Lawama; TXD306.

5.1 Introduction

Rice is chiefly consumed as whole grains after milling, a crucial primary processing operation of paddy grains. The processing entails dehulling, i.e., removing the hard outer layer (husk) to produce brown rice grains, followed by polishing, i.e., removal of the soft bran layer to obtain white rice grains.[1] The milling process affects the visual properties, such as the colour and size of the rice grains[1–3], which consumers perceive as essential indicators of the quality of milled rice, thus impacting the commercial value. Translucent white and head rice are preferred in most markets, and their price is almost triple that of the broken and grey or yellow grains[2,3]; head rice is the name used for milled rice grains having three quarters or more of their length before milling.[2] The milling process also affects the volatile profiles of rice grains by removal of the lipid-rich bran layer leaving the endosperm with lower levels of lipid oxidation products:[4,5] puffed corn flavour, raw rice flavour, wet cardboard flavour, hay-like flavour, and bitter taste are reduced while glossiness, plumpness, and sweet taste are increased with the degree of milling.

The effect of thermal treatment on volatile profiles and the colour of cereal-based products has been well documented. The loss of volatiles by vapourisation, the interaction of volatiles with the gelatinised matrix and lipid oxidation all contribute to changes in volatile profiles,[6–9] whereas non-enzymatic browning such as Maillard and caramelisation reactions are the major causes of changes in both colour and volatile profiles of processed cereal products.[10–13] Besides, several authors studied the effect of agronomic factors such as temperature in the field,[14,15] growth regulators and fertiliser applications,[16–18] soil salinity,[19,20] and maturity level[21] on the volatile profile of rice.

Heat processing of immature paddy rice such as *pepeta* has been associated with changes in visual and volatile profiles of processed end products. *Pepeta* processing consists of roasting fresh immature cleaned paddy grains, followed by immediate pounding to produce dehusked

and flattened grains called *pepeta*.[22,23] *Pepeta* processors use the change of grain colour to determine the optimum maturity to harvest rice for *pepeta* processing and to terminate the roasting process. Moreover, consumers use the colour of *pepeta* to distinguish the product from that prepared from mature rice grains. Consumers prefer a white-greenish *pepeta* product with a popcorn flavour from immature aromatic rice varieties over *pepeta* prepared from mature non-aromatic rice.[22]

To date, literature is lacking about how the visual properties and volatile profile of immature rice are related to *pepeta* processing. To gain the understanding that processors need to optimize their practices, grain maturity must be considered as it is linked to grain dimensions, colour, and volatile compounds.[21,24,25] The current study was performed to assess the effect of the maturity of rice on the visual properties (i.e., grain dimensions and colour) and volatile compounds of *pepeta* made from two Tanzanian rice varieties, namely Lawama and TXD306. TXD306 was also used to evaluate the effect of different soaking and roasting conditions during *pepeta* processing on visual properties (i.e., grain dimensions and colour) and volatile compounds of *pepeta*. Both paddy grains and polished milled rice were evaluated.

5.2 Materials and methods

5.2.1 Sample collection and preparation of rice-based products

Rice samples were collected in two phases following the rice production calendar of Kilombero and Ulanga districts in Tanzania (Fig. 5.1). The first phase was in the 2017/2018 production season. Samples of Lawama and TXD306 varieties were harvested according to the common practices of *pepeta* processors and rice farmers. Both are hybrid varieties commonly used for *pepeta* production.[22] Having a short growth cycle, the varieties can be cultivated twice annually, which guarantees the availability of immature rice grains. The collected samples were categorised into four maturity stages as described in our previous

study:[23] dough-grain stage (DGS, 15–21 days after 50% heading (DAH)), mature-grain stage (MGS, 22–28 DAH), fully-ripe stage (FRS, 29–35 DAH) and over-ripe stage (ORS, 36–43 DAH). The grains harvested before 29 DAH (DGS and MGS) were considered immature grains.[25]

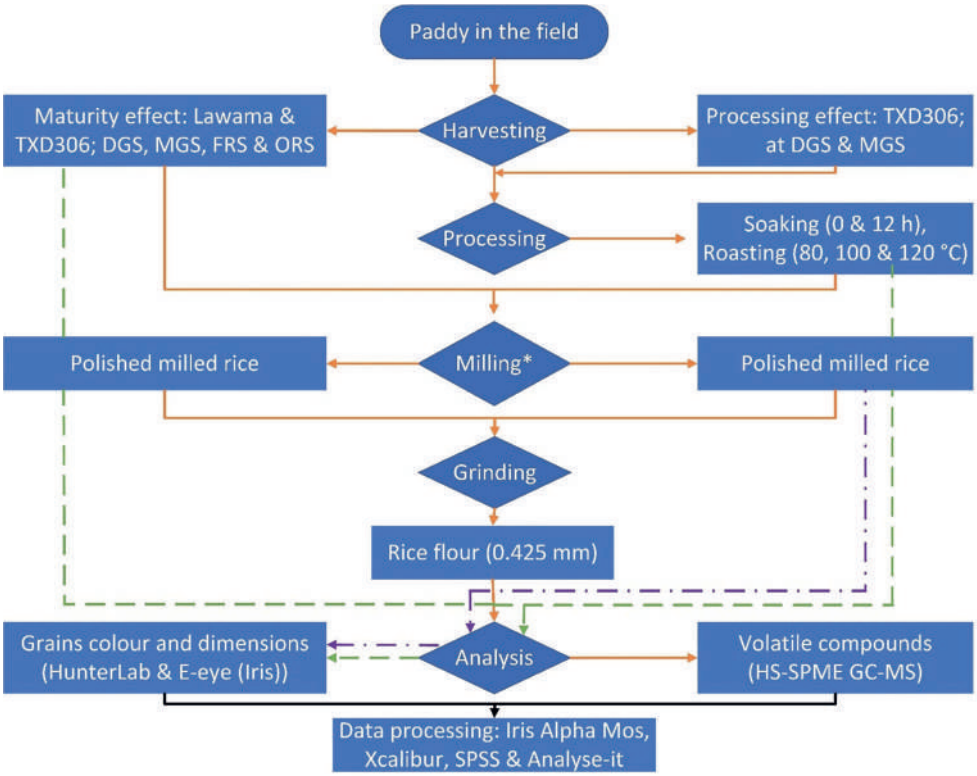

Fig. 5.1: Sampling and experimental plan of the study. Lawama & TXD306 – rice varieties, DGS – dough grain stage (15 – 21 days after 50% heading (DAH)), MGS – mature grain stage (22 28 DAH), FRS – fully ripe stage (29 – 35 DAH), ORS – over ripe stage (36 – 43 DAH). *Refer to dehusking and polishing of paddy grain.
– – – indicates grain colour and dimension analysis for paddy samples
– · – · indicates grain colour and dimension analysis for milled rice samples
——— indicates volatiles analysis for rice flour samples

The second collection phase concerned immature TXD306 rice, harvested at 18 and 26 DAH at Ulanga district during the 2018/2019 rice production season. Half of the harvested grains

were locally processed into *pepeta* by manually roasting the paddy grains on an open wood fire (181 – 270 °C) for 3 – 8 min, followed by immediate pounding to obtain the flattened grains after cleaning, as detailed in our previous study.[23] From both collection phases, portions of 250 g of harvested rice grains and *pepeta* were vacuum sealed (Princess®, S-492967-001, China) in plastic bags and temporarily stored at 4 °C before shipping to the Food Quality and Design (FQD) laboratory at Wageningen University, The Netherlands, where the samples were stored at -20 °C for further use.

Two-thirds of the second half of the grains harvested during the second collection phase were processed into various rice-based products, reproducing *pepeta* processing technology[22,23] under various cold-water soaking (0 and 12 h) and roasting temperature (80, 100 and 120 °C) treatments for 8 min using a hot air fluidised roaster (Toper, Optical Coffee Roaster, Turkey). All remaining material was dried to a moisture content of 11 – 13 % using a hot air dryer (TG 200, Retsch GmbH, Haan, Germany) at 50 °C and 90 fan power (i.e., an airflow approximating 185 m^3h^{-1}). Both roasted and dried samples were cooled at room temperature for approximately 6 h. Next, portions of about 100 g were 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. Samples were stored at -20 °C for further analysis. The processing treatments were then coded, indicating roasting temperature (°C) and soaking time (h), e.g., 80-0 means roasted at 80 °C and 80-12 means soaked for 12 h before roasting at 80 °C. In this study, three control samples from 16 and 26 DAH variety TXD306 were included: Fresh – undried paddy, Raw – dried paddy or polished milled rice, and *Pepeta* – locally prepared rice flakes.

5.2.2 Grain colour and dimension analysis

An electronic eye analysis of paddy and polished rice grains was performed using an IRIS VA400 visual analyser (Alpha MOS, France) for a detailed visual assessment of both colour and shape parameters. A picture of the sample surface (2 g for colour, and random but separately placed 50 whole grains for shape parameters) was taken using a high-resolution charge-coupled device camera (25 mm lens) with top and bottom-controlled white lighting conditions. The image background was filtered by adjusting colour threshold values (red, green, blue, RGB) of the pre-processing method (AlphaSoft software, v16) before further analysis. Shape parameters like area, height (i.e., length of the longitudinal line), and width (i.e., length of the dorsiventral line) were estimated using the "shape descriptors" function available in the software. The software modelled each grain as an ellipse E from which the major axis (longitudinal line) and minor axis (dorsiventral line) were extracted, so that shape descriptors (area, height and width) of each grain were calculated from the image taking into account the grain geometry and the variations to translation, rotation and change of scale of the grain in the image. The dimension descriptors represent the number of pixels of the grains and are unitless. For colour visualisation, the pre-processed images were decomposed in a 4096 colour spectrum, with the proportion of each colour in percentage. All analyses were performed at least in triplicate for each maturity stage and processing condition. For averaged colour values, HunterLab (CX2189, ColorFlex EZ®) was used to measure colour by illuminating 2 g of each sample (paddy and polished rice grains) using a xenon flash lamp. The component wavelengths (400-700 nm) of the reflected light from the sample were analysed to produce numeric L^*, a^*, and b^* colour values: L^* describes brightness from black (0) to white (100), a^* describes the red-green colour with positive values indicating redness and negative values indicating greenness, b^* describes the yellow-blue colour with positive

values indicating yellowness and negative values indicating blueness.[1] All analyses were carried out in triplicate.

5.2.3 Analysis of volatile organic compounds

Headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME GC–MS) was used to identify volatile organic compounds (VOCs) of polished rice flour (0.425 mm) samples. Briefly, 2 g of the sample was weighed in a 20 mL headspace (HS) vial and blank vial, crimped and incubated at 80 °C for 40 min. Subsequently, the vials were extracted for 25 min by 2 cm SPME DVB/CAR/PDMS fibre (Supelco, Sigma Aldrich, USA) and desorbed for 10 min in the GC. The extracted VOCs were chromatographed by using a stabilwax DA capillary column (30 m x 0.25 mm ID 0.25 μm thickness) with helium as the carrier gas at a constant flow rate of 1 mL min^{-1} in splitless mode. The oven temperature conditions for SPME injection were 40 °C for 2 min, then increased with 10 °C min^{-1} to 200 °C and next held at 200 °C for 5 min. Electronic ionisation at 70 eV was used; ion source and transfer line temperatures were 225°C and 260°C, respectively. Detection was performed in scan mode from 50 to 650 amu.

5.2.4 Data processing and statistical analysis

The grain dimensions (area, height and width) and colour (*L*a*b** values) data were subjected to one-way analysis of variance (ANOVA) with Tukey's post-hoc test at $p < 0.05$ using IBM SPSS® software to ascertain statistical differences among maturity stages and processing conditions. Data were treated independently for each maturity stage and processing treatment. To evaluate the effect of variety (i.e., Lawama and TXD306) and maturity on processed products, data were pooled together irrespective of their treatments. Two-way ANOVA was computed, treating “roasting” as a fixed effect and “soaking” as a

random effect to assess interactions between soaking and roasting conditions. For the GC-MS results, the Xcalibur 2.2 software (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to process the data. The VOCs were identified by comparing their mass spectra and relative abundances (similarity >80%) with the NIST spectral library database, and the retention indexes with those reported in the literature. Besides, the identified VOC peaks were blank corrected for SPME fibre and vial contaminants.[26,27] The VOC content was expressed as a percentage of a volatile peak area to the total sample peak area. The individual VOC and colour spectrum (percentage of the different visible colours by Iris Alpha Mos) data were further subjected to a nonparametric Kruskal-Wallis test ($p < 0.05$) to compare the similarity of distributions among experimental groups (i.e., maturity stages and processing conditions) since some of the data were not normally distributed (Shapiro–Wilk test < 0.05). Only significantly different colour spectra (Table 5.S1) and individual VOCs (Table 5.S2) were used to build the Principal Component Analysis (PCA) plots by the Analyse-it software (version 5.680.7620.32918, Addinsoft, New York, NY, USA) for Microsoft Excel® to compare sample information (maturity stages and processing conditions). The aligned rank transformation for nonparametric factorial ANOVA was performed using the ARTools software for Windows (version 2.1.0, University of Washington, Seattle, USA) to examine the main effect and interactions between roasting and soaking for individual VOCs.[28]

5.3 Results

5.3.1 Grains dimensions

Grain dimension descriptors (area, height, and width) changed during grain maturation, as shown in Table 5.1. Though the descriptors did not change significantly in TXD306 paddy grains, area and height slightly increased, whereas width decreased during grain development. This agrees with Fu *et al.*[29], who reported no significant differences in rice seed dimensions

during seed development after 15 DAH. By contrast, all descriptors in Lawama decreased (except at MGS) as paddy grains matured. Height and width showed significant highest (309) and lowest (85.7) values at MGS and ORS, respectively. This discrepancy could be due to differences in environmental factors and field management between farmers[30] as samples were collected from different farmers (section 5.2.1). No significant changes were observed between Lawama and TXD306 varieties.

Fig. 5.2 indicates the effect of rice processing on grain dimensions in TXD306 at 18 and 26 DAH. Processing significantly increased grains dimensions compared to raw rice; the sharpest increase in dimension values was observed in *pepeta*, probably due to the effect of pounding during *pepeta* processing.[23] The higher dimension values observed in roasted rice grains compared to raw rice could be due to changes in physical properties, including expansion of starchy endosperm resulting from gelatinised starch during roasting, which upon cooling, makes the starchy endosperm hard enough to withstand harsh milling conditions.[31] It is important to note that roasted and raw paddy grains were subjected to dehusking and polishing conditions (section 5.2.1). However, no significant change in dimension values (except area and height at 18 DAH) was observed among roasted products nor soaked products (except width at 120°C, 26 DAH) (Fig. 5.2). Though maturity (18 and 26 DAH) did not affect grain dimensions, an interaction effect between soaking and roasting was observed in grain dimensions for polished rice samples, i.e., a *P*-value of 0.005 (height) and 0.011 (width) at 18 DAH, whereas 0.000, 0.001 and 0.004 for area, height and width at 26 DAH, respectively.

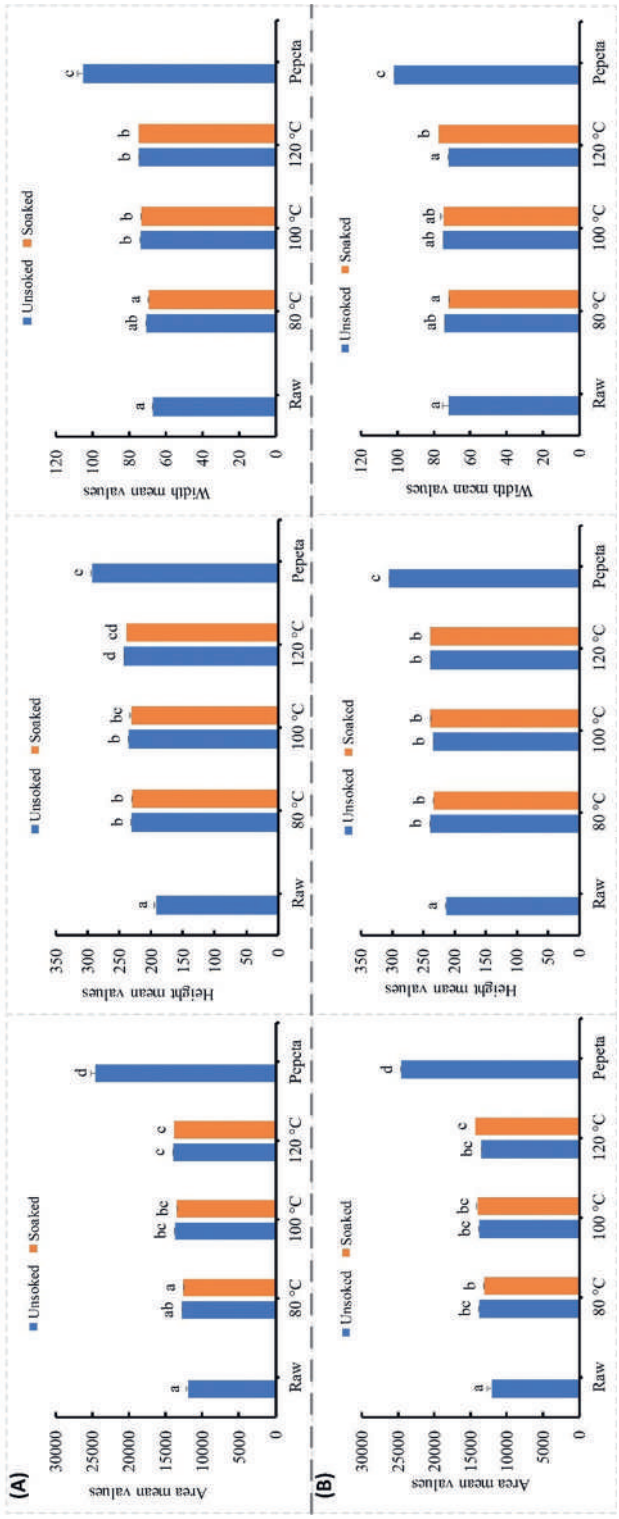

Fig. 5.2: Variability of dimension descriptors (area, height and width) of TXD306 rice at 18 days after 50% heading, DAH) (A) and 26 DAH (B) by different processing conditions. 80, 100 and 120 °C = roasting temperatures, *Pepeta* = locally prepared rice flakes, Raw = unprocessed rice, Soaked = water soaked rice at room temperature for 12 h prior roasting. Bar charts with different letters indicate a statistically significant difference ($p \leq 0.05$) among processing conditions for each dimension descriptor. Each bar chart represents mean and standard deviation of two replicates with 50 grains each. The dimension descriptors (area, height and width) represent the number of pixels of the object, are unitless and have no real physical significance but are used for comparison.

Table 5.1: Colour (*L* a* b** values by HunterLab) and dimension (Iris Alpha Mos) composition of paddy grains of Lawama and TXD306 varieties harvested at different maturity stages, expressed as mean ± standard deviation of triplicates.

Maturity stages	Colour values			Grain dimensions values†		
	Brightness *(L*)*	Redness *(a*)*	Yellowness *(b*)*	Area	Height	Width
Lawama						
DGS	50.53 ± 0.42^{c}	4.97 ± 0.22^{a}	27.13 ± 0.58^{a}	20827.7 ± 773.8^{a}	294.39 ± 6.87^{a}	90.23 ± 1.77^{ab}
MGS	47.65 ± 0.45^{b}	6.10 ± 0.40^{b}	29.22 ± 0.21^{b}	21409.1 ± 485.0^{a}	309.23 ± 3.35^{a}	91.55 ± 0.47^{b}
FRS	46.92 ± 0.71^{ab}	7.19 ± 0.37^{c}	29.93 ± 0.37^{b}	20407.2 ± 482.4^{a}	292.55 ± 1.85^{a}	88.86 ± 1.27^{ab}
ORS	45.52 ± 0.82^{a}	7.87 ± 0.04^{c}	29.67 ± 1.13^{b}	19558.5 ± 380.0^{a}	293.12 ± 3.91^{a}	85.72 ± 0.30^{a}
TXD306						
DGS	55.00 ± 0.46^{b}	4.16 ± 0.32^{a}	28.00 ± 0.24^{a}	20320.5 ± 74.36^{a}	295.54 ± 2.91^{a}	88.64 ± 0.51^{a}
MGS	54.47 ± 1.15^{b}	4.69 ± 0.59^{a}	28.24 ± 0.31^{a}	20397.4 ± 45.54^{a}	302.08 ± 1.87^{a}	86.61 ± 0.27^{a}
FRS	53.57 ± 0.57^{b}	6.36 ± 0.35^{b}	28.34 ± 0.26^{a}	20283.4 ± 157.4^{a}	300.69 ± 5.81^{a}	86.38 ± 1.56^{a}
ORS	49.77 ± 0.75^{a}	8.24 ± 0.06^{c}	29.38 ± 0.13^{b}	20477.5 ± 475.9^{a}	303.22 ± 1.93^{a}	86.92 ± 1.96^{a}

DGS – dough grain stage (15 – 21 days after 50% heading (DAH)), MGS – mature grain stage (22 28 DAH), FRS – fully ripe stage (29 – 35 DAH), ORS – over ripe stage (36 – 43 DAH). Values in each column bearing different superscripted letters are statistically different ($p \leq 0.05$) for each rice variety. †The grain dimension descriptors represent the number of pixels of the object, are unitless and have no real "physical" significance but are useful for comparison.

