TOWARDS HEALTHIER STAPLES:

YELLOW CASSAVA PASTA FORTIFIED WITH AFRICAN LEAFY VEGETABLES

OLURANTI MOPELOLA LAWAL

Propositions

1. Cassava pasta fortified with vegetables is a user-friendly food innovation contributing to food security.
   (this thesis)
2. Nutrition labels without determining the bioaccessibility of the nutrients are false.
   (this thesis)
3. A successful academic career without unlimited access to the internet is inconceivable.
4. A university is like a processing line, receiving the raw human materials and sending them out as finished products for the world.
5. Cycling during a heavy downpour is a most humbling experience.
6. Lockdowns and social distancing during a pandemic are more challenging for extroverts than for introverts.

Propositions belonging to the thesis, entitled:

Towards healthier staples: Yellow cassava pasta fortified with African leafy vegetables

Oluranti Mopelola Lawal
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Towards healthier staples: Yellow cassava pasta fortified with African leafy vegetables

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To 'Màámi' (Dorcas Olufunmilayo Aderibigbe)

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CHAPTER ONE

GENERAL INTRODUCTION AND THESIS OUTLINE

1.1 *Micronutrients as "mighty nutrients" to reduce the burden of hidden hunger*

The global community is becoming increasingly concerned about providing healthy and sustainable diets for all people living on this planet. The 3rd Millennium Development Goal (MDG), which is aimed at 'ensuring healthy lives and promoting well-being for all at all ages, emphasised that the regular intake of a healthy diet, i.e. a diet that meet the requirements of the human body, prevents several diet-related non-communicable diseases (NCDs) and micronutrient deficiencies (WHO, 2020). Micronutrient deficiency, also known as hidden hunger, is often unnoticed for a long time in individuals before the symptoms become apparent, thus the term 'hidden hunger'. It is a kind of undernutrition that arises when consumption and absorption of micronutrients (vitamins and minerals) such as vitamin A, iron and zinc are inadequate in sustaining good health and development (Gani *et al.*, 2019). It ravages most low-and-middle-income countries (LMICs) worldwide due to the low consumption of micronutrient-rich diets, heavy reliance on mainly starchy staple diets and the lack of diversity in the people's diet (von Grebmer *et al.*, 2014). As shown in Fig. 1.1, unhealthy diets, hidden hunger, and NCDs are interwoven and often closely linked (Branca *et al.*, 2019). About 2 billion people in developing countries, primarily children and women of reproductive age, are currently affected by hidden hunger, especially vitamin A, iron and zinc deficiencies (La Frano *et al.*, 2014).

Vitamin A deficiency is manifested by mild to acute systemic effects on innate and acquired host's resistance mechanisms to infection, an increased burden of infectious morbidity, mild to severe (blinding) stages of xerophthalmia, and an increased risk of mortality (Huang *et al.*, 2018). The earliest ocular manifestation of vitamin A deficiency is night-blindness and it is particularly prevalent in pregnant mothers. An inadequate diet in preformed or carotenoid precursors of vitamin A is the underlying cause of the deficiency, and adequate consumption of vitamin A-rich food may help reduce the incidence of the deficiency provided there are no underlying diseases or infections affecting absorption of vitamin A in the host. Since the human body cannot synthesise vitamin A, it must be obtained from the diet or through supplemental sources (Debelo *et al.*, 2017). Dietary vitamin A is consumed as either carotenoid from fruits and vegetables or retinoids from animal products. Thus, the World Health Organisation guidelines recommend a daily vitamin A intake of 400 mg RE for children aged 1-3 years, 500 mg RE for adult women, and 600 mg RE for adult men.

Iron deficiency also results in modifications of the human metabolic functions that could affect brain functioning, such as neurotransmitter metabolism, protein synthesis and organogenesis (Soetan *et al*., 2010). There are two forms of iron: haem and non-haem. Haem iron is found in animal sources, whereas non-haem iron is found in plants. Iron is needed to synthesise several enzymes and haemoglobin required to transport oxygen in the blood (Abbaspour *et al*., 2014). Absorption of non-haem iron can be enhanced by the presence of meat, poultry, fish and some acids (ascorbic, citric, malic, tartaric or lactic acids) while being inhibited by soy protein, calcium, phytates and polyphenolic compounds (Institute of Medicine, 2001). According to the Institute of Medicine Panel on Micronutrients, the daily Recommended Dietary Allowance (RDA) for all age groups of men and postmenopausal women is 8 mg; for premenopausal women, 18 mg while the median dietary intake of iron is approximately 16 to 18 mg for men and 12 mg for women.

The World Health Organization has also designated zinc deficiency as a significant disease contributing factor. It is estimated that 17.3 % of the world population has inadequate zinc intake, with the highest estimates in Africa (23.9 %). Zinc deficiency may lead to growth impairment, sexual dysfunction, inflammatory and gastrointestinal symptoms, or cutaneous involvement (Sanna *et al*., 2018). It can also result in an inadequate immune system (Chasapis *et al*., 2020). Zinc is involved in many processes in the human body, such as biochemical pathways and DNA replication and repair, gene expression and the regulation of cellular growth and differentiation (Cilla *et al*., 2019). Zinc also aids normal growth and development during gestation, infancy, and adolescence and is required for a proper sense of taste and smell (Maares & Haase, 2020). The daily Recommended Dietary Allowance (RDA) for zinc is 3 mg, for 0-3-year-olds, 8 mg for adolescents, 8 mg for women and 11 mg for men. However, the recommended daily requirements for zinc are challenging to meet for most LMICs due to the inadequate absorption of zinc caused by the interactions with inhibitors such as phytate and polyphenols generally abundant in plant-based foods (National Institutes of Health NIH, 2021).

1.2 Food biofortification as a sustainable dietary strategy against nutritional deficiencies

Many food strategies were adopted over the years to tackle the micronutrient deficiencies in LMICs, such as supplementation, fortification, dietary diversification and biofortification (Saltzman *et al*., 2016). Of these, biofortification has proven to be a sustainable and viable approach to tackling hidden hunger in the developing world (Bouis *et al*., 2011). Introduced about two decades ago, the biofortification strategy leverages on the regular daily consumption of substantial amounts of food staples by all household members, particularly women and children, who are most at risk for micronutrient malnutrition. Biofortification improves the quality of food crops either through conventional breeding techniques or genetically modified organisms. It involves breeding staple food crops to increase their micronutrient content levels, targeting staples consumed by low-income families in Africa, Asia, and Latin America (Hotz, 2013). Biofortification also provides an effective and affordable means of reaching malnourished rural populations with limited access to commercially marketed fortified foods and supplements (Bouis *et al.*, 2011). Biofortification of staple crops has considerable potential for long term impacts in correcting deficiencies of critically essential vitamins and minerals. Thus, several staple crops from sub-Saharan Africa such as maize, sweet potato, cassava, beans and pearl millet have been biofortified with either provitamin A, iron or zinc (La Frano *et al.*, 2014).

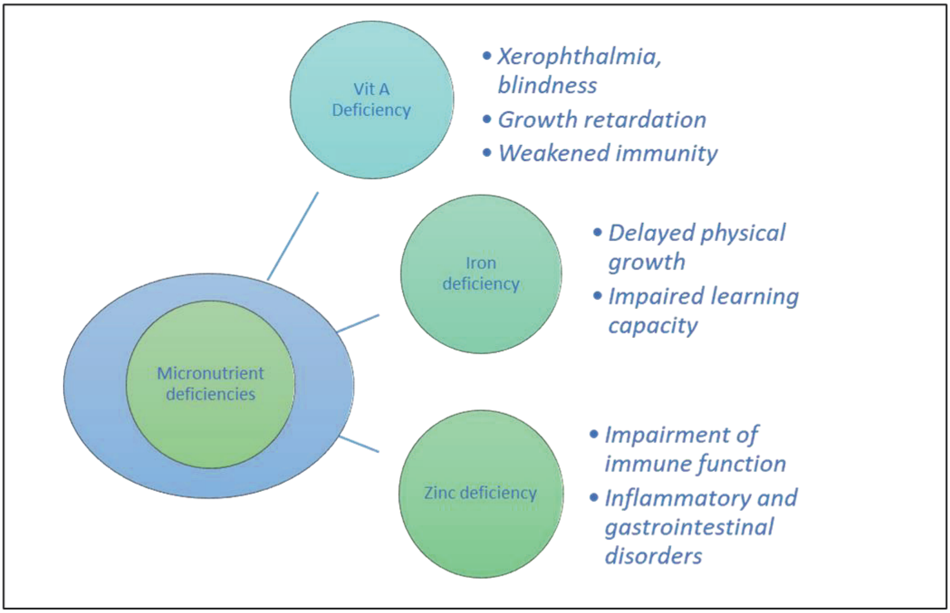

Fig 1.1 Health consequences of micronutrient deficiencies (adapted from Tulchinsky, 2010)

1.3 *Cassava for food security in Africa*

The sub-Saharan African region produces the largest quantity of cassava in the world; thus, cassava is foremost among the starchy staple diets of the people targeted for biofortification (Ilona *et al.*, 2017). Cassava (*Manihot esculenta* Crantz), a tropical crop also known as manioc or yucca, is one of the oldest roots and tuber crops providing dietary energy for two out of every five Africans (Nweke *et al.*, 2002). Cassava was domesticated from the Americas in the 16 to 18th century and utilized by humans for food, feed and beverages. It is strategically touted for food security in the tropics due to its affordability, availability and accessibility to the resource-poor (Hershey, 2017).