5.3.2 Colour composition and clustering

5.3.2.1 Colour compositions (*L* a* and b** values)

The average values (*L*, a*, b** by HunterLab) differed significantly among maturity stages (Table 5.1) and processing conditions (Table 5.2). Brightness (*L**) of paddy grains in Lawama and TXD306 significantly decreased as grains matured from DGS to ORS. By contrast, the redness (*a**) and yellowness (*b**) of paddy grains increased during grain development for both Lawama and TXD306 varieties. The results indicate that the levels of red and yellow pigments on the surface of the husk increased, whereas the counterpart green pigments decreased during grain development, as corroborated by Jimyang *et al.*[25] Only whiteness (*L**) values of paddy samples significantly differed between varieties; a higher value (53.2 ± 2.24) was observed in TXD306 than in Lawama (47.6 ± 1.91).

Drying and roasting of paddy grains significantly increased the fresh paddy grains' brightness at 18 and 26 DAH (Table 5.2). However, no significant changes (except for 18 DAH, at 80 °C) in brightness were observed between raw (dried paddy) and roasted paddy samples. Though the brightness increased with roasting temperature, no significant changes were observed among roasted paddy grains. The redness and yellowness did not change significantly upon drying (except redness at 26 DAH) of fresh paddy, whereas roasting significantly increased (except yellowness at 26 DAH) the redness and yellowness of fresh paddy with roasting temperatures as the consequence of drying. Soaking before roasting did not affect the brightness, redness, and yellowness of paddy grains, though slightly higher values of brightness and lower values of redness and yellowness were observed in soaked paddy grains compared to unsoaked paddy grains. This could be due to the high moisture content (i.e., free water molecules), which diluted the pigments responsible for redness and yellowness in soaked samples compared to corresponding unsoaked samples.

Table 5.2: Colour composition (L* a* and b* values by HunterLab) of paddy and polished rice grains of variety TXD306 processed by different practices expressed as mean ± standard deviation of triplicates.

Processing treatment codes	Colour values, 18 DAH			Colour values, 26 DAH		
	Brightness *(L*)*	Redness *(a*)*	Yellowness *(b*)*	Brightness *(L*)*	Redness *(a*)*	Yellowness *(b*)*
Paddy						
80-0	49.04 ± 0.99^{b}	3.72 ± 0.38^{a}	24.10 ± 0.77^{abc}	53.44 ± 0.93^{b}	5.99 ± 0.65^{ab}	26.84 ± 0.31^{a}
80-12	50.62 ± 0.27^{bc}	3.42 ± 0.18^{a}	23.32 ± 0.41^{a}	54.19 ± 0.33^{b}	5.97 ± 0.08^{ab}	26.67 ± 0.50^{a}
100-0	51.75 ± 1.22^{cd}	3.89 ± 0.21^{a}	25.25 ± 0.38^{b}	53.64 ± 0.28^{b}	6.21 ± 0.38^{b}	27.44 ± 0.69^{a}
100-12	53.69 ± 0.72^{d}	3.73 ± 0.13^{a}	25.02 ± 0.37^{bc}	53.69 ± 0.35^{b}	6.10 ± 0.19^{ab}	27.21 ± 0.69^{a}
120-0	52.07 ± 0.63^{cd}	5.48 ± 0.05^{b}	25.57 ± 0.42^{c}	55.08 ± 0.92^{b}	6.41 ± 0.39^{b}	27.74 ± 1.41^{a}
120-12	54.11 ± 0.07^{d}	3.99 ± 0.42^{a}	24.74 ± 0.76^{abc}	55.07 ± 1.43^{b}	6.24 ± 0.41^{b}	26.62 ± 0.57^{a}
Raw	52.13 ± 0.41^{cd}	3.37 ± 0.71^{a}	24.64 ± 0.34^{abc}	54.92 ± 0.43^{b}	6.56 ± 0.26^{b}	26.85 ± 0.71^{a}
Fresh	37.33 ± 1.52^{a}	2.89 ± 0.67^{a}	23.77 ± 0.63^{ab}	41.48 ± 4.43^{a}	5.02 ± 0.54^{a}	25.99 ± 1.35^{a}
Polished rice						
80-0	58.07 ± 0.40^{cd}	3.99 ± 0.14^{d}	24.76 ± 0.51^{c}	64.16 ± 0.18^{bc}	2.38 ± 0.12^{b}	22.13 ± 0.18^{c}
80-12	60.92 ± 0.14^{e}	3.28 ± 0.06^{cd}	24.61 ± 0.08^{c}	64.90 ± 0.32^{b}	2.35 ± 0.16^{b}	21.59 ± 0.17^{bc}
100-0	57.73 ± 0.31^{c}	3.68 ± 0.29^{d}	26.67 ± 0.39^{d}	63.12 ± 0.41^{c}	3.52 ± 0.09^{c}	24.81 ± 0.16^{d}
100-12	59.03 ± 0.42^{d}	2.99 ± 0.20^{c}	25.64 ± 0.17^{cd}	64.47 ± 0.40^{bc}	2.81 ± 0.95^{b}	23.49 ± 0.09^{d}
120-0	50.14 ± 0.66^{a}	11.8 ± 0.28^{f}	30.52 ± 0.39^{e}	55.64 ± 0.71^{a}	10.8 ± 0.26^{e}	30.33 ± 0.10^{e}
120-12	56.10 ± 0.37^{b}	7.49 ± 0.21^{e}	29.62 ± 0.61^{e}	56.76 ± 0.20^{a}	9.39 ± 0.13^{d}	29.53 ± 0.29^{e}
Pepeta	66.50 ± 0.34^{g}	1.12 ± 0.17^{a}	19.39 ± 0.36^{a}	69.25 ± 1.02^{d}	1.40 ± 0.41^{a}	18.98 ± 1.32^{a}
Raw	64.28 ± 0.44^{f}	2.02 ± 0.06^{b}	22.36 ± 0.19^{b}	67.91 ± 0.41^{d}	2.61 ± 0.20^{b}	20.58 ± 0.12^{b}

DAH = days after 50% heading. 80, 100 and 120 indicate rice roasted at the corresponding temperatures (°C), 0 and 12 indicate unsoaked and water-soaked rice at room temperature for 12 h prior roasting respectively, Raw = unprocessed paddy/rice, Fresh = undried raw paddy, *Pepeta* = locally prepared *pepeta*/rice flakes. Values in each column bearing different superscripted letters are statistically different ($p \leq 0.05$) for individual paddy and polished rice samples.

For the polished rice, roasting had a significant effect on the colour parameters of the white starchy endosperm; the brightness decreased while both the redness and yellowness increased with roasting temperature. This suggests inward diffusion of red and yellow pigments from the husk and bran into the endosperm[10] and/or non-enzymatic browning reactions such as caramelisation or Maillard browning induced by the heat treatment,[10,11] thereby diluting the white starchy endosperm. Both processes, i.e., inward diffusion of pigments and non-enzymatic browning reactions, are influenced by the extent of processing conditions, including roasting temperature.[32,33] Soaking before roasting had a significant effect on brightness (except at 26 DAH) and redness; high values of brightness and low redness values were observed in soaked samples when compared to corresponding unsoaked samples. Though no significant effect was observed on yellowness, soaking slightly decreased the yellow pigments compared to the corresponding unsoaked samples. The results suggest that water played a plasticiser role, diluting the reactants (i.e., amino acids groups, reducing sugars, and intermediate products), which thus become less concentrated; this affects the diffusion and decreases the rate of the Maillard reaction.[33] An interaction effect between soaking and roasting was observed in colour parameters (*L*, a*, b**) for polished rice samples only. Compared to other roasted products, *pepeta* showed higher brightness values and lower redness and yellowness values than raw rice for both 18 and 26 DAH. Technically, *pepeta* involves the mechanical pounding of roasted paddy into flattened grains immediately after roasting; this exposes the whitish, less pigmented inner endosperm fraction[1] on the outer surface of the *pepeta* that then dilutes the red and yellow pigments formed upon roasting.

5.3.2.2 Colour clustering by Principal Component Analysis

Colour separated the samples into four maturity stages in the Principal Component Analysis (PCA) plots in both varieties (Fig. 5.3). From the PCA loading plots (Fig. 5.3 Panels b and d),

the DGS in both Lawama and TXD306 were dominated by the colour codes for dark yellow (2982, 2983) and light yellowish-brown (3238, 3239, 3240) as indicated in Table 5.S1. In contrast to DGS, the MGS in Lawama resembled light olive-brown (codes 2691, 2692) and strong yellowish-brown (code 2693), whereas moderate yellow (codes 3510, 3255) was vivid in TXD306. FRS and ORS separated less well in both varieties indicating no further variation in colour after the grains were fully matured. Strong yellowish-brown (codes 2673, 2674, 2946, 2947) and dark orange-yellow (codes 2948, 3219, 3220) colours were observed in Lawama, whereas moderate orange-yellow (codes 3493, 3494, 3495, 3767) dominated in TXD306. This underpins processors' and consumers' claims of using colour changes of paddy grain to assess the optimum maturity for *pepeta* production and *pepeta* product selection, respectively.[22] Variation of rice grain colour during development has been well documented and related to grain maturity stages:[24,25] flowering stage (0-7 DAH) – the seed coat colour is green, milk grain stage (8-14 DAH) – the seed coat begins to change according to rice variety, dough grain stage (15-21 DAH) – the seed coat colour develops more intensely than in the previous stage, mature stage (22-28 DAH) – the seed coat colour becomes distinct by the rice variety, fully mature stage (29-35 DAH) – the seed coat colour represents that of the rice variety.

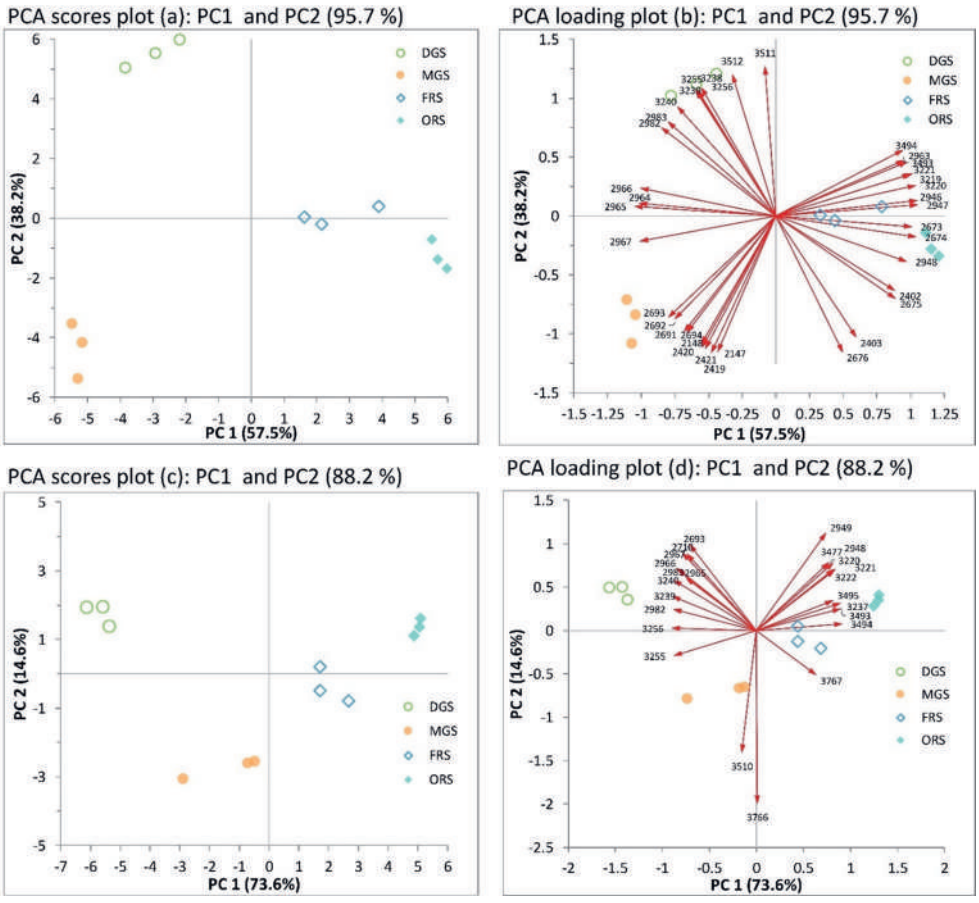

Fig. 5.3: Principal Component Analysis of Lawama (a, b) and TXD306 (c, d) rice varieties showing colour variability in paddy grains during grain maturation as perceived by humans using E-eyes (Iris Alpha Mos). The plots were derived from colour codes extracted from the samples (Panels b and d), which showed significant differences (Kruskal–Wallis test, $P < 0.05$) for maturity levels. DGS – dough grain stage (15 – 21 days after 50% heading (DAH)), MGS – mature grain stage (22 – 28 DAH), FRS – fully ripe stage (29 – 35 DAH), ORS – over ripe stage (36 – 43 DAH). For colour code interpretation refer to Table 5.S1.

Thermal processing and drying impacted the colour of paddy grains, the fresh paddy grains positioned relatively farther from others in the PCA plots in both 18 and 26 DAH (Fig. 5.4). The PCA loading plots (Fig. 5.4 Panels b and d) indicate that fresh paddy at 18 DAH is dominated by greyish yellow-brown colour (codes 1620, 1893, 2166), moderate orange-brown (codes 1619, 1892) and moderate orange (codes 1891, 1908), whereas in fresh paddy at

26 DAH colours dominated that are described as light olive (codes 2164, 2437), light olive-brown (codes 2165, 2438 and light brown (code 2967). A clear separation between fresh/undried paddy and other processing conditions, including raw paddy (dried product), complements processors' knowledge of using colour changes of paddy grains as one of the indicators to end the roasting process.[22] The effect of soaking paddy grains before roasting was prominent at 18 DAH, as soaked products clearly separated from non-soaked products. For soaked products, the colour of light olive brown (codes 2711, 2984) and greyish-yellow (codes 2983, 2985, 3256, 3257) corresponded to soaked products, while that of strong and light yellowish-brown (codes 2693, 2966, 3239) and light brown (codes 2694, 2967) were observed in 26 DAH. Less colour separation among processing conditions at 26 DAH (Fig. 5.4c and d) could be influenced by a more distinct brown colour of the paddy grain husk than at 18 DAH,[24,25] resembling that of roasted rice grains.

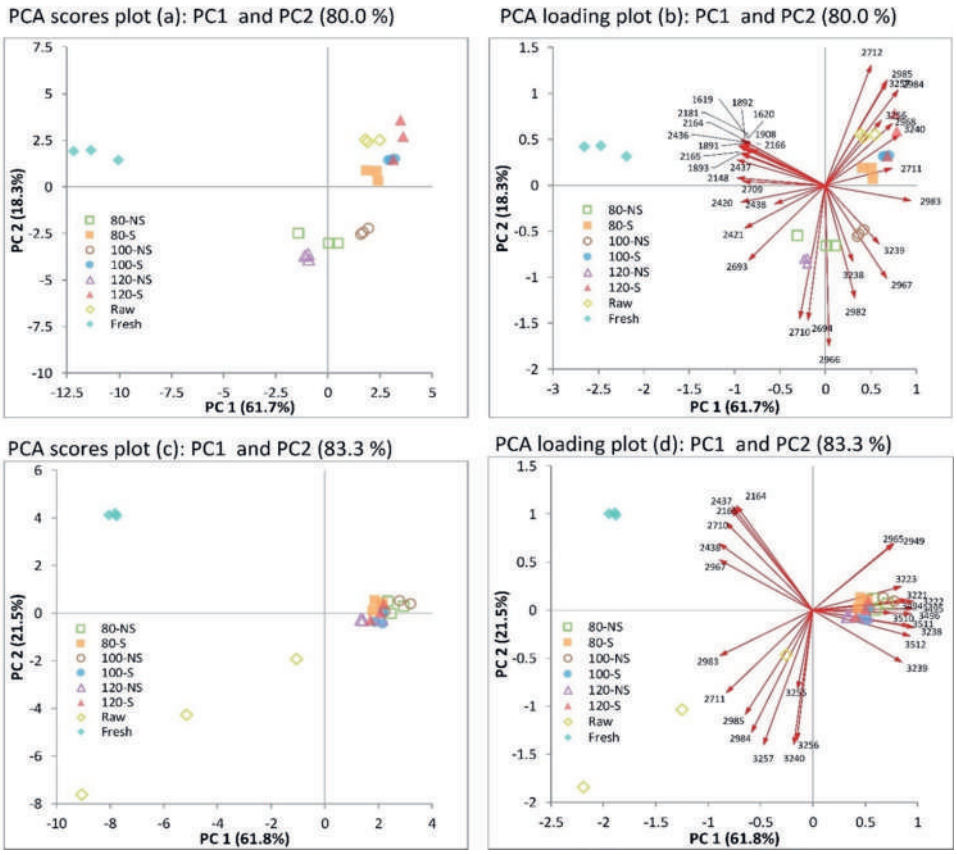

Fig. 5.4: Two-dimension PCA plots on the colour code data extracted from 18 days after 50% heading (DAH, Panels a, b) and 26 DAH (Panels c, d) processed paddy products by E-eyes (Iris Alpha Mos). The used colour codes (Panels b and d) showed significant differences (Kruskal–Wallis test, $P < 0.05$) among processing conditions. 80, 100 and 120 – roasting temperatures (°C), NS – not soaked, S- water-soaked paddy at room temperature for 12 h prior roasting, Raw – unprocessed dried paddy, Fresh – unprocessed wet paddy. For colour code interpretation refer to Table 5.S1.

In contrast to paddy grain, processing conditions separated for polished rice in the PCA plots (Fig. 5.5). The PCA loading plots (Fig. 5.5 Panels b and d) show that pale orange-yellow (codes 3786, 3785, 4058) and pale yellow (codes 3802, 4075, 4076) colours were abundant in both soaked and non-soaked products roasted at low temperatures (80 and 100 °C). Soaking before roasting at 120 °C caused a noticeable change in the colour of rice grains at 18 DAH. The colour codes of dark orange-yellow (2948, 3220, 3221), moderate orange-yellow (3493, 3494, 3767) and moderate orange (2949, 3204, 3222, 3477, 3768, 3784) were prominent in

non-soaked products while light yellowish-pink (4041, 4042) dominated in soaked products. Dry-heat moisture treatment of paddy rice (such as roasting of moistened paddy) led to physicochemical changes of the starchy endosperm, including the colour due to the formation of Maillard reaction products[(11)] and diffusion of husk and bran pigments into the starchy endosperm by the onset of the gelatinisation process.[(32)]

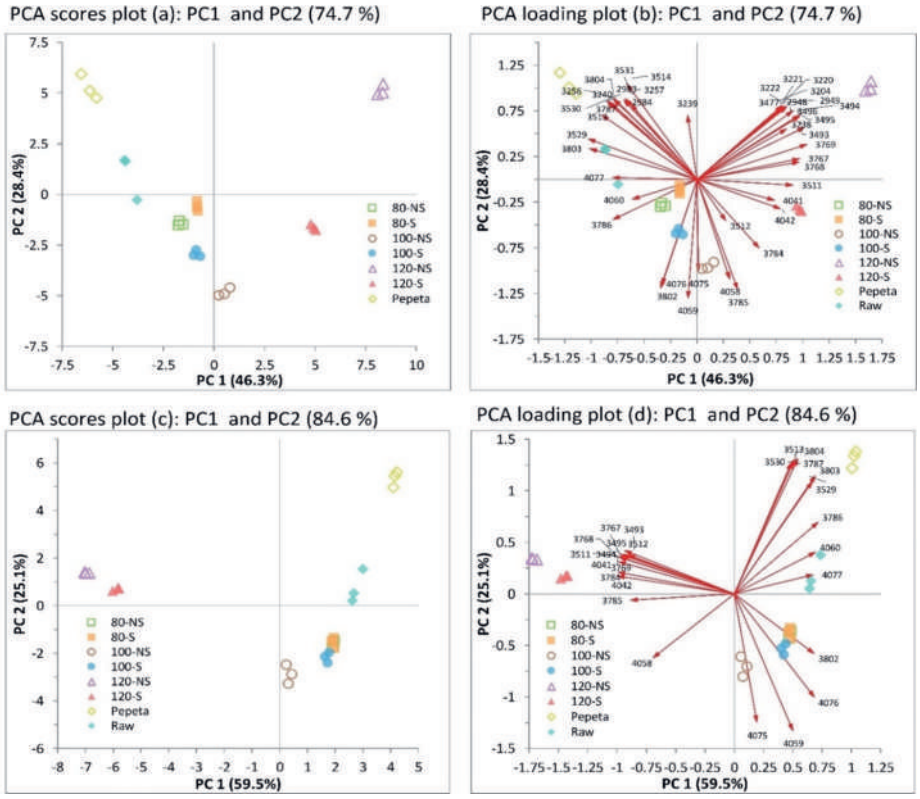

Fig. 5.5: Two-dimension PCA plots on the colour code data extracted from 18 days after 50% heading (DAH, Panels a, b) and 26 DAH (Panels c, d) processed milled rice products by E-eyes (Iris Alpha Mos). The used colour codes (Panels b, d) showed significant differences ($P < 0.05$, Kruskal–Wallis test) for processing conditions. 80, 100 and 120 – roasting temperatures (°C), NS – not soaked, S- water-soaked paddy at room temperature for 12 h prior roasting, *Pepeta* – locally prepared rice flakes, Raw – unprocessed dried paddy. For colour code interpretation refer to Table 5.S1.

5.3.3 Composition of VOCs at different maturity stages and processing conditions

Table 5.S3 details changes in VOC profiles, i.e., the formation and disappearance of some individual VOCs during grain maturation in Lawama and TXD306 rice varieties. A total of 52

and 53 VOCs were identified in Lawama and TXD306, respectively; hydrocarbons (24 in Lawama and 25 in TXD306 as well as aldehydes and ketones (9), alcohols (12), esters and ethers (4), and arenes, furans and isocyanides (3) were extracted for each Lawama and TXD306. Many volatiles were commonly found in both varieties and across different grain development stages with some exceptions. For example, volatiles such as dodecane, 3-methyl-5-propylnonane and 3-methylundecane were not detected at DGS, whereas 6-amino-2-methyl-2-heptanol was not detected at FRS in the Lawama variety. For the TXD306 variety, 3-methyl-5-propylnonane was only detected at MGS and FRS, while 6-dimethyl(isopropyl) silyloxytetradecane was only found at FRS and ORS. Other volatiles such as 2-hexyl-1-decanol, 6-amino-2-methyl-2-heptanol and 12-methyl-E,E-2,13-octadecadien-1-ol were not detected at ORS. The results agree with Hinge *et al.*[21], who reported changes in the volatile pattern across different rice grain development stages. Though no volatile profiles specific to variety were observed in this study, the abundance of total VOCs varied between varieties; TXD306 showed higher values (except at FRS) compared to Lawama (Fig. 5.6a). This is logical as TXD306 is a semi-aromatic variety, i.e., having higher amounts of VOCs than non-aromatic varieties such as Lawama. Several other studies[27,34–36] reported a higher amount of VOCs in aromatic rice than in non-aromatic counterparts. In variety TXD306, the abundance of VOCs decreased during grain development from DGS to ORS. Similar results have been reported by Hinge *et al.*[21], who found a significant decrease in VOCs as rice grain matured. In contrast, the abundance of VOCs in Lawama decreased from DGS to MGS, dramatically increased at FRS, then decreased again at ORS. The pattern in Lawama can be explained by further analysing individual VOC groups, as seen in Fig. 5.6b and Table 5.S3. Both hydrocarbon and arene groups increased from DGS to MGS and decreased at FRS before increasing at ORS, whereas the alcohols showed the opposite trend, increasing at FRS. This suggests that environmental stress (e.g., high temperature and drought) occurred, resulting in

the onset of pathways regulating VOC production[37,38] related to the conversion of hydrocarbon and arene groups to form alcohols. For TXD306, hydrocarbon and arene groups increased while alcohols decreased as rice grains developed from DGS to ORS (Fig. 5.6c). Though hydrocarbons were present in abundance, with a per cent share range from 38.1 – 54.1 (Lawama) and 42.5 – 54.9 (TXD306), respectively, of the total peak area across development stages, they do not contribute to rice aroma due to high perception threshold values.[39] Among the detected alcohols, 2-isopropyl-5-methyl-1-heptanol, 2-hexyl-1-octanol, 2-butyl-1-octanol, and 1-nonen-3-ol contribute to woody, sweety, fruity, floral, and green aroma in rice.[21,27,40] On the other hand, arenes and furans can contribute to aroma even in small concentrations.[21,39] Aldehyde and ketone volatiles were the second abundant group ranging from 16.1 – 18.9 (Lawama) and 15.8 – 18.9 (TXD306) per cent share of the total peak area across development stages. Identified aldehydes and ketones such as benzaldehyde, α-ethyl benzeneacetaldehyde, hexanal, nonanal, 6-methyl-5-hepten-2-one remained unchanged (except at DGS in TXD306) during grain development, and were the most important VOCs contributing to a nutty, bitter, floral, herbal, soapy, citrus, fruity, and green odour of rice.[21,35] Volatiles from the ester and ether groups were the least abundant with a per cent share of 2.9 – 5.1(Lawama) and 2.9 – 4.7 (TXD306) out of the total peak area, and slightly decreased at MGS and FRS compared to DGS and ORS in both TXD306 and Lawama. Two out of the four detected esters, i.e., methoxyacetic acid 3-tridecyl ester and 9-octadecenoic acid (2-phenyl-1,3-dioxolan-4-yl) methyl ester, suggest that early stages of enzymatic and microbial processes such as fermentation and sprouting occur during storage at 4 °C. These two compounds were among the most abundant volatiles found in rice bran sourdough[41] and buckwheat sprouts.[42]

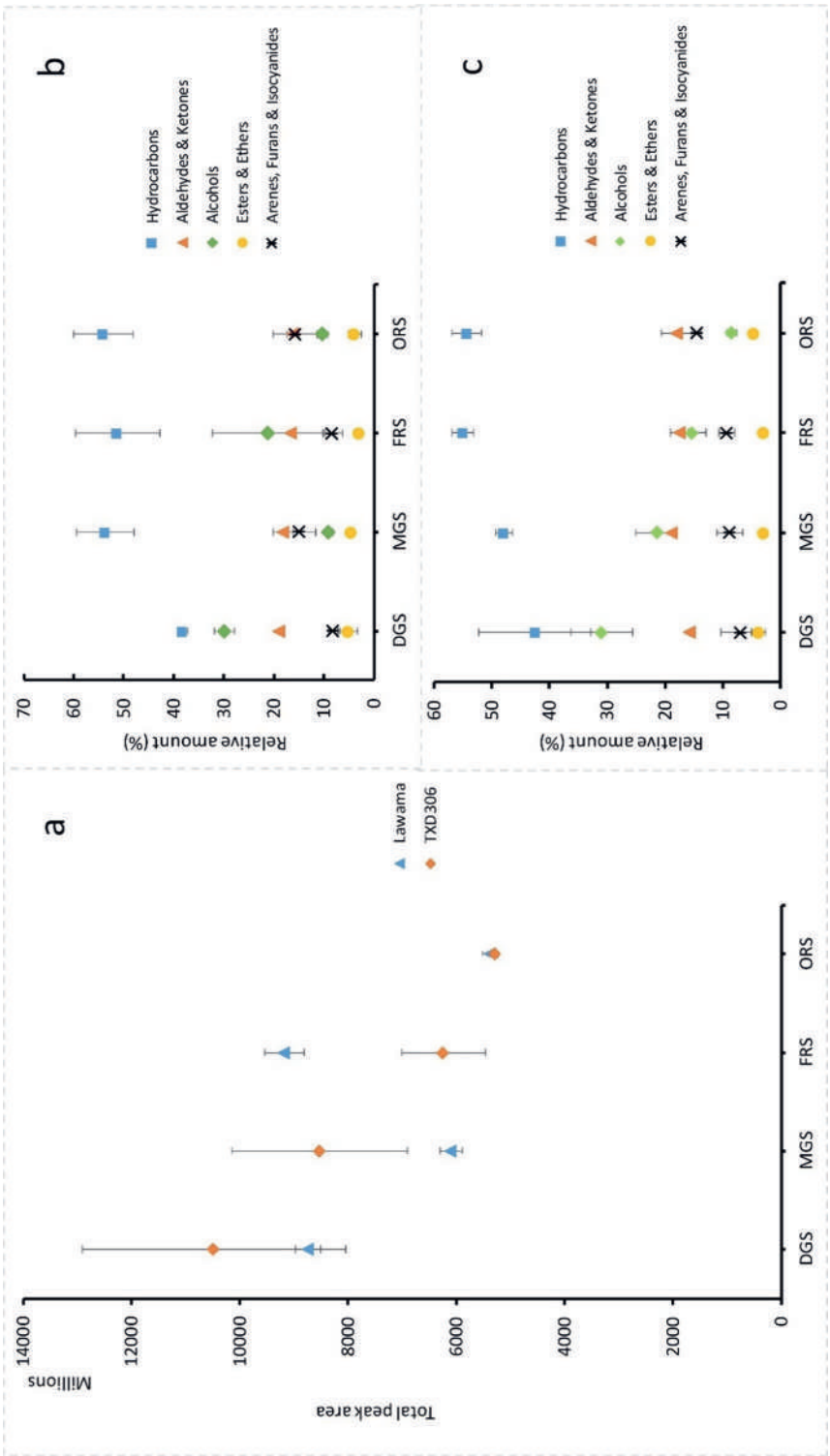

Fig. 5.6: Variability of volatile organic compounds in rice flour samples expressed as total peak area (a) and relative amount (peak area %) in Lawama (b) and TXD306 (c) during grain maturation. Data are presented as average of triplicates + standard deviation. DGS – dough grain stage (15 – 21 days after 50% heading (DAH)), MGS – mature grain stage (22 28 DAH), FRS – fully ripe stage (29 – 35 DAH), ORS – over ripe stage (36 – 43 DAH).

In general, changes in VOC profile during rice grain development reported in previous research,[15,21] have been related to alteration of endogenous hormones, resulting in primary and secondary metabolites as a response to biotic and abiotic stresses.[43] Furthermore, the VOC profile in plant materials such as cereals, is influenced by differences in harvest date and post-harvest handling such as dehulling and polishing.[26,44] It is important to note that paddy samples were collected from different farmers but grown in the same irrigation scheme and were dehulled and polished to remove bran before evaluating VOC profiles.

Processing had a significant effect on the VOC compositions (Table 5.S4 and 5.S5, Fig. 5.S1), with the formation and/or degradation of volatiles by different processing practices. The VOC profile of raw rice at 18 DAH (Fig. 5.S1a) and 26 DAH (Fig. 5.S1b) was abundant in esters and ethers (29-32%) and alcohols (28-29%), followed by hydrocarbons (17-18%), aldehydes and ketones (12-17%), and arenes, furans, and isocyanides (10-11%). These raw rice samples had a completely different distribution of the VOC classes though corresponded to the DGS and MGS of TXD306 variety in Fig. 5.6 (Panel c), respectively. This discrepancy could be due to environmental and farm management factors[14–21] since samples were grown during a different planting season (section 5.2.1). Besides, previous research reported alcohols as the second abundant VOC class in stored polished rice, after aldehydes, which are lipid oxidation products.[45,46] This is not the case in our study as rice samples were freshly harvested from the field, and only temporarily stored at 4 °C before analysis. Tables 5.3 and 5.4 further detail the VOC profile changes that occured upon roasting paddy grains. Roasting of paddy generated some volatiles such as 2,7,10-trimethyldodecane, 2,6,10,14-tetramethylheptadecane, 2,6,10-trimethyltetradecane and urs-12-ene, while other volatiles such as 4-methylundecane, 2,4,6-trimethyldecane, dodecane, 2,3,5,8-tetramethyldecane and undecane disappeared. The disappearance of volatiles as vapour upon heat treatment has been reported in a previous study,[9] which probably explains the observed behaviour in our rice samples during the

roasting process. The four hydrocarbons – 2,6,11,15-tetramethylhexadecane (0.28 – 1.17%), 3-methyl-5-propylnonane (0.15 – 2.92%), 5-tridecene,(z)- (2.95 – 7.15%) and urs-12-ene (7.21%) are examples of abundant volatiles identified in raw rice that increased upon roasting. Aldehydes, ketones and alcohols are VOCs considered as products of oxidative degradation of fatty acids,[12,45] contributing most to overall flavour because of their relatively low perception threshold.[12,40] As shown in Tables 5.S4 and 5.S5, significant differences in the aldehyde and ketone groups were observed in rice during roasting. Benzaldehyde, α-ethyl benzeneacetaldehyde, nonanal, and 6-methyl-5-hepten-2-one were the most abundant VOCs found in raw rice contributing to almond, nutty, bitter, floral, herbal, citrus, cucumber, fresh, grass, soapy, fatty and banana-like aromas.[39,47,48] These VOCs were identified in roasted rice and slightly decreased (except for 26 DAH at 80 – 100 °C) with the severity of processing conditions (Fig. 5.S1). This decrease could probably be due to further reduction of aldehydes and ketones to produce alcohol volatiles.[12] A significant increase with roasting temperature was observed in the alcohol group upon heat processing of rice (Table 5.S4 and 5.S5), more pronounced so at 26 DAH compared to 18 DAH (Fig. 5.S1). Detected octanol derivatives (2-butyl-1-octanol and 2-hexyl-1-octanol), 2-isopropyl-5-methyl-1-heptanol, and 2-methoxy-4-vinyl phenol were the main contributors to fruity, floral-like, woody, sweet-like, spicy and clove-like aromas[48] The 2-methoxy-4-vinyl phenol, which was abundant in roasted products, is considered as the product of thermal decomposition of p-coumaric acid and ferulic acid, generated during heating.[12]

The ester group indicates a pleasant, fruity, green and sweet-like aroma[49] derived from free fatty acid reaction products with some alcohols.[50] Propanoic acid butyl ester and 2-butenoic acid hexyl ester were the most abundant esters detected in both raw and roasted rice samples, showing inconsistent trends upon roasting at different temperatures (Table 5.S4 and 5.S5). These esters were also detected at a relatively high quantity in different fragments of cooked

rice.[46] The other two esters, namely butyl acetate and butyl propionate, added a unique aroma to the roasted rice products, and were apparently generated during roasting. Jin *et al.*[51] found similar VOCs produced during heat processing of oat grouts. Heterocyclic hydrocarbons such as arenes and furans play an essential role in the overall aroma of rice due to their relatively low odour perception thresholds.[12] 2-Pentyl-furan and 1,3-di-tert-butylbenzene were the only major heterocyclic hydrocarbons found in rice samples, which significantly increased upon roasting but slightly decreased with an increase in roasting temperature (Table 5.S4 and 5.S5). As furan derivative, 2-pentyl-furan was generated upon roasting, relating to lipid oxidation, Maillard and caramelisation reactions, and contributed to the nutty, green, almond, caramel, and popcorn-like aroma of roasted products[21,52] even in meagre amounts. The detected 1,3-di-tert-butylbenzene is considered a significant contributor to the aroma, especially in heat-treated food, formed by protein breakdown to produce Maillard reaction products.[12,21,52]

VOC clustering for the rapid identification of volatiles that are specific for different processing conditions was performed using the two-dimensional PCA (Fig. 5.S2). VOC profiles clearly separated among roasting temperatures at 18 DAH (Fig. 5.S2), with a noticeable impact of soaking before roasting observed at high roasting conditions (120 °C). Contrary to 18 DAH, the VOCs separated less clearly among processing conditions at 26 DAH. VOCs of processed samples at low roasting conditions (80 and 100 °C) clustered together but were relatively far from those at 120 °C and non-soaked products at 100 °C. However, the loading plots (Fig. 5.S2 Panels b and d) indicated that no discrimination was possible for the VOCs related to specific different processing conditions.

Though soaked products that were roasted at 120 °C emerged relatively far from their non-soaked counterparts in the PCA plots, soaking before roasting had no significant effect on the VOCs. The results contrast with Liu *et al.*[6], who observed fewer VOCs in millet porridge

upon an increase in water content, related to a smaller release of VOCs due to strong interactions with the gelatinised matrix.[8] Besides, a higher moisture content could have changed the kinetics of aroma generation during heating, i.e., some energy was used to evaporate water so fewer compounds were generated. This discrepancy can be due to a difference in moisture content between fresh immature paddy (33 – 41 %) and soaked paddy (48 – 50 %) used in the study. This resulted in the almost same degree of gelatinisation between soaked and counterpart non-soaked samples. No interaction effect (except for aldehydes at 18 DAH, and arenes at 26 DAH) between soaking and roasting was observed in VOCs. Furthermore, no distinctive pattern or significant amount of VOCs was observed to distinguish *pepeta* products from other roasted products.

5.4 Conclusion

For the first time, this study evaluated grain dimensions, colour, and volatile compounds of processed immature rice (*pepeta*) and its ingredients, i.e., immature rice grains. The results demonstrated that the visual quality (grain dimensions and colour) and volatile compounds of *pepeta* products are influenced by the degree of maturity of the rice grains and the roasting temperature. The pounding process improved the brightness of the *pepeta* product by diluting the yellow and red pigments formed during roasting, thereby impairing consumer acceptance of processed rice products. Considering that the visual quality and volatile compounds are critical quality parameters for consumers and processors, the results contribute to understanding consumer preferences for *pepeta* end products. The vast variability in both distribution and abundance of volatiles observed in immature rice grains and *pepeta* product counterparts, offer opportunities for further research on threshold values of active aroma compounds and identification of the association between consumer preference and the inherent volatile compounds. A monitoring study on off-flavour production during post-

harvest handling of freshly harvested immature rice grains and storage stability of *pepeta* products under tropical conditions, where the product is currently produced and consumed, is recommended.

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Conflicts of Interest

The authors declare no conflict of interest.

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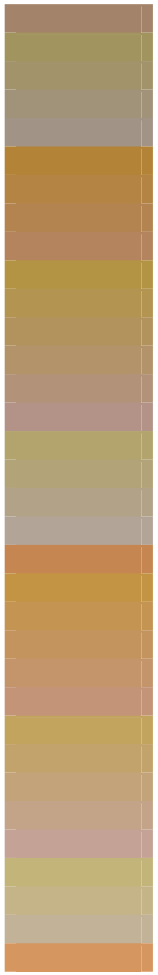
Appendix: Supplementary Material

Table 5.S1: List of colours of the paddy, rice and processed product samples and their appearance

Colour codes extracted	Probability values for maturity effect (paddy grains)*		Probability values for processing effect*				Colour name	Colour appearance
			Paddy		Milled rice			
	Lawama	TXD306	18DAH	26DAH	18 DAH	26DAH		
1619	-	-	0.011	-	-	-	moderate olive brown	
1620	-	-	0.019	-	-	-	dark grayish yellowish brown	
1891	-	-	0.007	-	-	-	moderate olive	
1892	-	-	0.009	**0.057**	-	-	moderate olive brown	
1893	-	-	0.032	-	-	-	grayish yellowish brown	
1908	-	-	0.006	-	-	-	moderate olive	
2147	0.016	-	-	-	-	-	strong yellowish brown	
2148	0.016	-	0.010	**0.085**	-	-	moderate brown	
2164	-	-	0.004	0.040	-	-	light olive	
2165	-	-	0.008	0.010	-	-	light olive brown	
2166	-	-	0.020	-	-	-	grayish yellowish brown	
2181	-	-	0.005	-	-	-	light olive	
2402	0.019	-	-	-	-	-	strong brown	
2403	0.022	-	-	-	-	-	strong brown	
2419	0.022	-	-	-	-	-	light olive brown	
2420	0.025	-	0.007	**0.158**	-	-	strong yellowish brown	
2421	0.025	-	0.006	**0.072**	-	-	light brown	
2436	-	-	0.020	-	-	-	light olive	
2437	-	-	0.003	0.009	-	-	light olive	
2438	-	-	0.013	0.009	-	-	light olive brown	
2673	0.024	-	-	-	-	-	strong yellowish brown	
2674	0.019	-	-	-	-	-	strong yellowish brown	
2675	0.016	-	-	-	-	-	strong yellowish brown	
2676	0.022	-	-	-	-	-	brownish orange	
2691	0.043	-	-	-	-	-	light olive brown	
2692	0.041	-	-	-	-	-	light olive brown	
2693	0.031	0.027	0.003	**0.111**	-	-	strong yellowish brown	

2694	0.024	**0.054**	0.006	**0.064**	-	-	light brown
2709	-	-	0.004	**0.166**	-	-	dark yellow
2710	-	0.044	0.003	0.017	-	-	dark grayish yellow
2711	-	-	0.004	0.019	-	-	light olive brown
2712	-	-	0.006	**0.060**	-	-	light grayish yellowish brown
2946	0.016	-	-	-	-	-	strong yellowish brown
2947	0.016	-	-	-	-	-	strong yellowish brown
2948	0.024	0.019	-	-	0.005	-	dark orange yellow
2949	**0.063**	0.016	-	0.033	0.004	-	moderate orange
2963	0.024	-	-	-	-	-	dark yellow
2964	**0.228**	-	-	-	-	-	dark yellow
2965	0.019	0.038	-	0.081	-	-	dark yellow
2966	0.024	0.024	0.004	**0.108**	-	-	light yellowish brown
2967	0.019	0.038	0.007	0.030	-	-	light brown
2968	-	-	0.003	**0.223**	-	-	light reddish brown
2982	0.016	0.016	0.004	**0.179**	-	-	dark yellow
2983	0.022	0.019	0.008	0.015	0.005	-	dark grayish yellow
2984	-	-	0.003	0.018	0.007	-	light olive brown
2985	-	-	0.005	0.041	-	-	yellowish gray
3204	-	-	-	-	0.005	-	moderate orange
3219	0.016	-	-	-	-	-	dark orange yellow
3220	0.016	0.016	-	-	0.002	-	dark orange yellow
3221	0.022	0.016	-	0.004	0.003	-	dark orange yellow
3222	**0.066**	0.019	-	0.035	0.003	-	moderate orange
3223	-	-	-	0.007	-	-	light brown
3237	**0.094**	0.016	-	-	-	-	moderate yellow
3238	0.024	**0.442**	0.005	0.004	0.003	-	moderate yellow
3239	0.022	0.016	0.003	0.016	0.010	-	light yellowish brown
3240	0.016	0.016	0.002	0.013	0.003	-	light yellowish brown
3241	-	-	-	0.104	-	-	moderate yellowish pink
3255	0.024	0.019	-	0.043	-	-	moderate yellow
3256	0.019	0.016	0.003	0.008	0.002	-	grayish yellow
3257	-	-	0.003	0.009	0.002	-	grayish yellow
3477	-	0.016	-	-	0.003	-	moderate orange