Cassava is the fourth most crucial calorie source after wheat, rice and maize in the human diet (FAOSTAT, 2019). Moreover, it is widely valued for its ability to yield well even on low-fertility soils and with periodic droughts, especially when other crops have failed (Hershey, 2017). Currently, over half (about 63 %) of the global production of cassava comes from Africa. Nigeria, with an annual production of about 60 million metric tons, is the largest producer and consumer in the world (Fig.1.2). As a result, nearly every person in Africa eats around 80 kg of cassava per year and the average intake of cassava in Nigeria is as high as 940 g per adult per day fresh weight (de Moura *et al.*, 2015).

The habitual consumption of cassava in Africa is common, not just due to low-income levels but also an age-long food habit of eating starch-based staples. Being a versatile staple, cassava is consumed in the form of a wide variety of cassava-based dishes (Table 1). Cassava is classified as either sweet or bitter, with the bitter cassava varieties containing higher amounts (>100 mg/kg) of cyanogenic glycosides. Thus, one of cassava's nutritional drawbacks is the presence of cyanides, which limits the ease of use for both human and animal consumption. Secondly, the cassava roots are highly perishable due to a high moisture content (33–72 % moisture) and thus have a short postharvest life (< 72 hours). Furthermore, conventional (white-fleshed) cassava varieties, though rich in starch (≈ 85 % dry weight and energy 16.5 MJ kg^{-1} DW) are poor in protein and micronutrients such as vitamin A, iron, and zinc (Montagnac *et al.*, 2009).

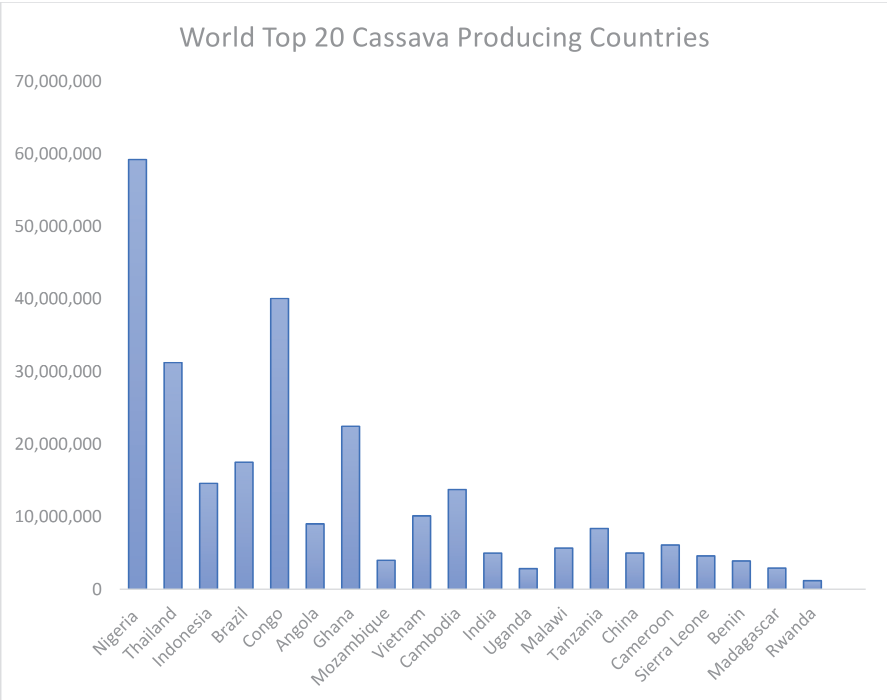

Fig.1.2. World top 20 cassava producing countries (Source: FAO, 2019)

Table 1.1 Cassava-based food products of the world

Category	Name/Type	Nature/Process	Region/Country	References
Staple	Whole roots	Raw, boiled, fried fresh starchy roots	Americas, Asia, and SSA	Shigaki, 2016
	Fufu, Dumpling, Mchuchume	Sticky dough	West Africa, Tanzania	
	Farina, pupuru,	Coarse roasted flour	South America, West Indies, Nigeria	
	Mucui	Boiled and fried with onions and meat	East Africa	Karuri *et al.*, 2001
	Landang (cassava rice)	Pelleted pulp of cassava in the form of rice	Philippines	Shigaki, 2016
	Macaroni (cassava noodles)	A composite of cassava flour, groundnut flour and wheat semolina	Asia	
	Gaplek	Dried cassava	Indonesia	
	Gari, Kpokpogari	Toasted fermented coarse savoury flour	West Africa	
	Attieke, Yakayake,	Fermented steamed cassava paste	West Africa	
	Meduame-M-bong	Boiled and fermented, eaten with leaves	Cameroon	
	Chickwange, Lafun, Ikivunde, Inyanga Alebo, Mokopa	Fermented sun or smoke-dried cassava	West Africa, Uganda, Rwanda	Halake & Chinthapalli, 2020
	Ugali, Mariwa	Fermented, dried, milled and made into porridge	Kenya	Karuri *et al.*, 2001
	Mukinwa	Mixed with maize and fried with onions	Kenya	
Desserts	Sago, tapioca pearls	Small ball-shaped dessert	Asia	Shigaki, 2016
	Cassava bread	Composite flour of cassava and wheat	West Africa	
	Jongkong, Kal or Awug	Cassava flour mixed with coconut and shredded palm sugar	Indonesia	
	Nagasari	Cassava mixed with maize, coconut and sugar; cooked with banana leaves	Indonesia	

	Cassava cake, pone Madeira	Grated cassava is mixed with coconut milk, eggs, and butter	Philippines, Papua New Guinea, Suriname, Solomon Islands and Vanuatu	Shigaki, 2016
	Udon	Cassava and wheat flour frozen udon noodles	Japan	
	Mie lethek	Cassava noodles	Indonesia	
	Tapioca noodle, Banh canh	Modified cassava starch		
	Cassa strips, Ajogun	Cassava milled with cowpea and fried	Africa	
Indonesian side dishes	Keripik singkong	A form of fried chips	Indonesia	Herminingrum, 2019
	Sawut	Steamed coarsely grated cassava		
	Lemet or utri	Steamed light meal, from grated roots		
	Ongol-ongol	Boiled and mixed with coconut milk, salt, and cane sugar		
	Lentho	Mixed with local spices and fried		
	Jemblem jrut	Fried after blending with grated coconut meat, salt and palm sugar		
	Miler or samiyer	A kind of chips		
	Singkong rebus	Directly steamed cassava roots		
	Singkong goreng	Soaked, steamed and fried street food		
	Tape pohung	fermented and steamed		
	Bubur anyep	The porridge is eaten to cool the digestive tract.		
	Cenil	Made from tapioca batter		
	Cassava fries	Cassava cut into strips and fried	Asia, Africa, South America	
Beverages	Cassava beer, mahewu	Fermented cassava	South Africa, Mozambique	Shigaki, 2016
	Busaa	Mixed with dry maize and fermented into beer	Kenya	Karuri *et al.*, 2001
	Banu or Uala	Distilled liquor	Uganda	
Salad	Abacha	Shredded fresh cassava roots	Nigeria	

Sauces	Cassava leaves, pondu, saka-saka	In various cooked forms as a leafy vegetable	SSA
	Cassareep	Boiled to form a thick, black syrup, with added spices	Guyana

1.4 *Provitamin A biofortified (yellow) cassava to the rescue?*

Cassava is an obvious choice for biofortification as it is consumed daily by large populations in sub-Saharan Africa, and thus a suitable food vehicle for delivering micronutrients (de Moura *et al.*, 2015). Provitamin A rich, naturally yellow-coloured cassava varieties were originally found in South America and then used to develop varieties with higher β-carotene concentrations through conventional plant-breeding techniques (Chávez *et al.*, 2005). After that, collaborative research efforts by several institutions {the International Center for Tropical Agriculture, Colombia (CIAT), the Brazilian Agricultural Research Corporation (Embrapa) and the National Agricultural Research and Extension Systems (NARES)} produced the yellow cassava, which was then introduced into Africa by the International Institute of Tropical Agriculture (IITA) in Nigeria. The breeding target is currently 15 µg/g provitamin A. Breeding efforts are ongoing in Brazil, DRC, Malawi, Nigeria, and Sierra Leone to achieve higher levels (Eyinla *et al.*, 2019).

In contrast to the conventional, white-fleshed bitter cassava, yellow cassava contains a lower level of cyanogenic glycosides (< 50 mg/kg). It is thus utilised as a gluten-free wheat replacement in several confectioneries, baked products, and more recently, pasta (Oladunmoye *et al.*, 2017; Lawal *et al.*, 2021a). Moreover, studies have shown that yellow cassava is nutritionally superior and a better source of beta carotene than the white variety (Ayetigbo *et al.*, 2018). It also has the potential of providing up to 25 % of the daily vitamin A requirements of children and women (Ilona *et al.*, 2017). According to Bechoff *et al.* (2018), consumer acceptance is higher for yellow cassava than for white in Africa and other countries where yellow cassava has been introduced. However, the retention of beta carotene varies between 10 % for boiled products to 87 % for highly processed yellow cassava products (Taleon *et al.,* 2019; Lawal *et al.*, 2015). Cassava is also known to be generally poor in other nutrients such as iron and zinc. Thus, consumers whose diets consist mainly of cassava, even when yellow, may still be vulnerable to micronutrient deficiencies (Talsma *et al.*, 2014). Therefore, there is a need to adopt additional nutritional measures to boost the micronutrient value of cassava food products.

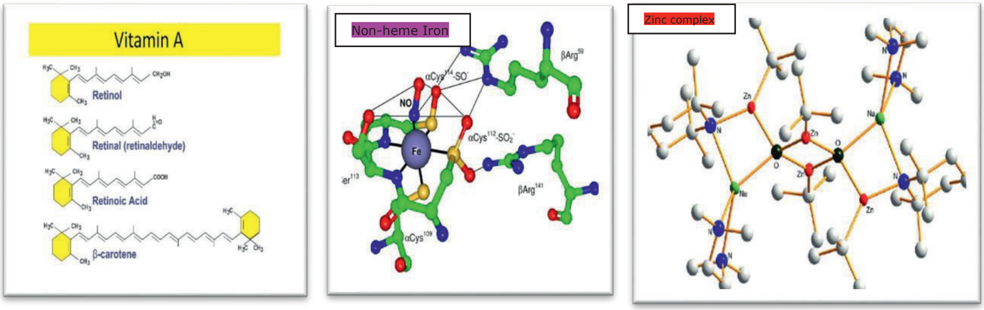

Fig 1.3 Structure of vitamin A, the iron-haem iron centre of iron-containing Fe-type NHases and zinc (Fennema, 2008)

1.5 African leafy vegetables for food-to-food fortification

Fortification of food with other food ingredients, also known as food-to-food fortification (F2FF), is an evolving food intervention adopted by food scientists to boost the nutritional value of foods (Kruger *et al.*, 2020). It is a blend of several food strategies (Fig 1.4). Food-to-food fortification has the advantage of simplicity and affordable technology. With the use of readily available and affordable ingredients, it is a sustainable strategy of improving the nutritional quality of food without losing acceptability, thus requiring a minimal dietary change of the consumers (Kruger *et al.*, 2020). Food-to-food fortification of starchy staples with vegetables rich in bioactive compounds incorporated in the food matrix has great potential to deliver healthy and nutritious foods to consumers in sub-Saharan Africa.

One of the most recently studied ingredients for food fortification is the African leafy vegetables. They are utilised for the development of functional and novel food products that confer specific health benefits on the consumers due to their high levels of beta carotene, iron and zinc. In this study, we identified about 150 leafy vegetables utilised as food in Africa (Appendix 1) with the potential to combat micronutrient deficiencies. Currently, the consumption of leafy vegetables in sub-Saharan Africa is low (27-114 kg per person per year), but several studies reported the increasing use as a functional ingredient (Table 1.2). Incorporating vegetable ingredients into food products is an innovative approach to boost vegetable intake (Perez-Moral *et al.*, 2018). For example, African leafy vegetable powder is used to fortify foods such as bread and pasta (Govender and Siwela, 2020) and mixed into smoothie drinks (Lawal *et al.*, 2021b; Managa *et al.*, 2021).

Leafy vegetables are reliable sources of nutrients; they contain vitamins, minerals and phytochemicals in significant quantities that can help in tackling hidden hunger (Gupta *et al.,* 2013; Oliviero & Fogliano, 2016). Besides, the dietary fibre content of vegetables modulates the function of the intestinal tract. Leafy vegetables also contribute to satiety and the prevention of constipation, while the proteins in vegetables are superior to those found in fruits (Hiza and Bente, 2007). Moreover, the inclusion of vegetables in the diet is protective against several diet-related and degenerative diseases and was inversely associated with type 2 diabetes (Cooper *et al.*, 2012) due to bioactive phytochemicals.

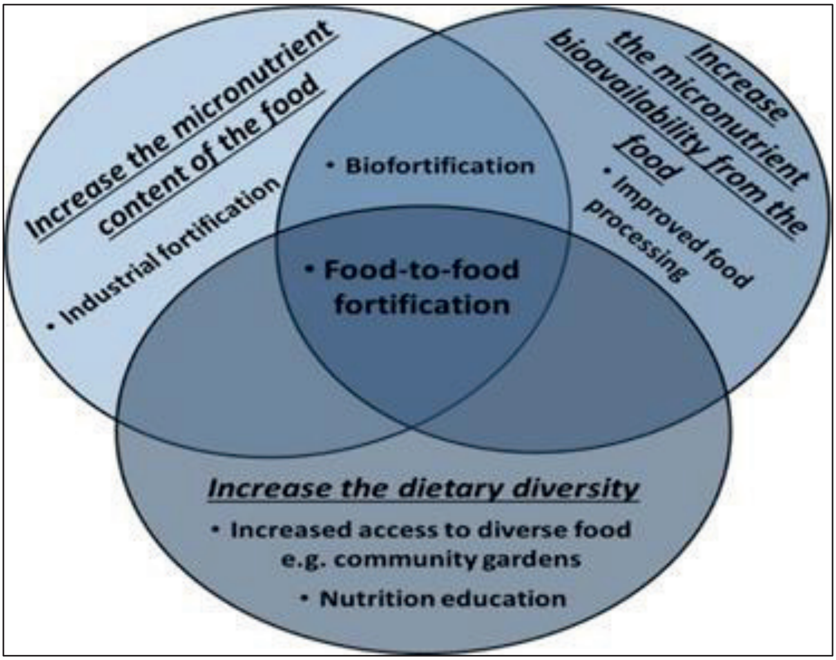

Fig 1.4 Food-based strategies to address micronutrient deficiencies (adapted from Kruger et al., 2020)

Several authors have studied the incorporation of staples with leafy vegetables (Table 2). For instance, van der Merwe *et al.* (2019), reported the inclusion of *Moringa oleifera* in pearl millet porridge, while Famuwagun *et al.* (2016), incorporated *Telfairia occidentalis* in wheat bread. Beswa *et al.* (2016), reported that the provitamin A, amino acid, and iron contents of biofortified maize improved with the addition of amaranth vegetable powder. However, studies about the bioaccessibility of the bioactive compounds in leafy vegetables to the human body after ingestion and digestion are still limited.

Table 1.2. Examples of food-to-food fortification with African leafy vegetables

	Vegetable fortificant	Food product	Level of fortification	Major outcomes	Reference
1	*Moringa oleifera* L.	Pearl millet porridge and dry leaf powder	10-30 %	At 10 % fortification, the content and bioaccessible iron increased by 56 % & 149 % and zinc by 66 % and 135 % both decreased with 30 % fortification.	Van der Merwe *et al.*, 2019
2.	*Moringa oleifera*	Pearl millet porridge and dry leaf powder	15 %	No change in bioaccessibility.	Oluyimika *et al.*, 2021
3	*Vigna unguiculata leaves*	Wholegrain maize porridge and dry leaf powder	40 %	Bioaccessible iron increased by 519 %, zinc by 295 %, no change in bioaccessibility.	Kruger *et al.*, 2020
4.	*Hibiscus sabdariffa*	Pearl millet porridge with dry leaves	10-30 %	At 10 % fortification, bioaccessible iron increased by 55 and 269 % and zinc by 32 and 142 %.	Van der Merwe *et al.*, 2019
5	*Moringa oleifera*	Extruded millet porridge with dry leaf powder	5 %	Increase in serum retinol 0- 11.6 mg/100g.	Ndiaye *et al.*, 2020
6.	*Moringa oleifera*	Wheat pasta fortified with leaf powder	5-15 %	The inclusion of leaf powder enhanced the phenol content, the antioxidant activity, and the mineral content of fresh pasta.	Simonato *et al.*, 2020
7	*Vigna unguiculata leaves*	Sorghum and maize porridge with cowpea leaf powder	3:2	Leaf powder inclusion increased the bioaccessible zinc 2-and 3-fold, respectively, and beta	Kruger *et al.*, 2018

				carotene contents from 10–13 mg/100 g.	
8	*Telfairia occidentalis*	Wheat bread with dry leaf powder	1-5 %	Iron and beta carotene content increased.	Sengev *et al.,* 2013
9	*Moringa oleifera*	Wheat bread with dry leaf powder	5 %	The inclusion of moringa increased the protein, crude fibre and ash contents of bread.	Chinma *et al.,* 2014
10	*Moringa oleifera*	Fermented cereal porridge with leaf powder	10-15 %	94 % increase in protein content with 15 % substitution of moringa leaf powder, iron content from 4.7 mg/100 g to 12.8 mg/100g, beta carotene from 121.7 to 1058.3 µg/100 g.	Abioye and Aka, 2015
11	*Moringa oleifera*	Complimentary food with leaf powder	5-15 %	Leaf powder fortification increased the protein and fibre content of maize complementary food.	Shiriki *et al.,* 2015
12	*Moringa oleifera*	Yam dough with leaf powder	2.5 %, 5 %, 7.5 % & 10 %	Protein and mineral contents increased with leaf powder fortification.	Karim *et al.*, 2013
13	*Solanum macrocarpon & Amaranthus cruentus*	Cowpea porridge with leaf powder	Not reported	The addition of leafy vegetables increased the mean haemoglobin and retinol concentrations of consumers.	Egbi *et al.*, 2018
14	*Telfairia occidentalis*	Wheat bread with leaf powder	1 %, 3 % & 5 %	Improvement of loaf weight.	Famuwagun *et al.*, 2016