3493	0.027	0.016	-	-	0.002	0.008	moderate orange yellow	
3494	0.024	0.016	-	0.005	0.003	0.003	moderate orange yellow	
3495	**0.086**	0.024	-	0.042	0.002	0.009	moderate orange	
3496	-	**0.055**	-	0.006	0.002	-	moderate yellowish pink	
3510	**0.086**	0.030	-	0.006	-	-	moderate yellow	
3511	0.016	**0.218**	-	0.009	0.003	0.005	moderate yellow	
3512	0.050	**0.055**	-	0.015	0.004	0.006	light yellowish brown	
3513	-	-	-	-	0.003	0.023	light yellowish brown	
3514	-	-	-	-	0.002	-	moderate yellowish pink	
3529	-	-	-	-	0.002	0.005	grayish yellow	
3530	-	-	-	-	0.003	0.002	grayish yellow	
3531	-	-	-	-	0.002	-	brownish pink	
3766	-	0.016	-	-	-	-	moderate orange yellow	
3767	-	0.042	-	-	0.002	0.003	moderate orange yellow	
3768	-	-	-	-	0.002	0.003	moderate orange	
3769	-	-	-	-	0.002	0.005	moderate yellowish pink	
3784	-	-	-	-	0.002	0.003	moderate yellow	
3785	-	-	-	-	0.003	0.003	pale orange yellow	
3786	-	-	-	-	0.004	0.003	pale orange yellow	
3787	-	-	-	-	0.002	0.003	moderate yellowish pink	
3802	-	-	-	-	0.003	0.002	pale yellow	
3803	-	-	-	-	0.002	0.003	pale yellow	
3804	-	-	-	-	0.002	0.003	pale yellowish pink	
4041	-	-	-	-	0.003	0.002	light yellowish pink	
4042	-	-	-	-	0.003	0.002	light yellowish pink	
4058	-	-	-	-	0.002	0.002	pale orange yellow	
4059	-	-	-	-	0.002	0.002	light yellowish pink	
4060	-	-	-	-	0.002	0.003	light yellowish pink	
4075	-	-	-	-	0.003	0.003	pale yellow	
4076	-	-	-	-	0.003	0.002	pale yellow	
4077	-	-	-	-	0.002	0.003	pale yellowish pink	

*The P-values (Kruskal–Wallis). P values marked in bold fonts are not significantly different ($P \leq 0.05$).

Table 5.S2: The *P*-values of the volatile compounds of immature rice harvested at different maturity stages and processing practices by headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME GC–MS) expressed as average peak area % ± standard deviation

	VOC codes	Maturity effect				Processing effect					
		Shapiro–Wilk test[a]		Kruskal–Wallis test[b]		Shapiro–Wilk test		Kruskal–Wallis test		ARTool test[c]	
		Lawama	TXD306	Lawama	TXD306	18DAH	26DAH	18DAH	26DAH	18DAH	26DAH
Hydrocarbons											
Decane	A	.322	.232	.083	.112	**.043**	-	-	-	-	-
4-Methyldecane	B	-	-	-	-	.292	.194	.310	.513	.569	-
2,2-Dimethyldecane	C	.095	.863	.184	.112	-	-	-	-	-	-
2,4,6-Trimethyldecane	D	.589	.509	.475	.198	.333	.375	.513	.513	-	-
2,3,5,8-Tetramethyldecane	E	.386	**.018**	.212	.841	**.034**	**.000**	**.002**	.288	**.000**	-
Dodecane	F	.302	.391	.565	.367	.051	.303	**.005**	.248	**.041**	-
2,6,11-Trimethyldodecane	G	**.004**	**.001**	.983	.104	-	.674	-	-	-	-
2,7,10-Trimethyldodecane	H	.334	.495	.841	.180	**.004**	.143	**.012**	**.050**	**.000**	**.000**
2,6,10,14-Tetramethylheptadecane	I	.153	**.049**	.572	.112	**.045**	**.029**	.785	.072	.642	**.015**
2,6,11,15-Tetramethylhexadecane	J	.660	**.000**	.321	.446	**.010**	.090	**.007**	.243	.052	.119
3-Methyl-5-propylnonane	K	.136	**.031**	.867	.439	**.006**	.160	.152	**.049**	.092	**.021**
6-Methyloctadecane	AQ	**.010**	.322	.244	.083	-	-	-	-	-	-
2,2,4,4-Tetramethyloctane	L	.245	.091	.083	.104	.226	.079	.054	.104	.520	**.037**
Tetradecane	M	**.005**	.465	.083	.129	**.029**	.062	.167		.466	
6,9-Dimethyltetradecane	N	.234	.335	.881	.280	.211	**.000**	.082	**.021**	.244	.082
2,6,10-Trimethyltetradecane	O	.110	.552	.446	.367	**.009**	.624	**.013**	.130	**.000**	.486
6-Dimethyl(isopropyl) silyloxytetradecane	P	-	**.025**	-	.121	.063	.167	**.050**	**.011**	**.000**	**.009**
Undecane	Q	.539	.550	.212	.198	.738	-	-	-	-	-
3-Methylundecane	R	**.017**	.472	.439	1.00	.415	.339	**.050**	.513	-	-
4-Methylundecane	S	.340	.252	.212	.184	.422	**.004**	**.047**	-	.472	-

1-Iodo-2-methylundecane	T	.506	.416	.160	.212	.628	.373	**.006**	**.050**	**.000**	**.000**
Hexamethylcylotrisiloxane	U	.460	.690	.083	.572	.873	**.001**	.807	.599	.179	**.011**
Pentacos-1-ene	V	.064	.399	.954	.139	.374	**.002**	.390	.076	-	.079
5-Tridecene,(z)-	W	.388	**.002**	.104	.367	.108	**.028**	**.015**	.099	.188	.898
4-Methyl-1-undecene	X	.346	.094	.198	.572		.469	-	**.029**	-	.101
Urs-12-ene	Y	**.001**	.073	.198	.083	.922	**.001**	.093	.070	**.000**	**.006**
Subtotal		.237	.115	.196	.139	.275	.415	**.010**	.052	.119	.061
Aldehydes and ketones											
Benzaldehyde	Z	**.003**	**.000**	.104	.280	**.000**	**.000**	.096	.237	.076	**.012**
3-Benzyloxy-2-fluoro-4-methoxy benzaldehyde	AA	.365	**.003**	.149	.121	.177	.014	.072	**.040**	**.012**	**.044**
α-Ethyl benzeneacetaldehyde	AB	.352	.638	.212	.343	.069	.212	.154	.058	.059	.051
3-Methylbutanal	AC	.176	.070	.244	.139	.081	**.001**	**.016**	.053	**.015**	.094
Hexanal	AD	.728	**.003**	.244	.244	-	-	-	-	-	-
Nonanal	AE	.761	.787	.212	.367	.091	1.000	**.013**	.115	.112	.560
6,10-dimethyl-5,9-dodecadien-2-one	AF	.064	**.008**	.418	.212	.777	.948	**.042**	.095	**.021**	**.047**
6-Methyl-5-hepten-2-one	AG	.558	.665	.212	.083	.344	.806	.098	**.034**	.055	.135
4-Hydroxybutan-2-one	BH	.127	.862	.244	.367	-	-	-	-	-	-
Subtotal		.377	.100	.244	.198	.054	.284	**.013**	.170	**.013**	.632
Alcohols											
2-Hexyl-1-decanol	AH	**.001**	**.004**	.261	.156	.671	.414	**.014**	.257	.671	.644
2-Octyl-1-decanol	AI	**.000**	**.000**	.212	.244	.135	.107	**.003**	**.026**	.493	.114
2-Ethyl-1-dodecanol	AJ	.077	**.000**	.160	.139	**.043**	.984	**.016**	.079	.206	.513
2-Octyl-1-dodecanol	AK	**.000**	**.000**	.112	.280	.646	.315	**.009**	**.029**	.106	.161
2-Isopropyl-5-methyl-1-heptanol	AL	**.000**	**.000**	.539	.539	**.038**	.882	**.007**	**.018**	**.000**	**.008**
6-Amino-2-methyl-2-heptanol	AM	.442	.362	.100	.156	-	-	-	-	-	-
-2-Hexyl-1-octanol	AN	**.002**	**.013**	.198	.160	.175	**.022**	**.017**	.063	.156	.410
1-Nonen-3-ol	AO	.247	**.012**	.367	.160	.857	.994	.439	1.00	**.018**	

2-Butyl-1-octanol	AP	**.001**	**.037**	.212	.104	**.000**	**.021**	.264	.123	.633	.143
1-Pentanol	AR	**.000**	**.000**	.083	.572	**.029**	-	-	-	-	-
-12-Methyl-E,E-2,13-octadecadien-1-ol	AS	.136	**.000**	.104	.565	.181	**.022**	.119	**.032**	.168	.178
2-Methyl-1-undecanol	AT	.871	**.007**	.475	.572	-	-	-	-	-	-
1-Methoxy-2-propanol	AU	-	-	-	-	**.029**	**.020**	.698	**.020**	.865	.111
2-methoxy-4-vinylphenol	AV	-	-	-	-	.454	.547	.507	**.032**	.949	.227
Subtotal		**.013**	.824	.129	.083	.978	.547	.059	.240	.134	.213
Esters and ethers											
Propanoic acid butyl ester	AW	**.000**	.597	.112	.139	.323	.768	**.034**	.098	.246	.231
Butyl acetate)	AX	-	-	-	-	**.020**	.377	**.023**	.329	.287	.524
2-Butenoic acid hexyl ester	AY	-	-	-	-	.906	**.011**	**.043**	**.019**	.873	.503
Butyl propionate	AZ	-	-	-	-	.066	.054	.083	.116	.052	.055
Methoxyacetic acid 3-tridecyl ester	BE	.254	.758	.083	1.00	-	-	-	-	-	-
2-Butenoic acid hexyl ester	BF	**.040**	**.022**	.244	.149	-	-	-	-	-	-
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-	BG	.194	.128	.083	.104	-	-	-	-	-	-
Subtotal		.655	**.028**	.367	.300	**.018**	.821	**.011**	**.020**	.402	.908
Arenes, furan and isocyanide											
Tetrahydrofuran	BA	**.033**	.850	.139	.112	-	-	-	-	-	-
1,3-Di-tert-butylbenzene	BB	**.016**	**.007**	.112	.761	.171	.168	**.004**	**.016**	.106	**.000**
2-Pentyl-furan	BC	-	-	-	-	.275	.904	**.018**	**.026**	.089	.094
Methyl isocyanide	BD	.223	.516	.367	.198	.742	.520	.658	.513	-	-
Subtotal		.397	.869	.149	.212	.071	.716	.073	**.008**	.373	**.006**

[a]Normality test.

[b]Nonparametric test equivalent to One Way ANOVA to evaluate similarity of distributions among experimental groups.

[c]The aligned rank transformation (ART) for nonparametric factorial ANOVA to examine interactions (roasting and soaking) effect.

P values marked in bold fonts are significantly different ($P < 0.05$).

Table 5.S3: Volatile compounds in Lawama and TXD306 rice varieties harvested at different maturity stages by headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME GC–MS) expressed as average peak area % ± standard deviation

	Lawama*				TXD306*			
	DGS	MGS	FRS	ORS	DGS	MGS	FRS	ORS
Hydrocarbons								
Decane	1.6 ± 0.1	3.7 ± 0.2	6.9 ± 0.9	3.3 ± 0.4	4.2 ± 0.3	3.4 ± 0.7	1.5 ± 0.4	3.6 ± 0.2
2,2-Dimethyldecane	19 ± 0.9	24 ± 3.7	21. ± 1.8	19 ± 0.7	17 ± 6.3	23 ± 1.9	28 ± 0.5	18 ± 1.1
2,4,6-Trimethyldecane	1.8 ± 1.0	2.6 ± 0.8	4.2 ± 0.9	4.9 ± 2.4	2.6 ± 1.8	1.7 ± 0.2	2.9 ± 0.4	5.4 ± 1.5
2,3,5,8-Tetramethyldecane	0.8 ± 0.0	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.0
Dodecane	-	3.7 ± 1.1	2.7 ± 1.2	3.9 ± 1.2	0.7 ± 0.1	2.8 ± 0.3	2.9 ± 1.0	1.9 ± 0.3
2,6,11-Trimethyldodecane	0.9 ± 0.5	1.1 ± 0.0	1.1 ± 0.1	0.9 ± 0.9	0.9 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	2.2 ± 1.1
2,7,10-Trimethyldodecane	0.5 ± 0.0	0.6 ± 0.5	0.4 ± 0.0	0.5 ± 0.4	0.3 ± 0.1	0.6 ± 0.3	-	0.7 ± 0.1
2,6,10,14-Tetramethylheptadecane	2.9 ± 0.2	2.6 ± 1.8	2.0 ± 1.5	0.6 ± 0.3	1.7 ± 0.2	0.2 ± 0.1	0.8 ± 0.2	3.9 ± 0.3
2,6,11,15-Tetramethylhexadecane	1.0 ± 0.0	1.2 ± 0.1	1.0 ± 0.3	1.3 ± 0.1	4.8 ± 0.6	1.0 ± 0.2	2.0 ± 0.7	1.2 ± 0.1
3-Methyl-5-propylnonane	-	0.2 ± 0.3	0.4 ± 0.3	0.5 ± 0.1	-	0.3 ± 0.2	0.3 ± 0.1	-
6-Methyloctadecane	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.5 ± 0.0	1.0 ± 0.0	0.6 ± 0.1
2,2,4,4-Tetramethyloctane	0.4 ± 0.1	2.4 ± 0.1	1.3 ± 0.3	2.0 ± 0.5	0.6 ± 0.1	1.4 ± 0.1	0.7 ± 0.2	3.0 ± 0.4
Tetradecane	1.5 ± 0.0	1.6 ± 0.0	0.9 ± 0.1	5.3 ± 1.0	1.1 ± 0.3	1.3 ± 0.1	2.1 ± 0.3	2.8 ± 0.8
6,9-Dimethyltetradecane	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.7	0.4 ± 0.2	0.5 ± 0.1	0.6 ± 0.1
2,6,10-Trimethyltetradecane	0.5 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.8 ± 0.2	0.5 ± 0.3
6-Dimethyl(isopropyl)silyloxytetradecane	-	-	-	-	-	-	1.0 ± 0.0	0.6 ± 0.1
Undecane	1.1 ± 0.0	2.3 ± 0.1	2.0 ± 0.4	2.5 ± 0.8	1.4 ± 0.6	2.4 ± 0.7	2.1 ± 0.3	2.7 ± 0.1
3-Methylundecane	-	-	0.3 ± 0.1	0.3 ± 0.1	-	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
4-Methylundecane	0.1 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	0.7 ± 0.2	0.2 ± 0.2	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.1
1-Iodo-2-methylundecane	1.5 ± 0.0	1.7 ± 0.2	1.5 ± 0.4	2.2 ± 0.0	1.0 ± 0.1	1.5 ± 0.5	2.0 ± 0.4	1.9 ± 0.2
Hexamethylcylotrisiloxane	0.2 ± 0.2	1.1 ± 0.5	0.6 ± 0.0	0.7 ± 0.1	0.4 ± 0.2	0.5 ± 0.3	0.4 ± 0.1	0.7 ± 0.1
Pentacos-1-ene	0.8 ± 0.0	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	0.5 ± 0.1	0.7 ± 0.1	1.2 ± 0.3	1.1 ± 0.4
5-Tridecene,(z)-	1.7 ± 0.0	1.2 ± 0.2	0.8 ± 0.1	1.4 ± 0.3	1.7 ± 0.8	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.0
4-Methyl-1-undecene	0.1 ± 0.0	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	1.0 ± 0.1	1.9 ± 0.2	1.3 ± 0.9	0.4 ± 0.0
Urs-12-ene	1.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.8 ± 0.3	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.0
Subtotal	38 ± 0.8	54 ± 5.8	51 ± 8.4	54 ± 6.0	43 ± 9.7	48 ± 1.4	55 ± 1.9	54 ± 2.6
Aldehydes and ketones								
Benzaldehyde	5.0 ± 0.2	1.5 ± 0.3	1.7 ± 0.3	1.2 ± 0.1	3.4 ± 1.9	1.8 ± 0.3	1.5 ± 0.2	1.6 ± 0.2
3-Benzyloxy-2-fluoro-4-methoxy benzaldehyde	0.6 ± 0.0	0.5 ± 0.2	0.2 ± 0.3	0.3 ± 0.2	0.6 ± 0.4	0.1 ± 0.1	-	-
α-Ethyl benzeneacetaldehyde	0.8 ± 0.1	0.4 ± 0.0	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.4 ± 0.0	0.7 ± 0.2	0.6 ± 0.1
3-Methylbutanal	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	0.4 ± 0.2

Hexanal	5.1 ± 0.3	6.9 ± 0.4	6.6 ± 0.9	7.2 ± 0.9	4.2 ± 0.7	7.7 ± 0.5	7.8 ± 0.2	7.7 ± 0.1
Nonanal	4.6 ± 0.2	6.7 ± 0.5	6.1 ± 0.8	5.0 ± 0.9	4.5 ± 0.5	6.4 ± 0.9	6.3 ± 0.8	4.7 ± 0.9
6,10-dimethyl-5,9-dodecadien-2-one	0.3 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	0.6 ± 0.3	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.8 ± 0.0
6-Methyl-5-hepten-2-one	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.7 ± 0.0
4-Hydroxybutan-2-one	2.0 ± 0.3	0.6 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	1.6 ± 0.6	1.4 ± 0.2	0.5 ± 0.3	1.1 ± 0.8
Subtotal	19 ± 0.0	18 ± 1.9	17 ± 0.3	16 ± 1.2	16 ± 0.1	19 ± 0.1	18 ± 1.6	18 ± 2.7
Alcohols								
2-Hexyl-1-decanol	3.6 ± 0.3	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	2.5 ± 1.3	0.6 ± 0.2	0.6 ± 0.2	-
2-Octyl-1-decanol	6.2 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	3.6 ± 0.4	0.6 ± 0.0	0.7 ± 0.2	0.2 ± 0.0
2-Ethyl-1-dodecanol	2.0 ± 0.2	0.3 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	1.2 ± 0.8	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
2-Octyl-1-dodecanol	3.6 ± 0.2	0.5 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	2.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.0	1.0 ± 0.4
2-Isopropyl-5-methyl-1-heptanol	1.6 ± 0.1	1.9 ± 0.4	7.2 ± 5.2	1.8 ± 0.6	4.9 ± 0.5	1.4 ± 0.2	2.2 ± 0.5	2.0 ± 0.6
6-Amino-2-methyl-2-heptanol	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	-	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	-
2-Hexyl-1-octanol	2.4 ± 0.0	0.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.2	1.7 ± 0.9	0.7 ± 0.2	1.1 ± 0.0	0.3 ± 0.0
1-Nonen-3-ol	0.9 ± 0.6	1.5 ± 0.1	1.0 ± 0.2	1.4 ± 0.3	0.6 ± 0.1	13. ± 1.6	2.3 ± 0.3	1.6 ± 0.0
2-Butyl-1-octanol	1.4 ± 0.0	1.5 ± 0.4	9.4 ± 2.4	1.2 ± 0.3	6.7 ± 2.9	2.8 ± 0.6	2.2 ± 0.3	1.3 ± 0.5
1-Pentanol	6.2 ± 0.3	0.3 ± 0.0	0.2 ± 0.0	0.7 ± 0.2	3.4 ± 0.4	0.3 ± 0.2	0.5 ± 0.1	0.6 ± 0.3
12-Methyl-E,E-2,13-octadecadien-1-ol	1.3 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	1.6 ± 0.3	1.0 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	-
2-Methyl-1-undecanol	0.4 ± 0.0	0.8 ± 0.0	0.6 ± 0.5	1.1 ± 0.4	2.6 ± 0.3	0.5 ± 0.1	4.3 ± 0.5	1.2 ± 0.2
Subtotal	30 ± 1.9	9.0 ± 0.6	21 ± 7.9	10 ± 1.1	31 ± 5.3	22 ± 3.6	15 ± 2.4	8.5 ± 0.9
Esters and ethers								
Propanoic acid butyl ester	2.1 ± 1.2	0.8 ± 0.0	0.5 ± 0.1	0.5 ± 0.2	1.1 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	0.5 ± 0.0
Methoxyacetic acid 3-tridecyl ester	0.1 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
2-Butenoic acid hexyl ester	2.2 ± 0.1	0.5 ± 0.0	0.5 ± 0.2	0.8 ± 0.1	1.9 ± 0.9	0.5 ± 0.0	0.4 ± 0.2	1.2 ± 0.1
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-	0.7 ± 0.1	2.8 ± 0.0	1.3 ± 0.2	2.3 ± 0.7	0.7 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	2.7 ± 0.4
Subtotal	5.1 ± 1.8	4.4 ± 0.0	2.9 ± 0.5	3.8 ± 1.4	3.8 ± 1.2	2.9 ± 0.2	2.9 ± 0.4	4.7 ± 0.2
Arenes, furan and isocyanide								
1,3-Di-tert-butylbenzene	1.2 ± 0.1	1.2 ± 0.9	0.2 ± 0.0	0.2 ± 0.1	0.8 ± 0.6	1.3 ± 1.0	0.4 ± 0.2	0.4 ± 0.1
Tetrahydrofuran	4.2 ± 0.8	11 ± 3.3	3.9 ± 0.7	10 ± 5.1	3.6 ± 0.7	5.8 ± 0.7	5.9 ± 1.5	8.7 ± 0.6
Methyl isocyanide	2.7 ± 0.1	2.4 ± 1.0	4.2 ± 1.0	5.3 ± 2.0	2.6 ± 1.3	1.7 ± 0.2	2.9 ± 0.4	5.4 ± 1.5
Subtotal	8.1 ± 0.9	15 ± 3.1	8.3 ± 2.0	16 ± 4.5	7.0 ± 3.4	8.8 ± 2.2	9.3 ± 1.4	14 ± 0.8

*No statistical difference (Kruskal–Wallis, $p \leq 0.05$) among maturity levels for both Lawama and TXD306 variety. DGS – dough grain stage (15 – 21 days after 50% heading (DAH)), MGS – mature grain stage (22 - 28 DAH), FRS – fully ripe stage (29 – 35 DAH), ORS – over ripe stage (36 – 43 DAH).