15	*Moringa oleifera*	Maize, millet porridge with leaf powder		Leaf addition increased the iron, zinc and beta carotene contents of porridge.	Arise *et al.*, 2014
16	*Amaranthus, Telfairia occidentalis & Solanum macrocarpon*	Wheat bread with leaf powder	1-3 %	Fortification enhanced the polyphenolic content of the bread.	Alashi *et al.*, 2018
17	*Amaranthus*	Wheat pasta with dry leaf powder	0-5 %	Higher protein, crude fibre, iron, zinc, magnesium and potassium with leaf powder addition.	Cárdenas-Hernández *et al.*, 2016
18	*Brassica oleracea var. italica*	Wheat bread with broccoli leaf powder	5 %	Leaf powder inclusion caused higher content of nutrients (proteins and minerals), as well as improved specific volume and bake loss.	Krupa-Kozak *et al.*, 2021
19	*Amaranthus*	Biofortified maize snack	0-3 %	Inclusion of leafy powder enhanced beta carotene and phenolic contents of the snacks.	Beswa *et al.*, 2016
20	*Amaranthus, Solanum macrocarpon, Telfairia occidentalis*	Wheat bread with leaf powder	0-3 %	Leaf powder inclusion increased the nutritional profile of the bread with fluted pumpkin rated highest.	Odunlade *et al.*, 2017
21	*Amaranthus*	Orange-fleshed sweet potato porridge with leaf powder	10 %	The inclusion of leaf powder improved the iron, zinc and beta carotene contents of formulated foods.	Onwuamaeze *et al.*, 2017

1.6 Emergence of cassava pasta as a vehicle for food fortification

One of the world's fastest-growing food segments is the pasta market. Originating from Italy from the first century BC, pasta is now popularly eaten worldwide as the quest for convenience and nutritious foods increases. Pasta is conventionally made with wheat and is well-appreciated for its palatability, convenience, low cost, shelf life and as an ideal vehicle for adding nutritious food ingredients. In recent times, as consumer interest in gluten-free/grain-free food products is growing, the substitution of wheat in the production of pasta products, particularly non-wheat gluten-free pasta made from grains (rice, corn, sorghum, pearl millet), pseudo-cereals (quinoa, amaranth, buckwheat), legumes (chickpea broad beans), roots and tubers (potato, cocoyam, plantain, cassava), is gaining attention (Gao *et al*., 2018; Chillo *et al.,* 2008a; Chillo *et al.,* 2008b). Wheat contains gluten proteins, mainly composed of gliadins and glutenins (80%), which give wheat pasta a firm and compact structure responsible for the slow digestibility of its starch content, thus resulting in a low glycemic index that is ideal for the management of type 2 diabetes. The World Health Organization (WHO) and the Food and Drug Administration (FDA) also recognised pasta as a good food matrix for nutritional enrichment. Sadly, the gluten network presents a health challenge for people with celiac disease or gluten intolerance, thus, the quest for wheat substitution in gluten-free pasta production (Sakurai *et al*., 2020). The nutritional enhancement of pasta using locally available gluten-free food ingredients is, therefore, an effective strategy to improve the health status of consumers. Cassava as a substitute for wheat in pasta production is increasing due to a rise in the consumption of instant noodles and spaghetti, particularly in Africa (Oladunmoye *et al*., 2017), and the need for efficient use of locally sourced raw materials. Moreover, the search for gluten-free pasta with additional health benefits is a major consideration for using yellow cassava in pasta production.

1.7 Bioaccessibility of micronutrients, an indication of the nutritional value of food

The fortification of staples with leafy vegetables is expected to enhance the nutritional status of consumers, but the availability of the nutrients for the human body needs to be confirmed to ensure the benefits. Several studies on the inclusion of leafy vegetables in functional foods lacked an investigation of the fate of the nutrients during and after digestion. The bioavailability of nutrients expresses the fraction of the ingested nutrient that reaches the systemic circulation and is ultimately utilised (Fig.1.6). Thus, depending on the compound in question, it has a certain physiological endpoint. Its determination requires sophisticated and cumbersome methods that consider endogenous nutrient losses through the enterohepatic circulation and incorporate nutrients into storage tissue (Fig.1.5). The simulation of the physiological conditions of the human gastrointestinal digestion via *in-vitro* studies are less expensive than the human studies and adopted as an indicator of micronutrient bioaccessibility as it indicates the micellization efficiency of the food. *In-vitro* bioaccessibility has been defined as the fraction of a compound released from its matrix in the gastrointestinal tract that becomes available for intestinal absorption.

Bioaccessibility must therefore be a criterion to be considered in the design of functional foods, given that any claim of health benefits established through the intake of these foods is not valid, except it can be proven that the bioactive component is absorbed efficiently (Aggett *et al*., 2005). Thus, it is not adequate to certify that the food contains a certain amount of nutrients; instead, it must be shown that the nutrient is digested and effectively made available to the body. In other words, a bioaccessibility study must be performed (Fernández-García *et al*., 2009).

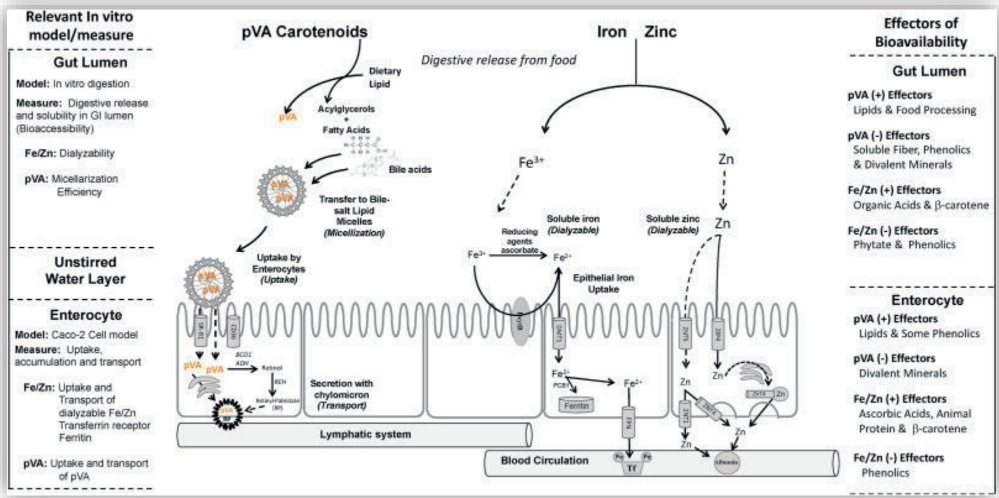

Fig. 1.5 The pathways and stages of human intestinal absorption of provitamin A, iron and zinc showing the major enhancers and inhibitors (adapted from Ferruzzi et al., 2020)

1.8 Bioaccessibility of beta carotene, iron and zinc

Studies have shown that processing generally affects the amount of bioaccessible beta carotene in food products, mainly due to exposure to heat, light, and oxygen (Berni *et al*., 2014), while the bioavailability of dietary zinc and iron is reduced by phytic acid and other inherent constituents such as tannin and fibre of plant foods (Guatam *et al*., 2011).

Iron bioaccessibility is dependent on its digestive extraction from the food matrix and its solubility in the gut lumen. Another key factor impacting the bioaccessibility of iron is the reduction of iron from ferric ($Fe3+$) to ferrous ($Fe2+$) forms by the action of gastric acid and ferric reductases in the intestinal lumen (Fig 1.3). The reduction of iron in the intestinal lumen enhances its solubility and subsequent interaction with transport systems at the brush border. Presence of chelators or reducing agents such as organic acids, ascorbate, histidine and cysteine in the food matrix or gut lumen are also known to enhance the bioaccessibility of iron and improve the absorption and bioavailability (Ferruzzi *et al.*, 2020).

Zinc is the second most abundant micronutrient in the human body after iron while dietary zinc absorption and bioaccessibility vary significantly between foods. The chemical and physical processing of food may affect zinc bioaccessibility and availability while studies have shown that protein appears to enhance overall zinc absorption in humans. The presence of phytate however shows an inhibitory effect on zinc bioaccessibility and absorption. Intestinal transport of zinc is controlled by the actions of zinc importers (ZIP4), which mediate the uptake of zinc from the intestinal lumen and efflux by transporters (ZnT1) from the enterocyte across the basolateral membrane, where it is ultimately exported into the systemic circulation (Fig. 1.5). The transporter ZnT5 may also play a role in intestinal uptake and transport of zinc (Maares and Haase, 2020).

1.9 Starch digestibility of cassava and its impact on health

The glycemic impact of starchy foods such as cassava depends on the rate and extent of digestion in the small intestine and is greatly influenced by the food ingredient type, product formulation and food processing conditions (Englyst *et al.*, 2018). A high rate of consumption of starchy foods such as cassava with a high glycemic index has been implicated in the incidence of type 2 diabetes and obesity (Thomas & Elliott, 2010). Therefore, the consumption of low glycemic index foods/diets is recommended to prevent and manage these diseases, as they favourably influence blood glucose and contain higher contents of resistant and slowly digestible starch (Brand-Miller *et al.*, 2003). Furthermore, including fibre-rich plant-based foods in the diet, such as vegetables, can improve glucose control and blood lipids for diabetics (Timm & Slavin, 2008). Many processing strategies are also developed aimed at controlling the glycemic response, such as selection of slowly digestible starch and incorporation of ingredients with high contents of phenolic compounds to inhibit carbohydrate digestive enzymes. It is thus essential to ensure the nutritional quality of new fortified foods by also considering their glycemic index to ensure they are beneficial to the consumers.