Table 5.S4: Volatile compounds in rice-based products of TXD306 harvested at 18 DAH in relation to different processing practices

Compounds name	Relative amount (average peak area % ± standard deviation)							
	Raw	80-NS	80-S	100-NS	100-S	120-NS	120-S	*Pepeta*
Hydrocarbons								
Decane[#]	-	-	-	0.80±0.39	-	-	-	-
4-Methyldecane	0.35±0.04	0.32±0.03	0.35±0.03	0.26±0.07	0.27±0.07	-	-	0.40±0.14
2,4,6-Trimethyldecane	0.11±0.01	0.10±0.02	-	-	-	-	-	-
2,3,5,8-Tetramethyldecane*	0.40±0.05	0.96±0.17	0.82±0.21	4.17±0.35	3.29±0.14	8.02±0.55	5.54±0.45	4.34±0.04
Dodecane*	0.29±0.02	0.49±0.08	0.41±0.02	0.28±0.04	0.31±0.02	0.19±0.03	0.21±0.00	0.23±0.03
2,7,10-Trimethyldodecane*	-	0.14±0.03	0.12±0.02	0.18±0.04	0.14±0.01	0.58±0.03	0.25±0.00	-
2,6,10,14-Tetramethylheptadecane	-	0.52±0.44	0.82±0.39	0.94±0.23	0.74±0.36	0.78±0.09	0.33±0.27	0.28±0.08
2,6,11,15-Tetramethylhexadecane*	1.17±0.34	1.34±0.94	1.22±0.66	2.75±0.67	4.16±0.68	1.74±0.08	3.77±0.10	0.66±0.05
3-Methyl-5-propylnonane	0.15±0.08	0.22±0.12	0.16±0.08	0.23±0.07	-	0.15±0.01	0.16±0.01	0.18±0.05
2,2,4,4-Tetramethyloctane	0.65±0.32	0.77±0.38	0.97±0.04	0.55±0.17	0.31±0.18	0.36±0.09	-	-
Tetradecane	1.68±0.67	2.27±0.47	1.25±0.62	0.91±0.45	0.90±0.05	1.10±0.14	1.12±0.06	1.80±0.37
6,9-Dimethyltetradecane	0.66±0.32	0.55±0.09	-	1.39±0.76	-	1.76±0.82	2.56±1.28	0.45±0.23
2,6,10-Trimethyltetradecane*	-	0.16±0.03	0.12±0.02	0.18±0.03	-	0.59±0.02	0.27±0.00	-
6-Dimethyl(isopropyl)silyloxytetradecane*	-	-	-	-	-	0.21±0.05	0.14±0.01	-
Undecane[#]	0.12±0.00	-	-	-	-	-	-	-
3-Methylundecane*	-	0.16±0.03	0.11±0.02	-	-	-	-	-
4-Methylundecane*	2.45±0.24	4.36±0.96	3.78±0.66	2.47±0.72	2.59±0.88	1.82±0.30	2.21±0.07	2.31±0.76
1-Iodo-2-methylundecane*	0.37±0.07	0.62±0.13	0.41±0.08	0.98±0.10	0.57±0.09	6.06±0.31	1.82±0.08	0.77±0.21
Hexamethylcylotrisiloxane	0.46±0.07	0.23±0.04	0.16±0.05	-	0.17±0.12	-	0.28±0.03	-
Pentacos-1-ene	0.16±0.06	0.10±0.02	-	-	-	-	-	0.11±0.05
5-Tridecene,(z)-	7.15±0.40	7.93±0.36	8.76±0.24	6.11±0.38	6.98±0.46	6.13±0.71	5.89±0.64	7.14±0.63
Urs-12-ene	-	1.56±0.10	2.07±0.46	1.06±0.23	1.19±0.59	1.68±0.28	-	-
Subtotal*	17.3±2.34	23.1±1.38	21.9±1.51	23.6±3.35	22.1±1.51	31.6±1.56	25.7±1.80	19.8±1.36
Aldehydes and ketones								
Benzaldehyde	8.22±0.27	0.20±0.04	0.16±0.01	3.19±0.78	5.23±2.56	-	7.16±0.20	8.29±0.38
3-Benzyloxy-2-fluoro-4-methoxy benzaldehyde	0.44±0.04	0.40±0.05	0.66±0.06	0.59±0.01	0.50±0.11	0.44±0.09	0.54±0.05	0.51±0.03
α-Ethyl benzeneacetaldehyde	3.07±0.89	2.69±0.74	1.84±0.53	3.01±0.70	2.07±0.79	1.75±0.39	3.58±0.88	4.02±0.73

3-Methylbutanal*	0.87±0.41	0.88±0.30	0.65±0.25	0.27±0.07	0.26±0.12	0.16±0.07	0.58±0.05	1.25±0.63
Nonanal*	3.29±0.95	2.33±0.46	1.94±0.18	3.49±0.21	3.33±0.17	3.23±0.31	2.48±0.06	1.97±0.11
6,10-dimethyl-5,9-dodecadien-2-one*	0.35±0.03	0.29±0.09	0.13±0.06	0.18±0.07	0.23±0.03	0.56±0.02	0.22±0.02	-
6-Methyl-5-hepten-2-one	0.41±0.10	0.50±0.11	0.28±0.08	0.34±0.06	0.23 ±0.05	-	-	0.31±0.15
Subtotal*	16.7±1.46	7.29±1.34	5.68±0.50	11.1 ±5.10	11.9±5.14	6.27±0.44	14.6±1.21	16.4±0.39
Alcohols								
2-Hexyl-1-decanol*	6.32±0.11	6.23±0.31	6.35±0.04	5.76±0.26	5.89±0.33	5.14±0.04	5.06±0.12	5.94±0.55
2-Octyl-1-decanol*	7.54±0.15	7.24±0.61	6.67±0.28	6.09±0.05	5.99±0.34	5.15±0.12	4.88±0.05	6.61±0.21
2-Ethyl-1-dodecanol*	1.50±0.05	1.86±0.38	1.12±0.46	3.39±0.28	3.30±0.16	3.21±0.31	3.27±0.07	3.02±0.71
2-Octyl-1-dodecanol*	3.25±0.27	3.19±0.37	3.37±0.28	2.49±0.39	2.59±0.38	2.37±0.32	1.78±0.17	3.04±0.01
2-Isopropyl-5-methyl-1-heptanol*	-	0.12±0.03	-	0.26±0.02	0.12±0.01	1.70±0.09	0.82±0.00	0.40±0.09
2-Hexyl-1-octanol*	3.34±0.19	3.34 ±0.35	3.14 ±0.05	2.86 ±0.08	2.82 ±0.14	2.98±0.06	2.68±0.02	3.15±0.34
1-Nonen-3-ol	-	-	0.66±0.59	-	-	0.57±0.52	-	-
2-Butyl-1-octanol	0.54±0.89	0.35±0.05	0.48±0.04	0.35±0.09	0.43±0.13	0.51±0.02	0.59±0.03	0.45±0.20
12-Methyl-E,E-2,13-octadecadien-1-ol	2.14±0.22	2.32±0.28	2.46±0.18	2.20±0.15	1.88±0.09	2.00±0.36	2.04±0.07	1.96±0.19
1-Methoxy-2-propanol	0.30±0.19	0.26±0.12	0.41±0.24	0.38±0.09	0.29±0.09	-	-	0.21±0.08
2-methoxy-4-vinylphenol	2.79±0.69	3.78±0.58	4.23±0.33	3.69±0.04	3.76±0.50	4.14±0.63	4.51±0.87	3.70±0.94
Subtotal	27.8±1.32	28.7±1.05	29.0±0.36	27.5±0.64	27.1±1.31	27.8±0.90	25.7±1.11	28.5±0.80
Esters and ethers								
Propanoic acid butyl ester*	17.3±0.28	16.9±2.12	18.5±1.44	14.8±0.79	16.2±1.13	14.6±0.83	13.6±1.30	16.6±0.43
Butyl acetate*	-	0.36±0.18	0.60±0.07	0.52±0.05	0.72±0.06	0.45±0.03	0.46±0.01	0.20±0.12
2-Butenoic acid hexyl ester*	10.1±0.22	12.2±1.66	13.4±1.44	11.6±1.62	11.9±1.20	10.4±1.31	11.4±0.23	9.33±0.43
Butyl propionate	-	0.37±0.09	0.28±0.04	0.20±0.06	0.28±0.14	0.15±0.02	-	-
Subtotal*	27.4±0.39	29.8±3.17	32.6±1.96	27.1±1.32	29.0±2.28	25.6±0.61	25.5±1.22	26.1±0.87
Arenes, furan and isocyanide								
1,3-Di-tert-butylbenzene*	3.22±0.11	4.72±0.88	4.38±0.14	4.78±0.48	3.95±0.14	3.49±0.20	3.54±0.09	4.12±0.65
2-Pentyl-furan*	3.54±1.65	6.23±0.31	6.35±0.04	5.76±0.26	5.94±0.33	5.14±0.04	4.88±0.05	5.05±0.13
Methyl isocyanide	4.12±0.01	0.10±0.01	-	-	-	-	-	-
Subtotal	10.9±0.24	11.1±1.13	10.8±0.15	10.6±0.76	9.89±0.24	8.67±0.25	8.46±0.11	9.17±2.18

*Statistical difference (Kruskal–Wallis, $p \leq 0.05$) among processing treatments, #No statistical test performed. DAH – days after 50% heading, 80, 100 and 120 indicate rice roasted at the corresponding temperatures (°C), NS – not soaked, S – water-soaked paddy at room temperature for 12 h prior roasting, Raw – unprocessed paddy/rice, *Pepeta* – locally prepared *pepeta*/rice flakes.

Table 5.S5: Volatile compounds in rice-based products of variety TXD306 harvested at 26 DAH in relation to different processing practices

Compounds	Relative amount (average peak area % ± standard deviation)							
	Raw	80-NS	80-S	100-NS	100-S	120-NS	120-S	*Pepeta*
Hydrocarbons								
4-Methyldecane	0.10±0.03	0.11±0.09	-	-	-	-	-	-
2,4,6-Trimethyldecane	0.12±0.02	0.12±0.08	-	-	-	-	-	-
2,3,5,8-Tetramethyldecane	0.21±0.04	0.68±0.06	-	-	-	-	-	0.10±0.03
Dodecane	0.10±0.08	-	-	-	-	-	-	0.11±0.04
2,6,11-Trimethyldodecane	0.12±0.01	-	-	-	-	-	-	-
2,7,10-Trimethyldodecane*	-	-	-	-	-	0.31±0.01	0.18±0.02	-
2,6,10,14-Tetramethylheptadecane	0.28±0.03	0.47±0.24	0.19±0.02	0.21±0.02	0.26±0.04	0.20±0.05	0.23±0.01	0.15±0.02
2,6,11,15-Tetramethylhexadecane	0.42±0.21	0.89±0.07	-	1.37±0.89	-	0.99±0.29	1.15±0.51	0.36±0.18
3-Methyl-5-propylnonane*	2.92±0.42	4.04±0.57	1.57±0.13	1.77±0.33	2.14±0.31	1.57±0.49	1.93±0.14	2.07±0.45
2,2,4,4-Tetramethyloctane	1.05±0.21	0.32±0.25	0.48±0.03	-	0.60±0.10	0.51±0.19	0.58±0.08	0.70±0.09
Tetradecane[#]	-	-	-	-	-	0.11±0.04	-	-
6,9-Dimethyltetradecane*	0.32±0.09	5.12±0.83	0.26±0.00	0.86±0.15	0.37±0.01	3.30±0.50	1.99±0.22	0.23±0.05
2,6,10-Trimethyltetradecane	-	0.62±0.29	0.57±0.08	0.64±0.32	0.85±0.14	0.60±0.05	0.75±0.05	-
6-Dimethyl (isopropyl) silyloxytetradecane*	0.38±0.07	1.77±0.99	0.84±0.40	3.63±0.66	1.68±0.59	3.41±0.62	6.07±0.56	1.76±0.71
3-Methylundecane	0.28±0.08	-	-	-	-	-	-	0.19±0.04
4-Methylundecane[#]	-	0.83±0.07	-	-	-	-	-	-
1-Iodo-2-methylundecane*	-	-	-	-	-	0.29±0.03	0.18±0.01	-
Hexamethylcylotrisiloxane	0.75±0.59	-	0.20±0.14	0.46±0.24	-	0.17±0.13	-	-
Pentacos-1-ene	0.60±0.06	1.60±0.73	0.70±0.03	2.16±0.28	0.75±0.30	0.96±0.52	0.80±0.19	0.52±0.07
5-Tridecene,(z)-	2.95±0.56	2.94±0.73	3.66±0.23	2.24±0.77	2.38±0.74	1.56±0.19	2.12±0.98	4.20±0.37
4-Methyl-1-undecene*	0.36±0.05	0.30±0.09	0.14±0.02	0.11±0.09	0.19±0.03	0.12±0.09	0.15±0.02	0.28±0.07
Urs-12-ene	7.21±0.21	6.45±2.93	12.3±0.56	7.10±0.45	8.13±0.15	7.14±0.40	6.75±0.69	8.42±1.86
Subtotal	18.4±1.54	22.6±7.96	21.2±0.57	20.9±1.73	17.8±0.34	21.5±0.77	23.2±0.32	19.4±1.46
Aldehydes and ketones								
Benzaldehyde	0.13±0.08	5.56±2.42	6.87±0.30	5.22±2.55	5.84±2.84	5.18±2.54	-	7.60±0.39
3-Benzyloxy-2-fluoro-4-methoxy benzaldehyde*	0.53±0.09	1.04±0.55	0.44±0.03	0.76±0.18	0.63±0.07	0.50±0.04	0.49±0.04	0.42±0.07
α-Ethyl benzeneacetaldehyde	2.79±0.07	3.02±0.19	4.40±0.58	3.55±0.95	2.83±1.26	1.69±0.08	5.10±1.30	4.31±0.12

3-Methylbutanal	1.46±0.85	3.10±2.23	0.20±0.07	0.18±0.06	0.67±0.18	0.46±0.11	0.60±0.03	0.94±0.42
Nonanal	1.49±0.75	1.76±0.30	2.28±0.31	2.97±0.08	2.79±0.59	2.88±0.69	3.28±0.18	2.05±0.99
6,10-dimethyl-5,9-dodecadien-2-one	2.88±0.09	-	-	0.14±0.04	-	0.28±0.06	0.16±0.08	-
6-Methyl-5-hepten-2-one*	2.58±0.04	2.02±1.57	0.31±0.06	0.36±0.08	0.29±0.05	-	-	0.17±0.02
Subtotal	12.4±1.97	16.5±2.56	14.6±0.26	13.2±2.59	13.1±1.98	11.1±3.76	9.77±2.07	16.1±1.05
Alcohols								
2-Hexyl-1-decanol	7.18±0.26	5.86±0.41	5.68±0.22	5.88±0.28	6.09±0.03	5.79±0.67	5.59±0.25	5.66±0.27
2-Octyl-1-decanol*	8.25±0.15	6.18±2.45	7.68±0.55	6.09±0.27	6.92±0.21	5.88±0.54	5.66±0.38	7.41±0.28
2-Ethyl-1-dodecanol	1.59±0.07	2.12±0.57	1.55±0.12	2.88±0.31	2.06±0.76	2.48±0.66	2.53±0.82	3.51±0.41
2-Octyl-1-dodecanol*	3.91±0.73	2.37±1.00	3.72±0.70	2.50±0.18	3.18±0.14	2.59±0.15	2.37±0.45	2.77±0.23
2-Isopropyl-5-methyl-1-heptanol*	-	0.18±0.03	-	0.32±0.07	0.11±0.02	1.94±0.05	1.44±0.10	0.11±0.01
2-Hexyl-1-octanol	2.39±1.06	3.66±0.09	3.09±0.05	3.01±0.12	3.28±0.26	3.22±0.18	3.22±0.21	2.76±0.24
1-Nonen-3-ol	-	0.44±0.05	-	-	-	-	0.45±0.23	-
2-Butyl-1-octanol	-	0.29±0.12	0.22±0.01	0.35±0.06	0.36±0.08	0.34±0.10	0.46±0.04	0.44±0.11
12-Methyl-E,E-2,13-octadecadien-1-ol*	2.65±0.08	1.78±0.80	1.39±0.25	2.23±0.20	2.18±0.21	2.24±0.23	2.52±0.13	1.59±0.36
1-Methoxy-2-propanol*	-	0.73±0.15	0.46±0.06	0.32±0.08	0.35±0.08	0.23±0.02	0.27±0.11	-
2-methoxy-4-vinylphenol*	1.50±0.03	3.07±1.35	4.71±0.36	5.52±0.86	4.49±0.74	5.10±0.72	5.74±1.09	4.28±0.12
Subtotal	27.5±1.01	27.9±4.92	28.6±2.04	29.1±0.76	28.9±65	29.9±2.06	30.3±1.01	28.6±1.87
Esters and ethers								
Propanoic acid butyl ester	18.1±1.43	13.5±3.80	18.8±1.72	17.0±0.71	18.8±1.17	16.9±0.02	16.4±1.45	16.9±0.51
Butyl acetate	-	0.32±0.04	0.25±0.02	0.31±0.01	0.14±0.09	0.18±0.10	0.24±0.12	0.12±0.06
2-Butenoic acid hexyl ester*	13.9±0.61	10.8±2.42	6.78±2.57	13.3±1.61	12.9±0.52	13.3±1.57	14.4±1.04	9.64±0.42
Butyl propionate	-	0.17±0.09	0.10±0.09	-	0.17±0.01	0.14±0.01	0.15±0.02	0.19±0.00
Subtotal*	32.1±1.73	24.8±2.08	25.9±2.88	30.7±2.29	32.1±1.37	30.5±1.55	31.1±2.60	26.9±0.98
Arenes, furan and isocyanide								
1,3-Di-tert-butylbenzene*	2.43±0.08	1.12±0.52	2.00±0.18	-	1.52±0.21	1.22±0.13	-	1.57±0.16
2-Pentyl-furan*	4.84±2.18	6.94±0.70	7.68±0.54	6.02±0.15	6.29±0.35	5.79±0.67	5.59±0.23	7.42±0.28
Methyl isocyanide	2.24±0.02	0.12±0.09	-	-	-	-	-	-
Subtotal*	9.51±0.63	8.18±0.51	9.68±0.66	6.02±0.15	7.81±0.19	7.01±0.80	5.59±0.24	8.99±0.47

*Statistical difference ($p \leq 0.05$) among processing treatments, #No statistical test performed. DAH – days after 50% heading, 80, 100 and 120 indicate rice roasted at the corresponding temperatures (°C), NS – not soaked, S – water-soaked paddy at room temperature for 12 h prior roasting, Raw – unprocessed paddy/rice, *Pepeta* – locally prepared *pepeta*/rice flakes.