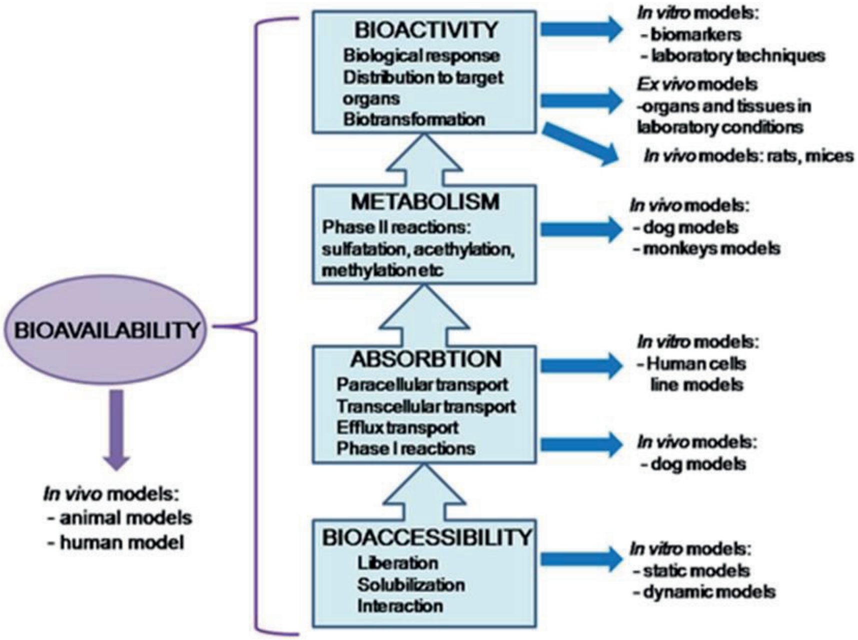

Fig.1.6 Distinction between bioavailability, bioaccessibility and bioactivity and the physiochemical events involved at each stage (adapted from Fernández-García et al., 2009).

1.10 Rationale and outline of the thesis

The nutritional outcomes of food-to-food fortification of yellow cassava with leafy vegetables are yet to be fully understood, and conflicting reports exist on the food matrix interaction effects. Moreover, existing studies had reported differing levels of losses during the processing and storage of biofortified cassava, but little information exists on the impact of incorporating leafy vegetables to enhance micronutrient content and the bioaccessibility of beta carotene from yellow cassava pasta.

This study thus pioneered the development of yellow cassava pasta with the primary objective of evaluating the functional food's nutritional, technological, and sensorial properties and the impact of fortification with leafy vegetables. Specifically, in this thesis, we aimed to achieve the following:

- Establish the overall consumer acceptability and sensorial properties of yellow cassava pasta.
- Assess the composition and nutritional values of the functional pasta.
- Evaluate the impact of leaf powder addition on the functional and cooking properties of the formulated pasta
- Investigate how much of the micronutrients are bioaccessible.
- Analyse the glycemic index of the yellow cassava pasta.

1.11 Outline of the thesis

In this thesis, we firstly describe, in **Chapter 2**, the consumer studies carried out to establish the attitudes, perceptions, and motives for consumption and perceived barriers of consumers for the yellow cassava pasta and leafy vegetables using focus group discussions and randomised face-to-face interviews. Liking, preference and ranking of the novel food were also established through consumer sensory perception. We confirmed consumer acceptance of yellow cassava pasta and identified the preferred leafy vegetables, i.e., amaranth and fluted pumpkin, for possible fortification.

Chapter 3 evaluates the proximate minerals, phytochemicals, and functional, thermal and cooking properties of the fortified yellow cassava pasta with amaranth leafy vegetables to gain insight into the nutritional benefits of food-to-food fortification of yellow cassava pasta with amaranth leafy vegetables. We also compare white and yellow cassava varieties to establish the impact of cultivar differences.

Chapter 4 evaluates the impact of the addition of fluted pumpkin leaf powder on the functional, textural, pasting, thermal, and cooking properties of the yellow cassava pasta. Here, we also studied the sensorial perception of consumers about the functional food and established modest overall acceptability and likelihood of purchase of the pasta.

Chapter 5 assesses the effects of leaf powder addition on the retention and bioaccessibility of beta carotene, iron and zinc in the vegetable-fortified yellow cassava pasta. At this point, we also evaluate the impact of the leaf powder addition on the starch digestibility and *in-vitro* glycemic index of the functional pasta.

The concluding chapter of this thesis (**Chapter 6**) provides a general discussion and summary of the studies described. In addition, suggestions for future research and experiences from scaling up are discussed.

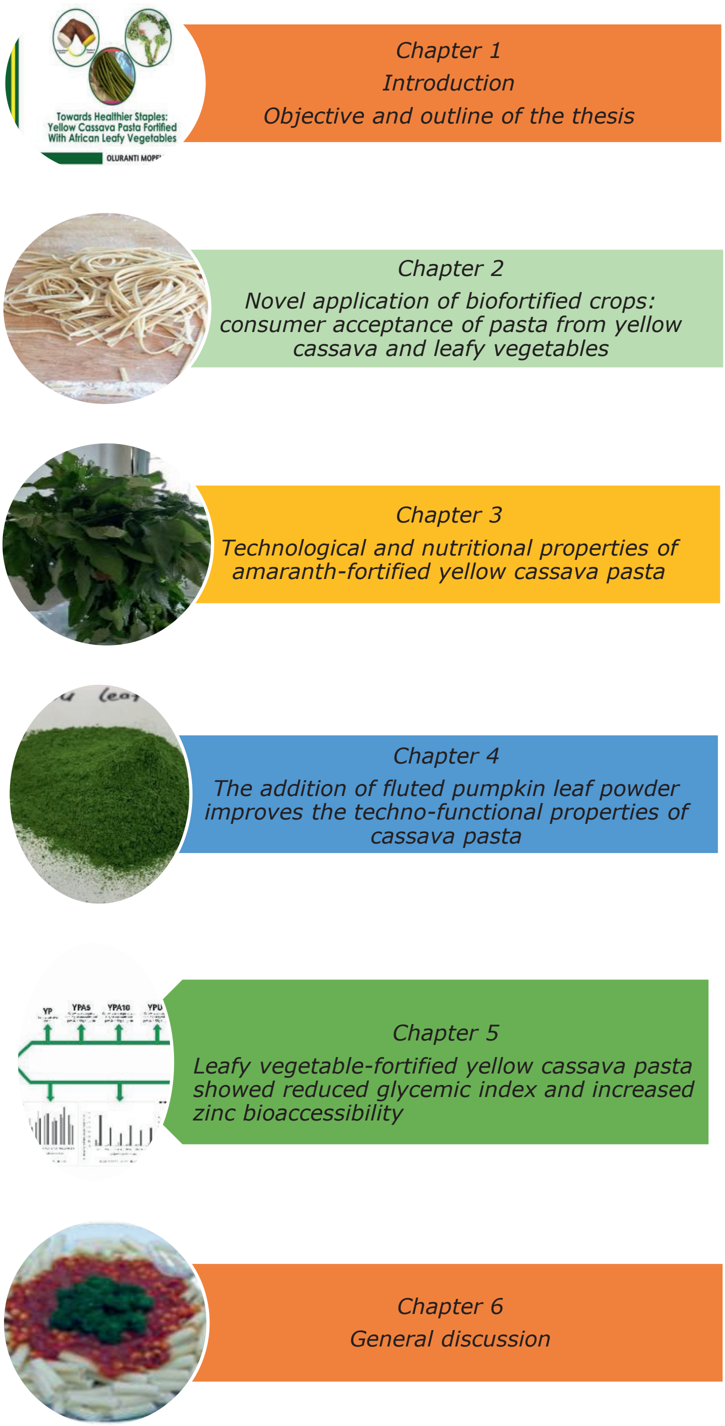

Fig. 1.7 Outline of this thesis

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CHAPTER TWO

NOVEL APPLICATION OF BIOFORTIFIED CROPS: CONSUMER ACCEPTANCE OF PASTA FROM YELLOW CASSAVA AND LEAFY VEGETABLES

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Abstract

Background: Newly developed yellow biofortified cassava has been adjudged as a cost-effective solution to vitamin A deficiency in low- and middle-income countries with high cassava intake such as Nigeria. In this study, yellow cassava was developed into a novel pasta enriched with amaranth vegetables and tested among consumers. Attitudes, perceptions, motives for consumption and perceived barriers were ascertained using focus group discussions and randomised face-to-face interviews, while liking, preference and ranking of the novel food were established through consumer sensory perception.

Results: Willingness to consume the new food, low food neophobia (32%), a health-driven consumption pattern, as well as an appreciable acceptance for the developed pasta, was established among the consumers. Ugwu (Telfairia occidentalis) was found to be the most preferred leafy vegetable. The yellow cassava pasta was ranked better than the conventional white cassava.

Conclusion: This study shows new avenues to valorise yellow cassava by which nutrition security can be improved in low- and middle-income countries of Africa.

Keywords: acceptance for leafy vegetables; pasta; preference; yellow cassava.

2.1 Introduction

Micronutrient deficiencies, known as hidden hunger, affect over two billion people worldwide.[1] In Nigeria, a high intake of white-fleshed cassava (up to 940 g per adult per day fresh weight) is associated with poor-quality meals, low dietary diversification and limited consumption of micronutrient-rich foods such as leafy vegetables.[2, 3] Hidden hunger is prevalent in Nigeria, with the world's largest cassava production.[4] Among children under 5 years, the prevalence of iodine deficiency, vitamin A deficiency and iron deficiency anaemia was 29.6%, 29.5% and 28%, respectively.[5, 6] Efforts to improve micronutrient intake are still expensive and inaccessible to most people.[7, 8] Dietary diversification is especially low among the poor.[9] Thus yellow-fleshed biofortified cassava was introduced in 2011, with a fresh root carotenoid content of 5–11 μg g^{-1}, as a cost-effective way of improving vitamin A intake.[7]

The new yellow-fleshed biofortified cassava varieties (henceforth referred to as yellow cassava) are good sources of pro-vitamin A carotenoids, but generally low in iron and zinc. Moreover, cassava contains hydrogen cyanide, which requires processing for elimination. The processing significantly causes carotenoid content loss in cassava products,[10, 11] from 10–20% in wet paste fufu up to 50–70% in roasted products such as gari, chikwangue and pupuru.[12-14] Other interventions are thus required to make up for the post-processing losses and improve the nutritional value of yellow cassava food products.[15] New foods from micronutrient-rich leafy vegetables (vitamin A, iron and zinc) and cassava would improve a starch-based diet's nutrient value. In Nigeria, over 40 different species of affordable leafy vegetables are consumed but in small quantities,[16-18] indicating the potential to reintroduce them in popularly eaten food products.