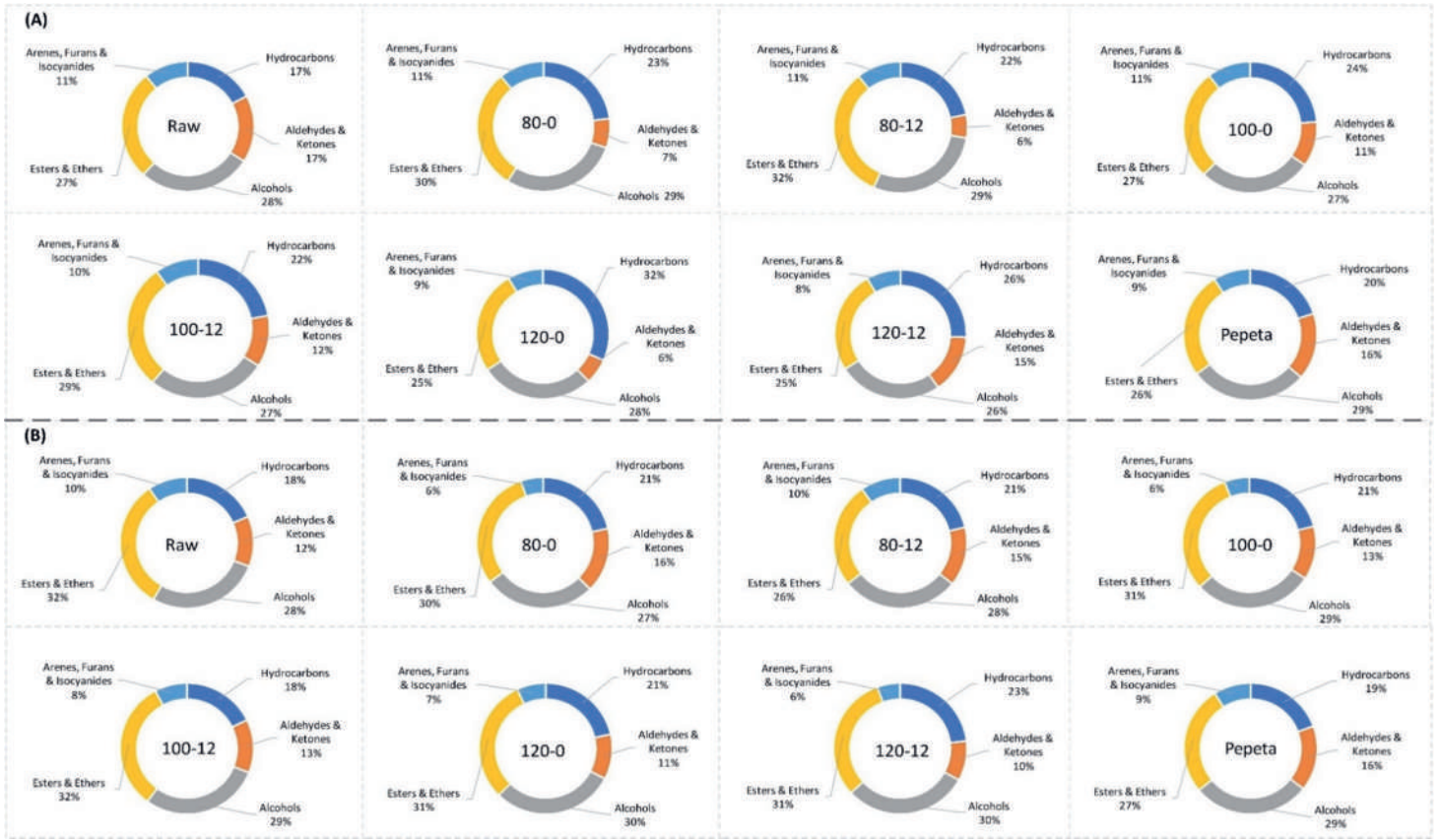


Fig. 5.S1: Variability of the volatile composition in rice flour at 18 days after 50% heading (DAH) (A) and 26 DAH (B) with different processing practices. 80, 100 and 120 indicate rice roasted at the corresponding temperatures (°C), 0 and 12 indicate unsoaked and water-soaked rice at room temperature for 12 h prior roasting respectively, Raw – unprocessed rice, *Pepeta* – locally prepared rice flakes. The bar charts represent the average percentage total peak area of triplicates for each group.

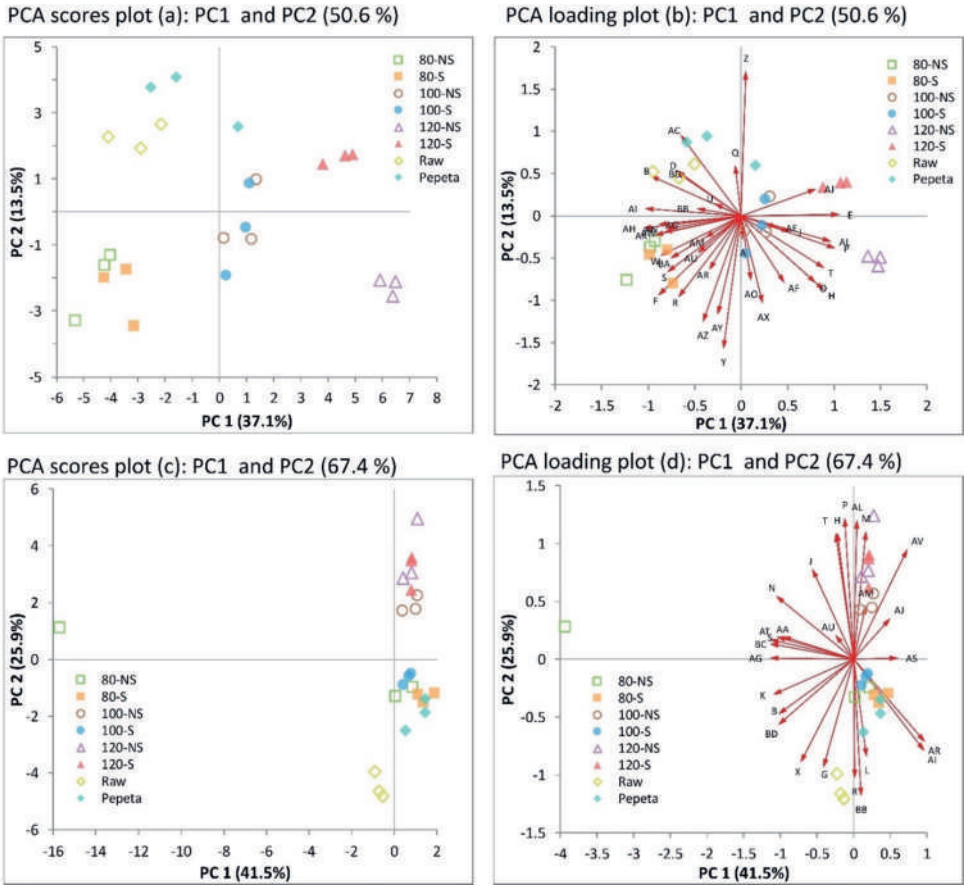


Fig. 5.S2: Two-dimension PCA plots on the volatile compounds of rice-based products extracted at 18 days after 50% heading (DAH, Panels a, b) and 26 DAH (Panels c, d) by HS-SPME GC–MS. Only volatiles compounds (Panels b, d) that showed significant differences ($P < 0.05$, Kruskal–Wallis test) among processing conditions are used in the PCA. 80, 100 and 120 – roasting temperatures (°C), NS – not soaked, S- water-soaked paddy at room temperature for 12 h prior roasting, *Pepeta* – locally prepared rice flakes, Raw – unprocessed dried paddy. For the interpretation of volatile compound codes refer to Table 5.S2.

CHAPTER 6.

General discussion

6.1 Introduction

Cereal grains are common staples in many countries, not only in Africa but also worldwide. Their production for dual purposes, i.e., as food and a source of income for the households across the Sub-Saharan Africa (SSA) countries, is rapidly growing. Typically, cereal grains are harvested when fully mature and consumed as a whole-grain meal or processed into various other cereal-based food products. However, most nutrients such as minerals, vitamins, and bioactive compounds decrease as cereal grains mature.[1–10] Therefore, enhanced local processing and utilisation of immature cereals to tap their potential nutritional benefits imply increased access to affordable, nutritious food. This will lead to improved nutrition and health status of the rural agricultural poor. This thesis project aimed to valorise a potentially nutritious though neglected immature cereal and its food products for enhanced food security in SSA. *Pepeta*, a locally processed immature rice product in Tanzania, was used as a case study to investigate changes in the nutritional, functional, and sensory properties upon processing immature rice. The thesis was designed in a context of malnutrition where most consumed foods are based on dehulled or polished cereal grains known to be nutritionally limited. The chapters in this thesis discuss the main findings (Table 6.1) and implications, relevance, opportunities, challenges, and future developments of the utilisation of immature cereal grains.

Table 6.1: Summary of main findings in this thesis

Aim	Main findings
Survey on pepeta processing knowledge	
• Gain insight into the *pepeta* processing knowledge and assess variations in the processing conditions and parameters across the study area. (**Chapter 2**)	• *Pepeta* contributes to food security as an early rice food • *Pepeta* is an essential treat for the ethnic groups in and around the study area. • Harvesting (i.e., maturity of the grains), roasting, and pounding were the most critical steps of *pepeta* processing. • General appearance, colour, texture and taste were associated with *pepeta* acceptability. • *Pepeta's* premium price contributes to the household earnings
Characterisation of immature rice and pepeta	
• Assess the effect of maturation and *pepeta* processing on the nutrient content and *in-vitro* digestibility of starch and protein of rice grains and *pepeta* products. (**Chapter 3**)	• Most nutrients decrease as the rice grains mature. • Maturity does not affect the *in-vitro* starch and protein digestibility. • *Pepeta* processing improves the nutritional properties of rice. • *Pepeta* processing conditions are adequate to fully gelatinise *pepeta* starch. • *Pepeta* has high and fast starch digestibility properties like that of cooked rice. • *Pepeta* can be a good source of highly digestible protein compared to cooked rice
Pepeta processing optimisation	
• Investigate the impact of roasting (dry-heat processing) at different conditions on the nutritional composition and *in-vitro* starch and protein digestibility of immature rice-based products. (**Chapter 4**)	• Dry heating improves vitamin and mineral content when compared to raw and cooked immature rice. • Dry heating reduces both protein and starch digestibility when compared to cooked immature rice. • Disulphide bonds play a vital role in heat-induced protein interactions in immature rice.
• Assess the effect of maturity and different processing practices on rice and immature rice-based products' visual quality and volatile profile. (**Chapter 5**)	• Maturity influences the colour and volatile profiles of rice grains. • Pounding affects the visual quality (grains size and colour) of *pepeta*. • Drying and roasting impact the colour of immature paddy grains. • Soaking before roasting limits browning. • Roasting modulates the volatiles' composition of immature rice.

6.2 Value addition along the rice value chain: locally processed rice-based food products

Cereals such as maize, rice, wheat, sorghum, millet, fonio and teff are popular and valuable components of the diet of millions of Africans, especially in SSA,[11,12] and the number of consumers is continuing to increase. They are the main source of nutrients and energy for many SSA household members as a staple food.[12] However, the cereal value chain, including rice, is characterised by high post-harvest losses of about 40 %, arising mainly from ineffective or inappropriate food processing technologies, careless harvesting and inefficient post-harvest handling practices.[13,14] These are among the significant factors constraining food and nutrition security in the developing countries of SSA, where seasonal food shortages and nutritional deficiency diseases are still a major challenge. In Eastern Africa, including Tanzania, rice is mainly consumed as milled white rice with limited processing into rice-based products,[15] whereas hydrothermal (parboiling) processing is the main paddy processing activity in West and Central African countries.[16,17] This calls for more efforts that would accentuate the upgrading, adoption, and utilisation of traditional food processing knowledge for improved food security in SSA.

In line with this rationale, the thesis project mapped out the locally prepared rice-based food products in Tanzania (**Chapter 2**). Fig. 6.1 highlights the main processing steps for each identified rice-based food product. This serves as a foundation step towards utilising traditional cereal processing knowledge in rural Tanzania to improve food security. We found that rice-based products prepared from mature rice grains dominated the list; they included cooked whole rice grains, coarse porridge or composite flour porridges (*uji*), stiff porridge (*ugali*), snacks, fermented products such as rice bread (*mkate*) and rice dough (*kitumbua*), and fermented drinks such as alcoholic beverages (*pombe*) and malts (*togwa*). Similar cereal-based products such as *uji*, *ugali*, bread, dough, as well as alcoholic and malt drinks have been reported for maize, wheat, sorghum, and millet in SSA.[18–24] Only three products prepared from immature rice grains were documented, namely *pepeta* (rice flakes), *bwimbwi* (*pepeta* in

powder form), and *ngalang'anyo* (a dehusked and polished immature parboiled rice). *Ngalang'anyo* is an intermediate product used to prepare cooked rice (*wali*) during the hunger period in Tanzania. Parboiled grains are cereal products prepared by roasting (dry heat parboiling) or steaming (wet heat parboiling) of soaked or wet grains to improve their nutritional and milling quality.[16,17,25] More traditional immature rice-based products are likely to exist, but may not be well documented and are therefore difficult to identify. Cooked white rice (*wali*) was the main form of consuming rice in Tanzania, while the popularity of other rice-based products varied across places and social-cultural groups (**Chapter 2**).

Comprehensive processing descriptions of the identified rice-based food products and their consumption patterns and nutritional properties are vital to any actionable step forward for enhancing processing technologies along the rice value chains in SSA. This would eventually put more food on the plates of producers and consumers alike. However, as a spin-off for this thesis project, i.e., valorisation of immature cereal-based products to enhance food security in rural vicinities, only *pepeta* processing knowledge and its product were used as a case study for in-depth research.

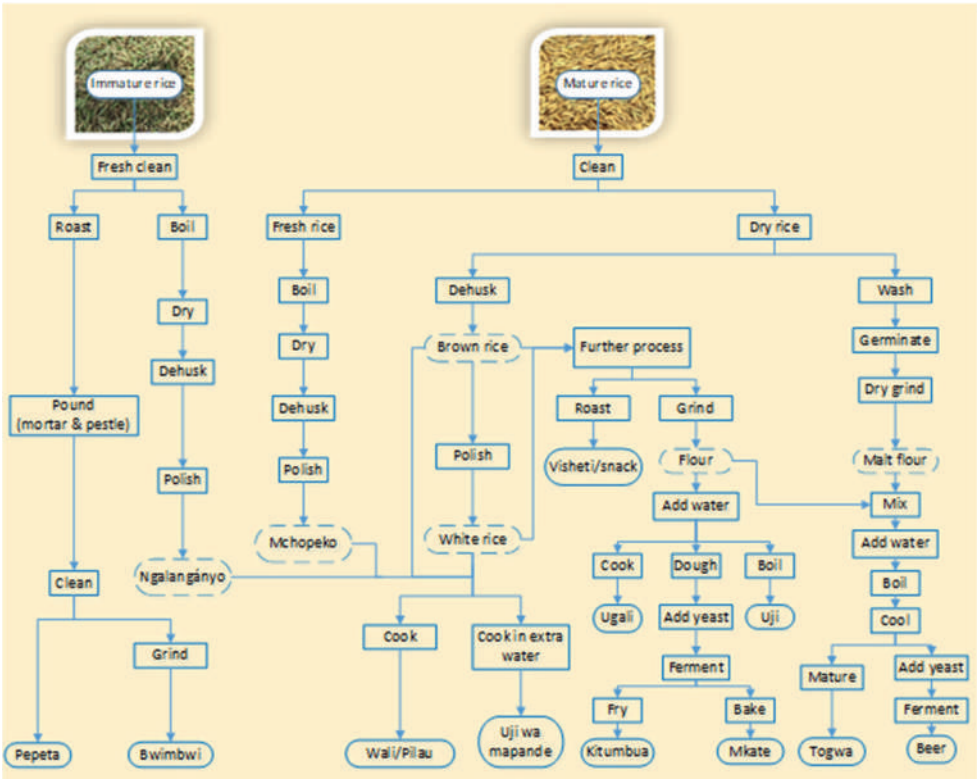


Fig. 6.1: Locally processed rice-based food products found in the study area (Tanzania), highlighting their main processing steps.

6.3 Processing and utilisation of *pepeta*: experience from indigenous knowledge

The potential for indigenous knowledge to contribute to the achievement of household food security is tremendous because the livelihood of the rural poor depends almost entirely on indigenous skills and knowledge, which are thus essential for their survival.[26] In addition, the traditional foods represent an essential part in the diet of many households in SSA because of their distinctive aroma, flavour and taste. It is well known that for any agricultural development and process to be effective, indigenous knowledge is of relevance; it has value not only for the culture in which it evolves but also for scientists and planners striving to improve conditions in rural localities.[27] Additionally, the recognition, promotion, and

utilization of indigenous knowledge, skills, and practices in food handling, processing, preservation, and storage are another way of attaining food security.[26]

Therefore, to position traditionally processed immature cereal-based products into the mainstream of efforts to reduce food insecurity, especially among the rural poor, **Chapter 2** surveyed the possible factors that can affect the *pepeta* value chain, as well as *pepeta* processing elements that interact with food and livelihood security. As we move on with the quest – *why the production of immature cereal-based products such as pepeta?* – we found that the *pepeta* product was used to break the hunger period, i.e., a source of early food when households have no food as the crops in the field are not yet fully mature. Interestingly, the use of immature cereal-based products such as green maize, green millet, green sorghum, green wheat, and green rice to break hunger is common in Africa,[18,19,28] where many countries are ranked low on the global food security index.[29] Therefore, the origin of most immature cereal-based products could be due to food scarcity, in line with Maxwell *et al.*[30], who theorised that harvesting grains at immature levels is often a sign of food insecurity.

Pepeta processing involves simple, low-cost, readily available materials and technology that fit the context of rural poor households. The optimum maturity of paddy grains, as well as the roasting and pounding, were found to be the most critical steps during *pepeta* processing; the maturity level and roasting conditions determine the colour and appearance of the *pepeta* product, whereas immediate pounding of hot roasted paddy grains ensures proper flattening of rice grains (i.e., the flaking process) as pounding of cooled grains would result in non-flattened rice grains (**Chapter 2**). Moreover, the use of immature paddy grains as a sole ingredient makes the *pepeta* product a potential natural, safe and healthy snack food, as an excessive use of cooking oil, salt and sugar has been associated with the increasing epidemic of noncommunicable diseases such as obesity, hypertension, diabetes, cardiovascular diseases, and certain types of cancer.[31,32] However, despite its popularity, *pepeta* production

was mainly at household level, varying among and within processors. The observed variations are not surprising but relatively common for many African foods based on indigenous knowledge since their prediction is based on an unwritten body of knowledge, held in different brains, languages and skills, varying across many groups, cultures and environmental conditions.[33] Generally, the observations show us the importance of indigenous knowledge, such as regarding *pepeta* processing, for increasing our scientific understanding as this encompasses knowledge that may be especially valuable in improving food security.

6.4 Product optimisation: laboratory simulation of *pepeta* processing technology

Pepeta processing knowledge can be assessed from various perspectives such as from a health and sustainability angle; focusing on the raw ingredients used, firewood consumption, processing time and tediousness, as well as the safety, sensorial and nutritional aspects of the final product. Sustainability is a broad concept addressing various aspects like ecology, economy and society, and its meaning varies depending on the context. According to the FAO,[34] sustainable diets are *"protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy while optimising natural and human resources."* From this point of view, the use of firewood, the high energy loss due to roasting on an open-air system and the labour intensiveness (**Chapter 2**) make *pepeta* processing technology less sustainable.

The changes that the nutritional and digestion properties of immature rice grains undergo upon processing into *pepeta* received further attention in **Chapter 3.** Generally, *pepeta* processing improved the nutritional properties of rice grains, demonstrating its potential use as a healthy snack or ingredient in cereal-based formulas. However, a very high and fast starch digestibility like that of cooked milled and polished rice, diminishes the health benefits of the *pepeta* product. The consumption of food having a fast and high starch digestibility

increases postprandial blood glucose levels associated with a high occurrence of type II diabetes.[35,36]

Therefore, these findings necessitate further improvement of *pepeta* processing techniques and the nutritional quality and functional properties of traditionally processed *pepeta* products. From this point of view, the processing conditions that improve the specific nutritional, functional and sensory properties of traditionally processed *pepeta* products (i.e., maturity and roasting conditions (soaking and temperatures), see Fig. 6.2) were the targets for optimisation under laboratory simulation of *pepeta* processing (**Chapters 4 and 5**).