Traditional food products of yellow cassava were acceptable to Nigerian consumers.[19] Oparinde *et al.*[20] studied consumer demand for yellow gari and eba, and consumer willingness to pay (WTP), while Bechoff *et al.*[21] examined acceptability and perception of traditional dough-like products from cassava (eba and fufu). Other studies were conducted mainly among farmers.[22] These authors reported positive responses to traditional yellow cassava products. Talsma *et al.*[23] tested the acceptance of boiled yellow cassava among school children in Kenya and reported likeness for the food and the need for additional food interventions.

To the best of our knowledge, the acceptability of novel food products from yellow cassava has not been studied. In this study, yellow cassava-based pasta enriched with the leafy vegetable amaranth was developed and tested. Pasta is a worldwide convenience food, which is suitable for adding ingredients rich in bioactive compounds. Pasta is gaining popularity in Nigeria and is eaten as spaghetti, macaroni and instant noodles. Several researchers investigated pasta development as functional food using non-wheat ingredients and vegetables.[24, 25] Yellow cassava vegetable enriched pasta is expected to provide consumers with a novel nutritious food that alleviates micronutrient deficiencies. However, consumer acceptance of such foods is pivotal. Bouis[26] also emphasised that acceptability is essential to the development of biofortified crops.

Consumers in low- and middle-income countries are becoming better educated, more demanding and less predictable in terms of purchase behaviour and more conscious about the health aspects of their food.[27] Thus changes in consumer tastes and preferences towards higher convenience food consumption attributed to urbanisation and rising income warrant further study.[28] Consumers can be ‘neophobic’ or ‘neophilic’ regarding taste, implying that neophobic humans have a natural tendency to dislike or even suspect new and unfamiliar foods, whereas neophilic people tend to accept novel foods.[29, 30] This study evaluated consumer acceptance of new foods from yellow cassava, pasta complemented with amaranth, and determined the main drivers of intended consumption across five states in Nigeria to improve nutrition security for the resource-poor.

2.2 Materials and methods

2.2.1 Sample preparation

The International Institute for Tropical Agriculture in Ibadan, Nigeria, provided yellow cassava variety TMS07/0593, with a carotenoid content of approximately 11 $\mu g\ g^{-1}$ (fresh weight).[7] The roots were peeled, washed, grated, de-watered, pulverised, dried and milled, according to Sanni *et al.*[31] to obtain high-quality cassava flour. The pasta was prepared by mixing 500 g cassava flour with boiled water in a 1:1 ratio by hand until a solid dough was formed. Pasta enriched with vegetables contained 5 g (w/w) freeze-dried amaranth powder. A mini extruder (Super Brev, Italy) was used to obtain pasta strands. Pasta samples were prepared according to good hygiene and manufacturing practices.[32]

2.2.2 Study area

This study was conducted between October and November 2019 in five states of Nigeria, representing four of the six geopolitical zones. Two zones were excluded because of pervading insecurity. The locations combined predominantly urban areas such as Lagos and Abuja, and mainly rural areas such as Bayelsa, Enugu and Akure (Ondo).

2.2.3 Study design

The study consisted of focus group discussions and a consumer acceptability study using questionnaires and a sensory perception evaluation among regular consumers of cassava products. The questions targeted (i) differences in acceptability among consumers for yellow cassava products and leafy vegetables, (ii) how demographic characteristics influence acceptability, consumer habits and individual preferences, (iii) the relationship between acceptability and sensorial differences perceived between the pasta samples, and (iv) possible food neophobia to assess potential consumption. There were slightly more males in the study than females, especially in the north, reflecting a society where culture makes men more socially accessible than women. Participants ranged from 18 to 75 years and were classified as Generation Z, Y, X, or baby boomers (mean age 33.5 years), with Abuja having the highest percentage of Generation Y (21–35 years old) and Lagos the highest number of Generation X respondents. Most of the respondents were single (50%), while the highest percentage of households with children below 5 years was in Bayelsa and Lagos had the lowest. 29% of respondents had higher education, while 15% had a form of post-secondary education. Bayelsa had a significantly higher percentage of respondents with post-university education (44%).

2.2.4 Focus group discussions

Three focus group discussions were conducted among 16 consumers: five rural men and women (32–61 years), seven urban women (22–50 years) and four urban men (26–40 years), who were familiar with cassava foods and leafy vegetables to investigate the habits, attitudes and perception of consumers as well as local utilisation of yellow cassava and leafy vegetables. Experienced participants who habitually consume cassava products were selected using local adverts. The focus group discussions helped the collection of data and were incorporated into the general questionnaire. Verbal consent was obtained from all respondents.

2.2.5 Consumer acceptability survey

Data on acceptance of yellow cassava products and leafy vegetables were collected through a consumer survey to assess the effect of various non-sensory factors (overview in Supporting Information Appendix A). For this study, 1437 people were randomly contacted. Of these, 575 met the requirements and participated in the survey. This purposive sampling indicated an awareness level of about 40% for yellow cassava in the study area. Questionnaires were administered face-to-face to the participants by trained university students. Respondents were recruited only if they had consumed yellow cassava and leafy vegetables before.

2.2.6 Questionnaire design

The items in the questionnaire were derived from the focus group discussions. The questionnaire consisted of three sections based on the conceptual framework of the three pillars of food acceptance and its drivers, namely (i) demographic characteristics of the participants using closed questions, (ii) food habits, food neophobia, lifestyle and consumption patterns of the participants rated on 7-point Likert scale with endpoints (strongly disagree–strongly agree) using an adapted version of the Food Choice Questionnaire,[33, 34] and (iii) barriers to consumption, previous eating experiences, familiarity with cassava products and leafy vegetables as well as a willingness to try newly developed cassava pasta.

2.2.7 Sensory perception

In a separate study, overall attractiveness, liking, smell, sweetness, saltiness, flavour, stickiness, firmness, and powderiness of the yellow cassava pasta samples were evaluated by 30 habitual consumers (16 males and 14 females) of cassava products, who were recruited by word of mouth and social media advertisements. The consumers' age range was 18–45 years. Four cooked cassava pasta samples (coded using three random numbers) were served in different random orders and tested, namely YFP (yellow cassava pasta), YFAP (yellow cassava pasta with amaranth), WFP (white cassava pasta) and WFAP (white cassava pasta with amaranth). The samples were cooked for 20 min, kept warm in a heating device and served using plastic cutlery. First, consumers were instructed and asked to rate the samples' attractiveness and overall liking (appearance, aroma, taste and texture) on a 9-point scale (1 = dislike extremely, 9 = like extremely). Next, consumers tasted the same pasta samples and ranked them on intensity (1 = least intense, 4 = most intense).

2.2.8 Statistical analysis

For the focus group discussions, qualitative data were generated on consumers' attitudes to yellow cassava, leafy vegetables and pasta products. The data guided the construction of questionnaires for the consumer survey. Cleaned data were analysed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). For the eight nominal and ordinal sociodemographic characteristics of consumers, percentages per class were given for each state. Tests on differences between states in these characteristics were done with chi-square tests. The habits of eating outside the home and food neophobia were described by relative frequencies of six levels on an ordinal scale ('never' to 'always') and nominal scale (no = 0, yes = 1), respectively. The differences between states, gender, age groups and the frequency of consumption were investigated using a Kruskal–Wallis test with paired comparisons. Consumers' attitudes and motives of choice for choosing their most preferred leafy vegetables were collected using 21 statements to which the respondents answered on a 1–7 Likert scale. Exploratory factor analysis was used to determine the underlying structure of attitudes and motives. For each factor, Cronbach's alpha was used to test for consistency. For sensory perception, Friedman's test and analysis of variance (ANOVA) were used to test for significant sensory attributes differences across the pasta samples judged by consumers.

2.3 Results and discussion

2.3.1 Focus group discussions

The focus group discussions revealed that participants had positive perceptions about the new yellow cassava–leafy vegetable pasta products and offered suggestions on several other new products that could be acceptable to them, such as snacks and breakfast products. '*Tapioca*' and '*Abacha*' (cassava-based salad) were also frequently mentioned. Several participants expressed willingness to consume the pasta products of yellow cassava and leafy vegetables with a statement such as '*Yellow cassava pasta is not available in our markets. If it was, I would like to buy*.' In contrast, the unavailability of yellow cassava in the market was generally perceived as a hindrance to consumption. The yellow cassava–vegetable pasta was generally liked among the participants. Participants also expressed a preference for vegetables they were familiar with, while the younger participants claimed ignorance of cooking methods for most of the vegetables mentioned as a reason for non-consumption (Supporting Information Appendix B).