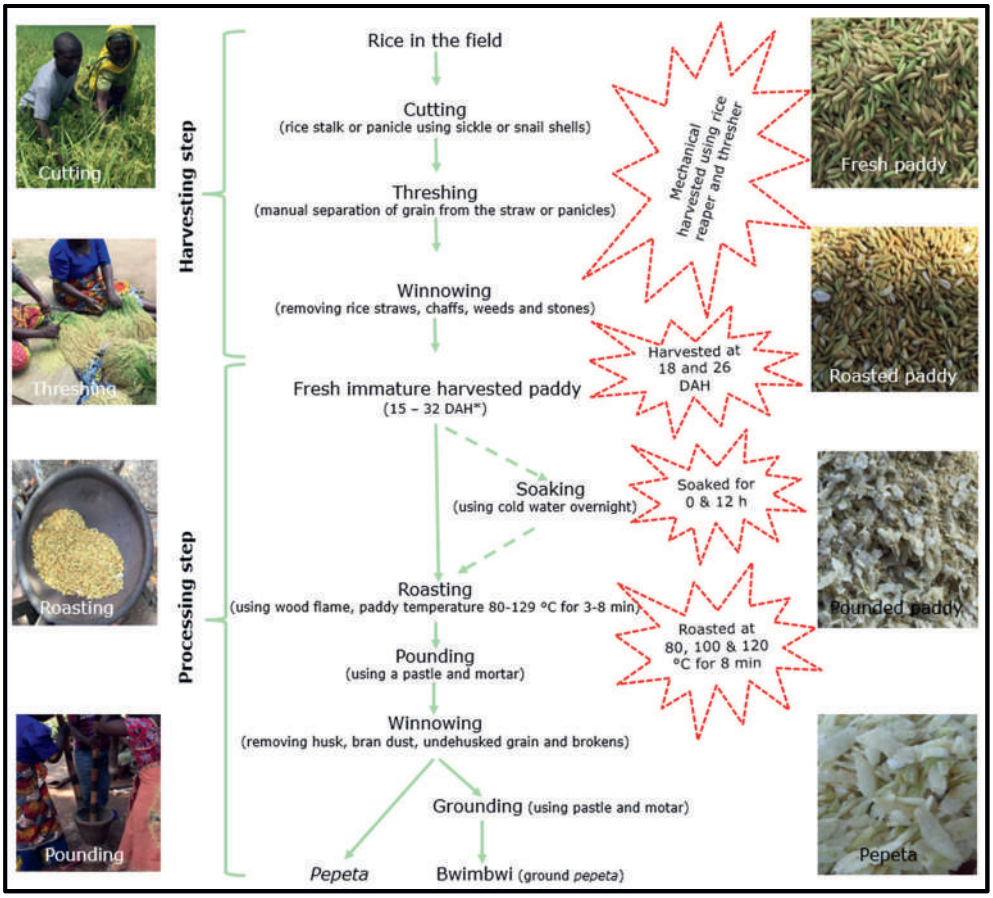


Fig. 6.2: *Pepeta* processing flow diagram indicating optimised processing steps (in red). *DAH – days after 50% heading

Three main *pepeta* processing steps were targeted for the optimisation study: (i) harvesting time to assess the optimum maturity (i.e., 18 and 26 DAH) of paddy grains used for *pepeta* production to validate our previous findings (**Chapter 3**); (ii) soaking (for 0 and 12 h using cold water at ambient temperature) – a preparation step used by processors to prolong the freshness of harvested paddy grains until the next day, as well as to soften and standardise the moisture content (**Chapter 2**) since grains do not mature evenly in the fields, not even on the same panicle[37,38] and (iii) roasting temperature at three levels (80, 100 and 120 °C), i.e., the extreme (minimum and maximum) and average temperatures used during traditional *pepeta* processing (80 – 129 °C, **Chapter 2**). The temperatures were also applied to produce *pepeta* using a sustainable technique, i.e., an electric hot air fluidised roaster, which is labour-saving and energy-efficient equipment. It is well known that starch gelatinisation of processed wet paddy grains is a function of temperature, greatly influencing starch digestibility properties.[39–41] Thus, understanding the impact of optimised *pepeta* processing conditions as compared to those from cooked milled white rice regarding starch digestibility properties was crucial for producing a healthier *pepeta* product.

6.5 The role of maturity on nutritional quality and health-related functional properties of cereal grains

Cereal grains are generally harvested and consumed fully mature. However, recent research has focused on the nutritional properties of immature grains, aimed at assessing the nutritional and functional profiles of grains at different stages of maturation. As immature grains have potential nutritional benefits compared to their mature counterparts, this thesis investigated the effect of maturity on the nutrient content of rice grains harvested for *pepeta* processing and the *in-vitro* digestibility of their starch and protein (**Chapter 3**). The maturity of harvested rice grains destined for *pepeta* production ranged from 15 – 28 DAH (i.e., days

after 50 % heading), whereas the optimum harvest maturity for maximum grain yield and head rice recovery is 30 – 36 DAH.[42] The immature rice grains had a higher amount of most macronutrients such as lipids, protein and ash, and soluble and insoluble dietary fibre compared to corresponding fully mature rice grains. However, maturation did not affect the *in-vitro* starch and protein digestibility (**Chapter 3**). Similar results were also reported for protein content in maize[1] and sorghum,[43,44] lipid content in wheat,[2] and dietary fibre in wheat[2] and barley[3,4,45] grains during maturation, showing the potential of immature grains to be used as functional food ingredients. Higher amounts of total sugars, glucose, maltose and fructose were found in immature grains of crops such as rice,[5,46] wheat,[3,6,7] maize[1] and barley[45] compared to their mature grains, because sugars are used in starch synthesis during maturation. However, the association between sugar and starch content was not established in this thesis, as no data were collected for sugar content. Moreover, starch slightly decreased as rice grains developed from 15 – 43 DAH (**Chapter 3**), which was associated with increased protein since proteins are involved in starch synthesis and accumulation in developing grains.[47,48]

The amounts of micronutrients in the cereal grains were also affected by maturation. Higher amounts of B group vitamins such as thiamine, nicotinic acid, nicotinamide were observed in immature rice compared to its mature counterparts (**Chapter 3**). Similar results in rice were reported by Ji *et al*.[49] as well as for wheat and barley.[2,6] Likewise, mineral content decreased during maturation of grain crops such as rice,[49] wheat,[50,51] maize[9,10] and sorghum,[52] depending on differences in grain varieties, cultivation conditions and compounds,[53] with some exceptions. For instance, iron and zinc increased as rice grain matured in this thesis (**Chapter 3**), while calcium and zinc were relatively stable for wheat,[51] as well as copper and phosphorus for rice.[49]

6.6 The impact of roasting conditions on nutritional, functional and sensory properties of processed immature rice grains

Roasting has been practised to prepare cereal snacks for hundreds of years as it is the simplest, cheapest and quickest traditional method of the application of dry heat. For instance, roasting is the intermediary process in the production of expanded and popped rice-based products.[54] A relatively well-known and widely used traditional process of preparing flaked rice involves roasting soaked paddy before a flaking step,[55] similar to the *pepeta* processing technology although soaking of the freshly harvested, immature paddy is optional. Roasting of freshly harvested paddy or soaked paddy grains resembles a heat-moisture or dry-heat parboiling process that influences the nutritional, functional, and sensory properties of cereal-based foods, where the impact highly depends on the severity of the processing conditions.[39–41,56] Thus, the evaluation of nutrient bio-accessibility, sensory properties and product functionality is essential to be able to optimise the processing of heat-processed food products. As such, the impact of the optimised conditions on the nutrient and digestibility properties of laboratory simulated *pepeta* products received particular attention in **Chapter 4**. We found that the optimal roasting conditions (i.e., soaking and temperatures) differently affected the nutrient content and product functionality. For example, among the evaluated B vitamins, riboflavin and nicotinamide increased while thiamine decreased with an increase in roasting temperature. The increase in micronutrients due to inward diffusion into the starchy endosperm and loss of some micronutrients due to heat is well established for thermal processing of whole cereal grains such as paddy.[25,57]

Soaking of cereals and legumes is commonly employed as an innovative strategy to increase the bioavailability of micronutrients, including iron and zinc, from plant materials containing high phytate levels.[58] This increase of micronutrients bioavailability due to reduction in phytic acid caused by wet processing such as soaking is based on a combination of leaching of

phytates and the activity of endogenous phytase.[59,60] In this thesis, soaking before roasting greatly increased the iron content compared to zinc, thiamine, riboflavin and nicotinamide (**Chapter 4**). This indicates the possibility for processed immature cereal products that were soaked before roasted to combat iron deficiency. We further observed that, although total dietary fibre decreased with increasing roasting temperature, the ratio of soluble fibre to insoluble fibre increased. Similar results were also reported for lupin grains,[61] indicating the potential of *pepeta* processing technology to enhance the health functionality of fibre for immature cereal-based products.

Dry autoclaved wheat products,[62] dry parboiled paddy[39] products and dry roasted bambara groundnut products[63] were digested more slowly *in-vitro* than completely gelatinised products such as boiled samples, indicating that the more severe the processing condition, the more rapid the digestion of starch. We found similar results in this thesis (**Chapter 4**), where the *in-vitro* starch digestibility of roasted immature products increased with the severity of the processing conditions. However, the starch digestibility was lower than that of cooked immature rice, which provides options to manipulate roasting temperature to produce immature rice-based products with a desired glycemic index. Concerning protein, both roasting and cooking improved *in-vitro* protein digestibility of immature rice-based products; cooking provided a high protein value compared to roasting. Similar results were reported by Mubaiwa,[63] who found higher *in-vitro* protein digestibility values in boiled bambara grits compared to both dry roasted, and combined soaked and roasted grits. However, within the roasted products, *in-vitro* protein digestibility did not lineraly increased with roasting temperature in this thesis; digestibility increased when immature rice was roasted at lower temperatures (80 and 100 °C) while it greatly decreased upon roasting at a higher temperature (120 °C). Reports have suggested that thermal processing (including dry heating) reduces protein digestibility of cereals, attributable to the formation of disulphide and hydrophobic-

bonded aggregates between various proteins.[64–66] However, no association was found between protein digestibility and protein aggregations in the current research (**Chapter 4**).

Roasting of starchy foods, including cereals, imparts desirable taste and flavours, thereby directly affecting the evaluation of the sensory quality and consumer acceptability.[67] Volatile compounds such as furans, pyrazines, aldehydes, and ketones greatly contribute to the characteristic flavour of roasted products.[68] Only furans and phenolic volatile compounds increased with roasting temperature in this thesis (**Chapter 5**). Conceivably, using a tightly closed roasting system to prevent evaporation of volatile compounds and immediate assessment of the volatile compounds without dehulling of the roasted cereals could have enhanced the quantity of volatiles of the roasted products, including pyrazine compounds. However, this would be elaborating on a different food product, not a traditional roasted *pepeta* product. Furthermore, although we identified the distribution and abundance of volatiles in the processed immature rice products, the threshold values of active aroma compounds, and the relationship between consumer preferences and the inherent volatile compounds were not established. To attain this, we suggest further research using a more sensitive method such as gas chromatography-tandem mass spectrometry (GC-MS/MS) combined with gas chromatography (GC)- olfactometry and trained panellists.

6.7 Processed immature rice-based products as a food-based approach to combat micronutrient deficiencies

Many poor households in the SSA region rely on agriculture for their livelihoods – many of whom suffer from undernutrition, e.g., inadequate micronutrient intake resulting from monotonous starchy staple diets based mainly on energy-dense food products. A food-based strategy is a sustainable, long-term community-focused approach to meet micronutrient needs. An essential element to food-based approaches involves improving food processing,

preservation and preparation techniques that enhance the nutrient density of cereal-based diets.[69] Increasing production and consumption of micronutrient-dense cereal-based foods is associated with an increased household individual probability of adequate micronutrient intake in developing countries as poorer consumers often allocate a larger share of their income to food types with high-calorie contents and a lower cost per calorie.[70]

Additionally, starchy foods tend to have high phytate and fibre contents that affect the bioavailability of micronutrients such as iron and zinc.[71,72] Germination, fermentation, and soaking can reduce the phytate content of cereals and legumes,[58–60,73] thereby enhancing micronutrient bioavailability. In this thesis, we found a significant increase of iron content in products that were soaked before roasting (**Chapter 4)**, which makes a case for optimised *pepeta* processing knowledge to possibly combat dietary iron deficiency affecting children and women of reproductive age. This line of reasoning is based on the recommended nutrient intakes (RNI's) for iron from meals with a dietary iron bioavailability of 5 % (i.e., a monotonous cereal diet containing a preponderance of iron absorption inhibitors).[74] As shown in Table 6.2, 250 g of soaked-roasted product is enough to provide the maximum requirement of dietary iron intake per day, upon consumption for infants (0.5 – 1 year), children (1 – 10 years), male adults (above 18 years) and women at post-menopausal age (above 50 years), whereas more than 50 % is contributed to the required RNI for boys (10 – 18 years), and women at reproductive age (18 – 50 years) and lactating. *Bwimbwi*, a powdered *pepeta* product (Fig. 6.1), can be the alternative formulation to *pepeta* as a complete iron-rich weaning food for infants and children, served after mixing with hot water or milk. Contrary to iron, zinc was only marginally affected by roasting conditions (i.e., soaking and temperatures). Consumption of 250 g of processed immature rice-based food will not give 100 % of the RNI, based on the low zinc bioavailability (15%) due to high phytate contents.[75]

However, the dietary iron and zinc bioavailability depends not only on the content in the meal but also on its composition, i.e., the balance between their absorption inhibitors (tannins and phytates) and absorption enhancers such as vitamin C present in the diet.[76–78] The content in tannins and phytates was not determined in the present thesis, but we recognise that it is useful for any implementation action. Furthermore, the enrichment of immature processed cereal-based food products such as *pepeta* and *bwimbwi* with, for instance, locally available wild fruit pulp juices rich in vitamin C and organic acids, such as monkey orange (*Strychnos* spp.),[79] baobab (*Adansonia digitata*)[80,81] and tamarind (*Tamarindus indica* L.)[82], can be a good strategy to enhance iron absorption. The high contents of organic acids of the juices impart a fresh flavour and lower the pH, which contributes to food preservation and safety. Moreover, the juices affect the mouthfeel, making consumers perceive a reduced thickness of the final product.[79]

Table 6.2: Contribution (in %) of the intake of 250 g of processed immature rice-based products to the recommended nutrient intake (RNI) of iron and zinc.

Age group (years)*	Fe (mg/kg, db)	5 % bioavailability** RNI** (mg/day)	5 % bioavailability** % contribution	Zn (mg/kg, db)	15 % bioavailability** RNI** (mg/day)	15 % bioavailability** % contribution
Infant (0.5-1)						
– Soak & roasted	118	18.6	**159**	11.9	6.6	45.2
– Roasted	82.2	18.6	**110**	11.2	6.6	42.4
– *Pepeta*[#]	62.1	18.6	83.5	14.5	6.6	54.9
– Raw	27.5	18.6	37.0	13.1	6.6	49.8
Children (1-10)						
– Soak & roasted	118	17.8	**166**	11.9	11.2	26.6
– Roasted	82.2	17.8	**115**	11.2	11.2	25.0
– *Pepeta*	62.1	17.8	87.2	14.5	11.2	32.4
– Raw	27.5	17.8	38.7	13.1	11.2	29.3
Boys (10 – 18)						
– Soak & roasted	118	37.6	78.5	11.9	17.1	17.4
– Roasted	82.2	37.6	54.6	11.2	17.1	16.4
– *Pepeta*	62.1	37.6	41.3	14.5	17.1	21.2
– Raw	27.5	37.6	18.3	13.1	17.1	19.2
Girls (10 – 18)						
– Soak & roasted	118	65.4	45.1	11.9	14.4	20.7
– Roasted	82.2	65.4	31.4	11.2	14.4	19.4
– *Pepeta*	62.1	65.4	23.7	14.5	14.4	25.2
– Raw	27.5	65.4	10.5	13.1	14.4	22.8
Male (18^{+})						
– Soak & roasted	118	27.4	**108**	11.9	14	21.3
– Roasted	82.2	27.4	75.0	11.2	14	20.0
– *Pepeta*	62.1	27.4	56.7	14.5	14	25.9
– Raw	27.5	27.4	25.1	13.1	14	23.5
Female Menstruating (18-50)						
– Soak & roasted	118	58.8	50.2	11.9	9.8	30.4
– Roasted	82.2	58.8	34.9	11.2	9.8	28.5
– *Pepeta*	62.1	58.8	26.4	14.5	9.8	37.0
– Raw	27.5	58.8	11.7	13.1	9.8	33.5
Post-menopausal (50^{+})						
– Soak & roasted	118	22.6	**131**	11.9	9.8	30.4
– Roasted	82.2	22.6	90.9	11.2	9.8	28.5
– *Pepeta*	62.1	22.6	68.7	14.5	9.8	37.0
– Raw	27.5	22.6	30.5	13.1	9.8	33.5
Lactating						
– Soak & roasted	118	30	98.3	11.9	19	15.7
– Roasted	82.2	30	68.5	11.2	19	14.7
– *Pepeta*	62.1	30	51.7	14.5	19	19.1
– Raw	27.5	30	22.9	13.1	19	17.3

% contributions are based on normative storage requirement estimate and low bioavailability diet (5 % for iron and 15 % for zinc), for consumption of 250 g food product per day. [#]Immature rice-based product locally roasted and pounded. *Highest RNI among age sub-group was considered. **from WHO[83]

On the other hand, the preparation of cooked rice (*wali/pilau*, Fig. 6.1), which is the main form of consuming rice in Tanzania, involves washing and soaking rice grains in water, leading to loss of water-soluble vitamins such as thiamine, riboflavin and niacin. This does not occur in the processing of immature rice-based products such as *pepeta* as no water is used. Considering the benefits of these components in the diets, their contribution (in %) to the recommended nutrient intakes (RNI) was calculated (Table 6.3). The calculations show that the assessed immature rice-based food products in this thesis are rich sources of water-soluble B group vitamins. Consumption of 250 g of the products (soaked & roasted, roasted and *pepeta*) will provide more than 100 % of the RNI for riboflavin (B_2), and more than 50 % of te required RNI for niacin-nicotinic acid (B_3) and niacin-nicotinamide ($B_{3'}$). *Pepeta* product is assumed to meet the RNI for thiamine. The provided amount of niacin did not exceed the tolerable upper intake level (UL) of 15, 30 and 35 mg/day for children (1-10 years), boys/girls (10-18 years), and adult men/women (above 18 years),[84] respectively. No calculations on the UL were done for thiamine and riboflavin as there is no established toxic level.

Table 6.3: Contribution (in %) of the intake of 250 g of several processed immature rice-based products to the recommended nutrient intake (RNI)) of thiamine (B_1), riboflavin (B_2) and niacin (nicotinic acid B_3 and nicotinamide $B_{3'}$)

	B_1 (35 % bioavailability)[#]			B_2 (60 % bioavailability)[$]			B_3 (24 % bioavailability)[†]			$B_{3'}$ (24 % bioavailability)[†]		
Age group (years)	Content*	RNI**	%***	Content	RNI	%	Content	RNI	%	Content	RNI	%
Children (1-10)												
– Soak & roasted	0.1	0.3	37.9	18.8	0.4	**7061**	18.1	4.0	**271**	15.2	4.0	**227**
– Roasted	0.1	0.3	26.3	14.6	0.4	**5486**	16.5	4.0	**247**	13.9	4.0	**208**
– *Pepeta*	2.0	0.3	**575**	64.0	0.4	**23989**	17.6	4.0	**264**	18.4	4.0	**275**
– Raw	0.5	0.3	**137**	24.5	0.4	**9184**	19.9	4.0	**298**	2.1	4.0	32.0
Boys (10 – 18)												
– Soak & roasted	0.1	0.9	12.6	18.8	0.9	**3138**	18.1	12.0	90.4	15.2	12.0	75.8
– Roasted	0.1	0.9	8.80	14.6	0.9	**2438**	16.5	12.0	82.4	13.9	12.0	69.3
– *Pepeta*	2.0	0.9	**192**	64.0	0.9	**10662**	17.6	12.0	87.9	18.4	12.0	91.8
– Raw	0.5	0.9	45.7	24.5	0.9	**4082**	19.9	12.0	99.5	2.1	12.0	10.7
Girls (10 – 18)												
– Soak & roasted	0.1	1.1	10.3	18.8	1.3	**2173**	18.1	16.0	67.8	15.2	16.0	56.8
– Roasted	0.1	1.1	7.20	14.6	1.3	**1688**	16.5	16.0	61.8	13.9	16.0	52.0
– *Pepeta*	2.0	1.1	**157**	64.0	1.3	**7381**	17.6	16.0	65.9	18.4	16.0	68.8
– Raw	0.5	1.1	37.4	24.5	1.3	**2826**	19.9	16.0	74.6	2.1	16.0	8.00
Men (18$^+$)												
– Soak & roasted	0.1	1.2	9.50	18.8	1.0	**2825**	18.1	16.0	67.8	15.2	16.0	56.8
– Roasted	0.1	1.2	6.60	14.6	1.0	**2195**	16.5	16.0	61.8	13.9	16.0	52.0
– *Pepeta*	2.0	1.2	**144**	64.0	1.0	**9596**	17.6	16.0	65.9	18.4	16.0	68.8
– Raw	0.5	1.2	34.3	24.5	1.0	**3674**	19.9	16.0	74.6	2.1	16.0	8.00
Women (18$^+$)												
– Soak & roasted	0.1	1.1	10.3	18.8	1.1	**2568**	18.1	14.0	77.5	15.2	14.0	64.9
– Roasted	0.1	1.1	7.20	14.6	1.1	**1995**	16.5	14.0	70.6	13.9	14.0	59.4
– *Pepeta*	2.0	1.1	**157**	64.0	1.1	**8723**	17.6	14.0	75.3	18.4	14.0	78.6
– Raw	0.5	1.1	37.4	24.5	1.1	**3340**	19.9	14.0	85.2	2.1	14.0	9.10
Women lactating												
– Soak & roasted	0.1	1.2	9.50	18.8	1.6	**1765**	18.1	17.0	63.8	15.2	17.0	53.5
– Roasted	0.1	1.2	6.60	14.6	1.6	**1372**	16.5	17.0	58.1	13.9	17.0	48.9
– *Pepeta*	2.0	1.2	**144**	64.0	1.6	**5997**	17.6	17.0	62.0	18.4	17.0	64.8
– Raw	0.5	1.2	55.0	24.5	1.6	**2296**	19.9	17.0	70.2	2.1	17.0	7.50

*Content in mg/100g dry basis, **RNI in mg/day from WHO,[83] ***Calculated % contribution based on bioavailability by [#]Khokhar and Kappoor,[85] [$]Dainty et al.[86] and [†]Cartel and Carpernter[87] for consumption of 250 g food product per day.