2.3.2 Demographic characteristics associated with neophobia and consumer attitude across the states

Results showed that most Nigerian consumers are generally neophilic towards new foods (68 %). Food neophobia was higher among females (35 %) than males (31 %), with significant differences across states ($P < 0.001$). Respondents in Bayelsa had the highest neophobic tendencies, with 42 % not willing to try new food. The most food neophilic age group was Generation Z (20 ≤ years), with 57 % claiming they like to try new foods. However, baby boomers had higher food neophobic characteristics than other age groups (Fig. **2.1**), in line with studies by Meiselman *et al.*,[35] who established a neophobic tendency among older US consumers and associated older age groups with higher food neophobia. Ullrich *et al.*[36] also measured 'food adventurousness' with a single question: 'How often do you try unfamiliar foods?' and reported higher levels for the Z and Y generations. The comparatively higher level of food neophobia found for many elderly Nigerians may affect their acceptance of new cassava pasta food products. Cross-cultural differences in food neophobia were also observed in all the states, with a relatively higher food neophobia in Bayelsa. Furthermore, a higher education level was related to the willingness to try new food as more educated respondents tended to be more food neophilic. Therefore, less literate individuals seem more likely to reject new foods from yellow cassava and leafy vegetables. Significant differences were also observed across the states in consumers' frequency of food consumption outside the home (Fig**. 2.2).**

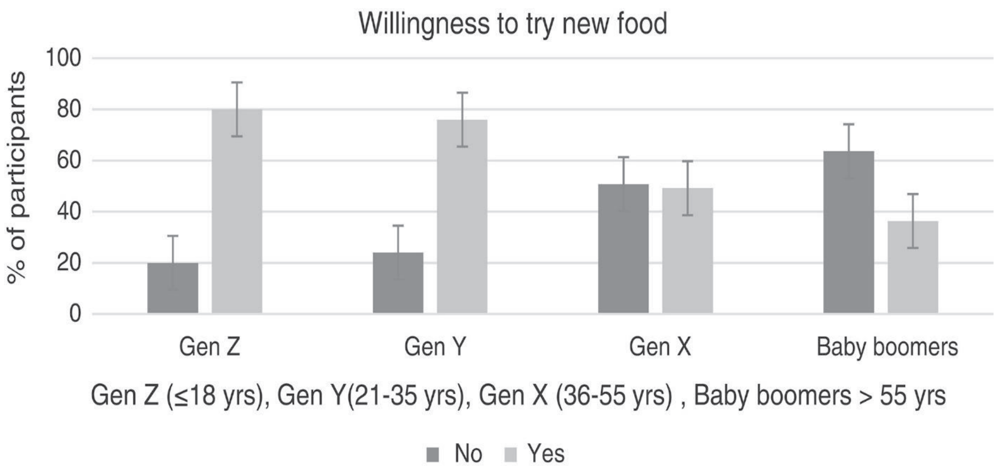

Figure 2.1. Food neophobia across four Nigerian states by age group

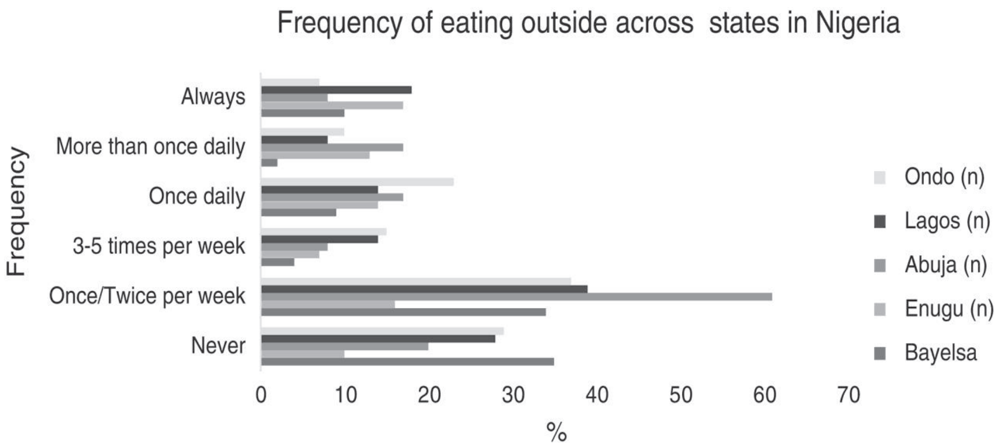

Figure 2.2 Attitude of consumers to eating outside the home across the states in Nigeria

2.3.3 Cassava and leafy vegetable consumption, habits and attitudes

A high frequency of consumption of yellow cassava and leafy vegetables was observed in all the states, in line with several other reports on Nigerians' food habits.[37] Most consumers who had already consumed yellow cassava for more than 1 year also ate it with leafy vegetables. Respondents claimed that vegetables are commonly milled, cooked, steamed or stir-fried and are sparingly eaten but usually not in the raw form. The frequency of cassava and leafy vegetable consumption was significantly different across the states (Table 2.1), while 82 % of consumers habitually consume cassava at least once every week with a form of vegetable. This finding is consistent with focus group discussions, indicating an increased intake of vegetables with starchy staples such as gari, fufu and lafun. As shown in Table 1, most consumers reported eating cassava daily or more than twice a week, with the highest consumption frequency among Bayelsa consumers (67 %). The study also showed that consumption frequency was higher for leafy vegetables than cassava, especially in Enugu and Ondo, where about 35 % of consumers reported a daily intake of leafy vegetables (Appendix G).

Table 2.1 Consumption frequency of yellow cassava and leafy vegetables across the states

	Yellow cassava consumption frequency					Leafy vegetable consumption frequency				
	Everyday	>Twice/ wk.	Twice /wk	Once/ wk	Once/2wks	Everyday	>Twice/ wk.	Twice /wk	Once/ wk	Once/2wks
Bayelsa (n)	8	52	12	8	10	26	54	9	4	1
%	8.9	57.8	13.3	8.9	11.1	27.7	57.4	9.6	4.3	1.1
Enugu (n)	0	10	8	7	48	27	33	9	3	2
%	0.0	13.7	11.0	9.6	65.8	36.5	44.6	12.2	4.1	2.7
FCT (n)	11	21	26	17	42	21	43	13	18	13
%	9.4	17.9	22.2	14.5	35.9	19.4	39.8	12.0	16.7	12.0
Lagos (n)	4	15	8	10	47	29	42	16	14	13
%	4.8	17.9	9.5	11.9	56	25.4	36.8	14.0	12.3	11.4
Ondo (n)	13	18	12	24	30	43	36	24	12	8
%	13.4	18.6	12.4	24.7	30.9	35.0	29.3	19.5	9.8	6.5
Total (N)	36	116	66	66	177	146	208	71	51	37
%	8.0	25.0	14.0	14.0	38.0	28.5	40.5	13.8	9.9	7.2

The self-reported higher intake frequency for leafy vegetables than cassava among consumers may be attributed to the heightened awareness of the importance of vegetable consumption as advocated by the local authorities and reported in the focus group discussion (Supporting Information Appendix B) – an indication of the positive influence of nutrition education on the acceptance of food by consumers. It is thus expected that increased nutrition education across the country may lead to a higher frequency of consumption. Some studies on commonly consumed leafy vegetables in Nigeria rated Amaranthus spp. as number one.[38,39] However, in this study, *Telfairia occidentalis* (ugwu/ fluted pumpkin) was found to be the most preferred leafy vegetable, at 29 % consumer preference across the states (Appendix D) and an especially high preference in Lagos, a predominantly urban location, in line with the report of Olatona *et al.* [16,17] The most preferred leafy vegetables from this study (fluted pumpkin, amaranth and bitter leaf) were also reported by Adewoyin *et al.* [40]

2.3.4 Consumer attitudes and motives for leafy vegetable consumption

Major determinants of consumer attitudes and motives of choice for leafy vegetables were ascertained using exploratory factor analysis (EFA). This analysis revealed the underlying structure of the questionnaire. EFA indicated five factors influencing consumption based on the screen plot of the eigenvalue. These factors explained 52.4% of the variance; the rotations converged in 19 iterations (Appendix K). These results differ from the nine factors presented by Steptoe *et al.*, [33] as only five factors had items loading 0.50 or more. Our output (Table 2.2) revealed that Factor 1 was related to the health and nutritional aspects of leafy vegetables.

Table 2.2 Motives driving leafy vegetable consumption among consumers

	Loading	Mean	SD	Cronbach Alpha
Factor 1 Health and Nutritional aspects				0.87
Has medicinal benefits	0.61	6.33	1.18	
Keeps me healthy	0.59	6.35	1.13	
High in fibre	0.65	6.06	2.09	
Factor 2 Familiarity				0.87
It is commonly eaten by my tribe	0.94	6.30	1.22	
It is the food I have been used to	0.88	6.02	1.32	
Factor 3 Convenience				0.86
Can be easily purchased	0.55	6.15	1.33	
It is easy to wash	0.59			
Can be cooked very simply	0.56	6.18	1.44	
Factor 4 Sensory Appeal				0.87
It is very tasty	0.76	6.10	1.43	
Factor 5 Natural Content				0.86
It is free from contaminations (e.g., pesticides/fertilizers)	0.78	6.15		
It is handled hygienically	0.68	6.02		

This dimension represented a desire to live and eat healthily. By contrast, Factor 2, familiarity, indicated adherence to established and long-term habits about food. This segment may tend towards aversion for unfamiliar leafy vegetables. Factor 3 represents the convenience and simplicity of preparation and is equally important to these consumers. Factor 4, sensory appeal, represents how important it is for the consumers to enjoy the food. Factor 5, natural content, is mainly concerned with the naturalness of the food – foods without artificial ingredients. The results show that the main motives of consuming leafy vegetables are related to health, as also reported by other authors.[17] This result suggests that consumers for whom health considerations are most important will most likely accept yellow cassava with vegetable pasta products.