6.8 Opportunities and limitations for utilisation of immature cereal grains: focus on small and medium-sized enterprises in SSA

In SSA countries, many high-yielding varieties of staple cereals such as rice, maize, millet and sorghum have been released by the NARS (National Agricultural Research System) in collaboration with the CGIAR (Consultative Group on International Agricultural Research) centres. However, despite their popularity for their high yields, many varieties are not preferred for consumption by consumers due to their poor cooking quality and are thus rated as low-grade varieties.[88–90] Considering their nutritional potential, the use of immature grains offers the opportunity to better exploit less attractive hybrid varieties, hence tapping their underutilised high-yield potential for food security. Furthermore, various processed products can be enhanced with immature cereals, which increases the possibilities. Most traditional processed cereal-based products in SSA countries are produced at a household level and a few at a small scale. Generally, they have a short shelf life.[18,19] Improvement of traditional value-added immature cereal-based products such as *pepeta*, can create jobs in rural areas and will help to make the products compete globally as import substitutes or even for export to niche markets.[89]

The practicability of simple, low-cost and labour-saving food processing technologies such as for producing ready-to-eat snack foods by, for example, extrusion cooking, popping, puffing and flaking in SSA are promising. These technologies can improve the functional and nutritional quality of processed cereal-based products by enriching or completely preparing foods with immature grains and thus their acceptability through adopting local recipes using a consumer-oriented approach. They are the bedrock of small-scale food processing enterprises that are crucial to rural development, reducing post-harvest food losses and increasing food availability in SSA. By generating employment opportunities in the rural areas, small-scale food industries reduce rural-urban migration and the associated social problems.[94] However, poor management, inadequate working capital, and limited access to potable water, cheap and

reliable energy, as well as banks and other financial institutions, high-interest rates and low-profit margins hamper the adoption of these technologies.[91,92] Besides, policies and harsh regulatory requirements are among the factors external to the small and medium-scale enterprises that hinder their growth and development in SSA countries. For instance, agricultural produce cess, i.e., a levy charged by local authorities at a percentage of the farm-gate price on products sold by the farmer to traders, is used in several SSA countries, especially in Eastern Africa. According to Ricome *et al.*[93], the produce cess reduces farmers' revenues significantly and consequently their incentive to produce more commodities. This eventually hastens food insecurity and poverty.

On the other hand, increasing exposure and raising public awareness through disseminating processing knowledge and information on the nutritional benefits of immature cereal grains, as well as training and encouraging small and medium cereal-based enterprises, will drive the adoption of immature cereal-based products, thus improving food security in SSA. Moreover, as Vipham *et al.*[94] well put it, *"there is no food security without food safety*." This means, to improve food security, SSA countries should also strengthen their capacity and investments toward improvements of the nutritional quality and safety of food, and a reduction in food- and water-borne illnesses. Regrettably, the pace of such investments is not satisfactory, leaving a huge burden on the SMEs when they want to upgrade or shift to a formal business, i.e., a colossal cost not only in acquiring processing machinery and storage facilities but also to adhere to national, regional or international food quality and safety standards.

6.9 Towards new immature cereal-based foods: the way forwards

Food insecurity remains prevalent in SSA countries and is closely associated with poverty whereby household members suffer from a lack of access to sufficient food, leading to hunger, malnutrition and illnesses. As the primary sector for the production of foods, agriculture plays a major role in food security, i.e., by ensuring food availability. For decades,

governments and other food system actors have implicitly and explicitly emphasised actions that promote agricultural production of abundant cheap food as the key means to end hunger and eventually food insecurity problems, e.g., the dissemination of "Green Revolution" technologies.[95] However, the conceptual linkages between hunger and the dimensions of food security (food availability, access, and consumption) were not clearly established, i.e., food availability is necessary but on its own insufficient towards achieving the ultimate food security goal of nutritional well-being. This is evident in the fact that micronutrient deficiencies (hidden hunger) remain a public health issue in SSA and worldwide, despite the substantial increase in the number of people having access to sufficient food. Today more support is also provided for research and development, and value addition on enhancing the quality of staple foods (e.g., through product diversification, fermentation, composite flours, pre-gelatinisation, germination, soaking) and improving the nutrient content and composition of cereal grains (e.g., low glycemic index crops, micronutrient biofortified crops) to enhance the health of populations.

Equally, the use of immature cereal grains has the potential to bring agriculture and nutrition together to supply more nutritious food. The nutritional and functional benefits of immature grains are plausible pathways for promoting the transformation to immature cereal-based foods. The issue of dietary quality has become increasingly important in Africa, especially for those affected by relatively abrupt changes in lifestyle, for example, due to civil disruption, migration, urbanisation and modernisation. Moreover, processed immature cereal-based food products (such as *pepeta*) are culturally accepted and manifest in an SSA context. However, the realisation of nutritional health impacts will depend on how the transformation enablers such as innovations and technologies, and market and trade, are tackled, considering the current SSA food preparation and consumption practices.

6.9.1 Adapting existing technologies to the specific properties of immature grains

Research on processing innovations and technologies of immature cereal-based products is limited. Many of the compounds (nutrients) have only been investigated by a single study and have not yet been challenged or confirmed by other studies. Most commonly and widely used technologies to process cereal grains into food products need to be validated for immature cereal grains since nutrients can behave differently during processing. For instance, inward diffusion of nutrients and gelatinisation can occur during the roasting of freshly harvested immature paddy grains with more than 30 % moisture (**Chapter 3 and 4**). This is very unlikely for fresh mature grains (with less than 27 % moisture) since gelatinisation requires at least a 30 % moisture content.[96] A study by Casiraghi *et al.*[97] on pasta enhanced with immature wheat grain found that a considerable amount of fructo-oligosaccharides, which was potentially added by the immature wheat, leached out during cooking. Conversely, a high amount of lipids in immature grains impairs the shelf life and flavour of immature cereal flours, thus limiting their applications. Therefore, more studies on technological solutions to enhance shelf life and consumer acceptability of processed immature cereal-based food products, as well as on the retention of micronutrients and their bioavailability, are highly recommended and should be adaptable to rural areas.

Furthermore, harvesting immature cereals reduces grain yield due to the high amount of unfilled and shrivelled grains compared to harvesting at optimum maturity.[42,98] Also, dehusking or polishing of immature cereal grains before thermal processing produces a low yield and high amount of broken grains compared to mature grains as they are too weak to withstand the mechanical forces during processing due to a high amount of milky-white chalkiness.[99] These yield losses can be compensated with a premium selling price of immature grains compared to their mature grains counterparts (**Chapter 2**). The premium selling price could be promoted as an economic incentive for the valorisation of immature

cereals towards better cereal-based nutrition. Additionally, a comparative cost-benefit analysis of immature and mature cereal-based food production chains is highly recommended to provide empirical evidence of the involved costs and benefits.

6.10 Closing remarks

The potential nutritional and functional benefits of immature cereal grains offer an alternative solution to the nutritional improvement of diets of resource-limited communities that depend on monotonous starchy food as their main source of dietary energy and nutrients. This thesis work was focused on finding ways to improve the nutritional quality of indigenously processed immature rice-based products by using scientific approach to valorise their utilisation. The present work has increased the scientific knowledge on understanding the nutritional quality, and *in-vitro* starch and protein digestion properties of immature grains, as well as the changes they undergo upon processing into so-called *pepeta*.

Optimisation of the processing conditions can greatly improve the nutritional quality of the products and provides possibilities for commercialisation. Generally, our findings suggest that the optimised *pepeta* processing conditions cannot be viewed as a *one-size-fits-all solution* for improving the nutritional quality and functional properties of traditionally processed *pepeta* products to enhance food security in SSA. However, a customised approach for specific product properties and target groups in the community to improve the nutritional status via optimised *pepeta* processing conditions is feasible. Specifically, the best *pepeta* processing conditions for improvement of specific nutritional and functional properties were recommended in **Chapter 4**; harvesting at 26 DAH is optimum for *pepeta* production to reduce rice yield loss, roasting at 100 °C for production of *pepeta* with high-quality protein and low starch digestibility properties, and soaking before roasting at 120 °C for production of *pepeta* with a high amount of micronutrients.

Finally, opportunities for utilisation of immature cereal grains, as well as limitations and the way forward towards their utilisation, were discussed. All in all, this thesis has demonstrated that immature rice-based food products can play a beneficial role in supplying essential nutrients to resource-constrained communities, and these findings can be applied to other immature cereal grains as well.

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Summary

Summary

Cereals contributes a substantial proportion of the food supply for humans and animals globally, including in the SSA where they form a major source of dietary energy and protein intakes as staple foods. Beside their importance to food supplies and health, they contribute to improved income among the most food-insecure population groups in SSA countries. However, most of cereals are consumed as refined products (dehusked or polished) to improve their sensory properties. This renders the processed cereal-based products of poor nutritional quality as the refining process also removes important nutrients. On the other hand, there is increasing trend of consumers wanting more cereal-based healthy foods containing specific health-promoting properties. In this sense, immature cereal-based products such as *pepeta* (a locally processed immature rice flakes from Tanzania), appear to be among the many alternatives to pursue the goal of better cereal-based nutrition due to their nutritional potential benefits. It has been found that immature cereals and their products contain higher amount of numerous nutrients beneficial to health, including dietary fiber, vitamins, minerals and phytochemicals, compared to their mature counterparts. However, the information about the effect of processing on the nutritional and sensory properties and the bioavailability of nutrients in immature cereal grains and their products such as *pepeta*, is limited. Therefore, it is important to assess the traditional knowledge of *pepeta* product and its quality to improve the processing conditions and products' nutritional, sensory, and digestibility properties.

In **Chapter 2**, factors that can affect the *pepeta* value chain, as well as *pepeta* processing elements that interact with food and livelihood security such as availability of immature rice, *pepeta* processing techniques, storage practices, product characteristics, preferred product attributes, as well as *pepeta* trade supply chain and problems, were surveyed among the key players of *pepeta* processing chain: rice farmers – producer of sole raw materials, i.e.,

immature paddy grains used in *pepeta* processing; processors – local indigenous knowledge practitioner with vast knowledge on *pepeta* processing; and consumers - *pepeta* end-users with or without *pepeta* processing knowledge. Results indicated the potential role of *pepeta* to reduce food insecurity, especially among the rural poor as *hunger breaker*, i.e., a source of an early food when the crops are yet to reach maturity, and households' food supply is inadequate. Moreover, as a traditional processing knowledge, the pepeta processing parameters and conditions were not standardised, affecting consistently production of good quality *pepeta* products.

Since *pepeta* processing knowledge involve roasting of immature rice grains, it was of paramount importance to investigate changes in nutrients content and digestibility during maturation of rice grains, as well as upon processing into pepeta products (**Chapter 3**). Generally, immature rice grains destined for *pepeta* production had a higher amount of most nutrients such as lipids, protein, ash, soluble and insoluble dietary fibre, as well as B group vitamins such as thiamine, nicotinic acid, and nicotinamide compared to mature counterparts. Though pepeta processing improved the nutritional properties of rice, a very high and fast starch digestibility comparable to that of cooked milled and polished rice, diminish the health benefits of the *pepeta* product.

Therefore, these findings necessitate further improvement of *pepeta* processing techniques and the nutritional quality and functional properties of traditionally processed immature rice-based products such as *pepeta*. From this point of view, the following chapters of this thesis were designed to optimise the processing conditions that improve the specific nutritional, functional and sensory properties of traditionally processed *pepeta* products (i.e., maturity and roasting conditions (soaking and temperatures)). In **Chapter 4**, the laboratory simulation of *pepeta* processing was employed to investigate the impact of dry-heat processing at different conditions on the nutritional composition and *in-vitro* starch and protein digestibility

of immature rice-based products. The results indicate that the nutritional profile of immature rice products can be modulated by processing conditions, dry heating improves vitamin and mineral content when compared to raw and cooked immature rice. Soaking before roasting greatly increased the iron content compared to zinc, thiamine, riboflavin and nicotinamide, indicates the possibility for processed immature cereal products that were soaked before roasted to combat iron deficiency. Moreover, dry heating reduces both protein and starch digestibility when compared to cooked immature rice, where disulphide bonds play a vital role in heat-induced protein interactions in immature rice.

The effect of maturity and different processing practices on rice and immature rice-based products' visual quality and volatile profile was assessed under laboratory simulation, as well (**Chapter 5**). It was found that both the maturity and roasting influences the colour and volatile profiles of rice grains, whereas soaking before roasting limits non-enzymatic browning process such as Maillard and caramelisation reaction, and diffusion of bran pigments into rice starchy endosperm. The results are vital towards understanding the sensory properties of immature cereal-based products such as *pepeta* which are critical for the product's acceptability by consumers.

All the findings of this thesis were integrated and discussed in **Chapter 6.** The discussion focused on the role of immature cereals and their products towards improving the nutritional and sensory properties of cereal-based food products where most consumed products are based on refined cereal grains known to be nutritionally limited. Furthermore, an overview of the opportunities, challenges, and future developments of the utilisation of immature cereals grains and their products was highlighted.

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April 2022

Wageningen, the Netherlands.

About the author

Curriculum Vitae

Kulwa Furahisha Miraji was born in Urambo, Tanzania, on 27 June 1985. He graduated from Sokoine University of Agriculture (SUA) in Morogoro, Tanzania, with a BSc Food Science and Technology degree 2010. Afterwards, he worked in the food industry for two years as Food Quality Controller before joining the Tanzania Agricultural Research Institute (TARI) in 2012 and is employed there to date as Researcher. In the same year 2012, Kulwa progressed with his studies for an MSc degree in Food Science at SUA. During his master thesis, he assessed factors affecting the milling quality of rice (*Oryza sativa, L*), a major staple cereal which is second after maize (*Zea mays, L*) in Tanzania. He returned to TARI after his graduation in 2014 and served as AfricaRice country focal person under *Africa-wide Postharvest and Value addition Task Force*: the mechanism established by AfricaRice organisation to work with partners across sub-Saharan Africa to develop or adopt machineries and practices that reduce post-harvest losses and enhance the quality and market value of rice, rice-based products and by-products. In 2015, he was awarded a VLIR-UOS merit scholarship to attend a six months International Training Program – Food Safety, Quality Assurance Systems and Risk Analysis at Ghent University, Belgium. He did a *Quantitative assessment of the Aflatoxin risk of maize from farm to table: case study of ugali consumption in Tanzania*, as the requirements for the certificate. He started his sandwich PhD in 2017 at Food Quality and Design Group of Wageningen University and Research in the Netherlands, funded by NUFFIC (grant award CF13182/2017). He carried out his research in Netherlands and Tanzania between 2017 and 2021. He also supervised diverse topics of MSc/BSc theses students of different background and nationalities. This thesis presents the results of the scientific research, which have been published in peer reviewed journals.

List of publications

This thesis:

- **Miraji KF**, Linnemann AR, Fogliano V, Laswai HS, Capuano E. Nutritional quality and in vitro digestion of immature rice-based processed products. *Food Funct* **11**:7611–25 (2020). https://doi.org/10.1039/D0FO01668C

- **Miraji KF**, Capuano E, Fogliano V, Laswai HS, Linnemann AR. Utilization of Pepeta, a locally processed immature rice-based food product, to promote food security in Tanzania. *PLoS One* **16**:e0247870 (2021). https://doi.org/10.1371/journal.pone.0247870

- **Miraji KF**, Linnemann AR, Fogliano V, Laswai HS, Capuano E. Dry-heat processing at different conditions impact the nutritional composition and *in-vitro* starch and protein digestibility of immature rice-based products. Food Funct **12**:7527-7545 (2021). https://doi.org/10.1039/D1FO01240A

- **Miraji KF**, Capuano E, Laswai HS, Linnemann AR. Degree of maturity and dry-heat processing affect visual quality and volatile profile of roasted immature rice grains. (*Submitted for publication)*

Others:

- **Furahisha K**, Chove LM, Chaula D. Effect of Final Moisture Content , Cooling Time and Paddy Variety on Milling Quality of Rice (Oryza sativa , L.). *J. Agric. Sci. Food Technol.* **2**, 169–179 (2016).

- Ndindeng SA, Candia A, Mapiemfu DL, Rakotomalala V, Danbaba N, **Furahisha K**, Houssou P, Mohammed S, Jarju OM, Coulibaly SC. Valuation of Rice Postharvest Losses in Sub-Saharan Africa and Its Mitigation Strategies. *J. Rice Sci*. **28**(3): 1-5 (2021). http://www.ricescience.org/EN/Y2021/V28/I3/1

Overview of completed training activities

Overview of completed training activities

A: Discipline specific activities

Courses

- Healthy and sustainable diets: Synergies and trade-offs (VLAG, Wageningen, *The Netherlands*, 2017)
- Sensory perception and food preference: The role of context (VLAG, Wageningen, *The Netherlands*, 2018)
- Healthy food design (VLAG, Wageningen, *The Netherlands*, 2018)
- Confocal microscopy analysis practical training (WLMC, Wageningen, *The Netherlands*, 2019)
- Symposium Population dynamics (SKOV, Wageningen, *The Netherlands*, 2019)
- Summer Course Glycoscience: online edition (VLAG, Groningen, *The Netherlands*, 2021)

Conferences and meetings

- 8th World Sustainability Forum Conference, online event (MDPI, Basel, *Switzerland*, 2020)*
- 34th EFFoST International Conference, online event (ELSEVIER, Haifa, *Israel*, 2020)*
- 9th European Conference on Sensory and Consumer Research, online event (ELSEVIER, Rotterdam, *The Netherlands*, 2020)*
- Bonding science and policy to accelerate food systems transformation, online event (MUSE, Paris, *France*, 2021)
- 16th ICC Cereal and Bread Congress, online event (ICC, Canterbury, *New Zealand*, 2021)
- Virtual International Conference on Food Digestion (INFOGEST, Dublin, *Ireland*, 2021)
- East Africa Rice Conference, online event (IRRI, Nairobi, *Kenya*, 2021)
- Virtual Research Conference Plant-Based Foods & Proteins Europe (BRIDGE2FOOD, Bilthoven, *The Netherlands*, 2021)

B: General Courses

- Reviewing a Scientific Paper (WGS, Wageningen, *The Netherlands*, 2018)
- Scientific Publishing (WGS, Wageningen, *The Netherlands*, 2018)
- Research Data Management (WGS, Wageningen, *The Netherlands*, 2018)
- Searching and Organizing Literature (WUR – Library, Wageningen, *The Netherlands*, 2018)

- VLAG PhD week (VLAG, Baarlo, *The Netherlands*, 2018)
- Philosophy and Ethics of Food Science and Technology (VLAG, Wageningen, *The Netherlands*, 2019)
- Introduction to R (VLAG, Wageningen, *The Netherlands*, 2019)
- Applied Statistics (VLAG, Wageningen, *The Netherlands*, 2019)
- Making Impact: Increasing the relevance of research through science-society interaction, online event (WGS, Wageningen, *The Netherlands*, 2021)

C: Assisting in teaching and supervision activities

- Supervising 2 BSc and 3 MSc theses (FQD, Wageningen, *The Netherlands*, 2017-2021)

D: Optional activities

- Preparation of research proposal (FQD, Wageningen, *The Netherlands*, 2017)
- Organizing field trip for MSc student (TARI-Ifakara, Morogoro, *Tanzania*, 2019)
- Reviewing scientific article: *Evaluation of coatings for application in raffia big bags in conditioned storage of soybean cultivars in seed processing units.* DOI: https://doi.org/10.1371/journal.pone.0242522 (PLOS ONE, Cambridge, *United Kingdom*, 2020)
- Meetings and colloquia (FQD, Wageningen, *The Netherlands*, 2017-2021)

**Poster presentation*

FQD: *Food Quality and Design*

ICC: *International Association for Cereal Science and Technology*

INFOGEST: *International Network of Excellence on the Fate of Food in the Gastrointestinal Tract*

IRRI: *International Rice Research Institute*

MDPI: *Molecular Diversity Preservation International*

MUSE: *Montpellier University of Excellence*

TARI: *Tanzania Agricultural Research Institute*

VLAG: *Graduate School for Nutrition, Food Technology, Agrobiotechnology and Health Sciences*

WGS: *Wageningen Graduate School*

WLMC: *Wageningen Light Microcopy Center*

WUR: *Wageningen University and Research*

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