2.3.5 Consumers' perception of yellow cassava pasta among participants

The initial perception of consumers for the pasta product was tested by rating their general impression of the product on a 6-point ordinal scale from (1) 'very positive' to (6) 'negative'. Overall, 71 % of the 476 respondents gave a positive/very positive score (Fig.2.3), an indication that consumers will likely welcome a cassava pasta product. Over 50 % of Abuja and Ondo consumers were very positively inclined towards the yellow cassava product, while Abuja also had the highest percentage (20 %) of neutral to negative consumers.

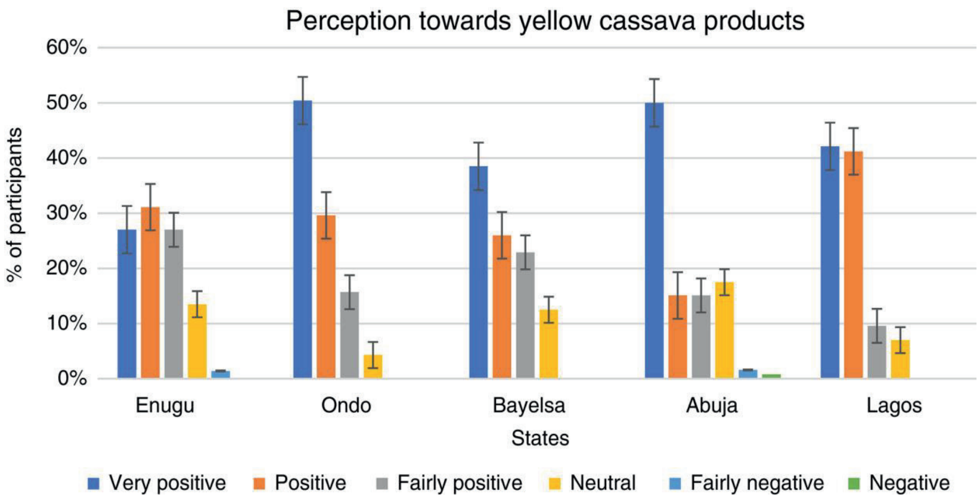

Figure 2.3. Perception of consumers towards yellow cassava food products across the states.

2.3.6 Barriers to the consumption of yellow cassava and green leafy vegetables among participants

The most frequently mentioned barriers to the consumption of yellow cassava were 'Availability', 'Taste', 'Convenience' and 'Health' (but all less than 10 %), while the main perceived barrier to vegetable consumption was 'Health concerns. As shown in Fig. 4, the primary barrier to the consumption of yellow cassava observed in this study was availability, but still only at less than 10 %. The barrier associated with the availability of yellow cassava was also reported by Talsma *et al.* [23] among Kenyan consumers. Results from the consumer survey corroborated the complaint of focus group participants about the scarcity of yellow cassava in the market and the low awareness for yellow cassava observed during participants' recruitment. The distribution of yellow cassava across Nigeria appears to be low, as confirmed by the responses of consumers. Participants also indicated that the barriers to consuming leafy vegetables were related to taste, familiarity, personal preference, convenience, preparation and health concerns (purging/diarrhoea). Several leafy vegetables with bitter tastes are laborious to prepare, thus reducing their consumption. Moreover, poor preparation techniques of some vegetables were also said to result in health issues. However, the main barrier to consumption was related to health concerns, as some respondents (29 %) who had issues with the consumption of leafy vegetables claimed that some vegetables cause diarrhoea. Due to the high fibre content of leafy vegetables, some cruciferous vegetables may cause bloating and gas pains or induce diarrhoea when consumed too frequently.[41,42] This barrier to consumption was similarly reported among mid-Western African American women.[43] Generally, the barriers to vegetable consumption mentioned by Nigerian consumers were also similar to the findings of Lucan *et al.* [44] among African Americans residing in the USA. They reported the main barriers to consumption – taste, cost, health, convenience and availability – whereas Santos *et al.* [45] reported 'cost 'and 'familiarity' as main barriers to vegetable consumption.

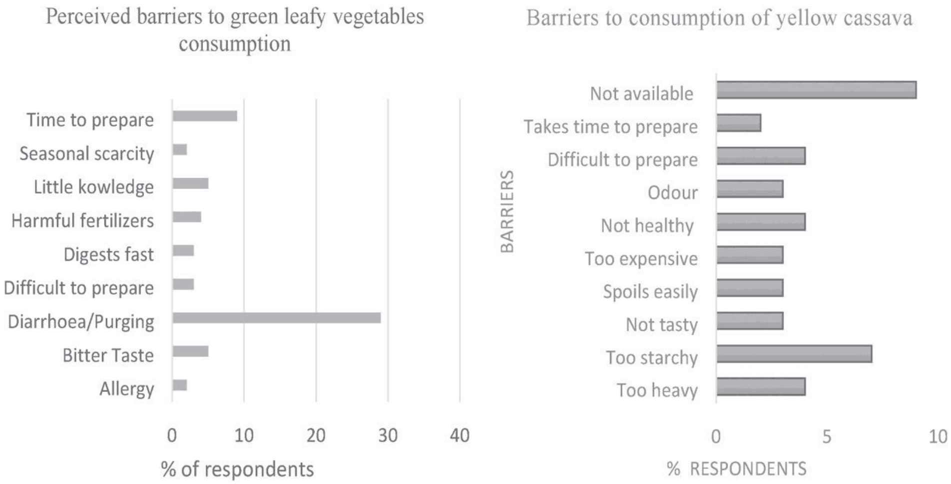

Figure 2.4. Perceived barriers to consumption of yellow cassava and leafy vegetables.

2.3.7 Preferred new products from yellow cassava among consumers

Pasta products, such as macaroni, spaghetti and noodles, were mentioned most frequently as a preferred new yellow cassava product by 25 % of the respondents, followed by breakfast cereals (20 %) (Supporting Information Appendix I). As shown in Fig. 2.5, pasta products were consumers' choice across the states, particularly in urban locations, confirming that pasta products are fast gaining acceptance among Nigerian consumers. Other products mentioned by the respondents were 'Abacha' (a form of cassava-based salad) and tapioca or mingau (a cassava-based pudding). In Bayelsa, many respondents (39 %) expressed the desire to have yellow cassava made into porridge, puddings and local beverages.

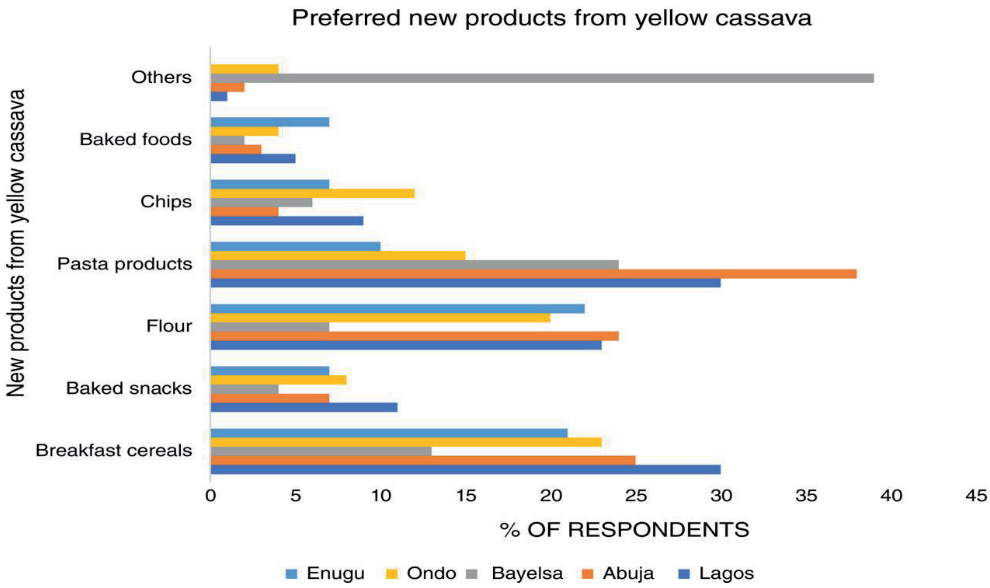

Figure 2.5. Preferred new food products from yellow cassava by consumers across the states.

2.3.8 Sensorial differences among samples

ANOVA of the ranking of test results indicated some significant differences between pasta samples for the evaluated attributes at $P < 0.05$ (Fig. 2.6). YFP was ranked significantly higher on attractiveness ($P = 0.00$) and overall liking ($P = 0.00$) than the other samples, indicating higher acceptability, while YFAP did not differ significantly from the other samples. A significant difference ($P = 0.041$) was observed between the samples in terms of stickiness. YFP was ranked as most sticky, while WFAP was ranked least sticky. A significant difference ($P = 0.001$) between the samples in terms of firmness was also established. The sensory results show that YFP was ranked highest in firmness. No significant differences were found in sweetness, saltiness, flavour intensity and powderiness among the samples. Study limitations A limitation of this study was the predominant use of student interviewers. However, this high number of students reflects Nigeria's demographic structure, where 50.3 % of the population is between the age of 15 and 54 years and the literacy level are at 62 %. A high literacy level was observed in this study and may have impacted the low food neophobia observed among the study population, as studies have shown that neophobia is lower among better-exposed individuals.[35] This study indicated that at present ugwu may have become the most preferred leafy vegetable in Nigeria, contrary to existing literature. Thus further research is needed on the acceptability of a yellow cassava pasta combined with the most preferred leafy vegetable, ugwu.

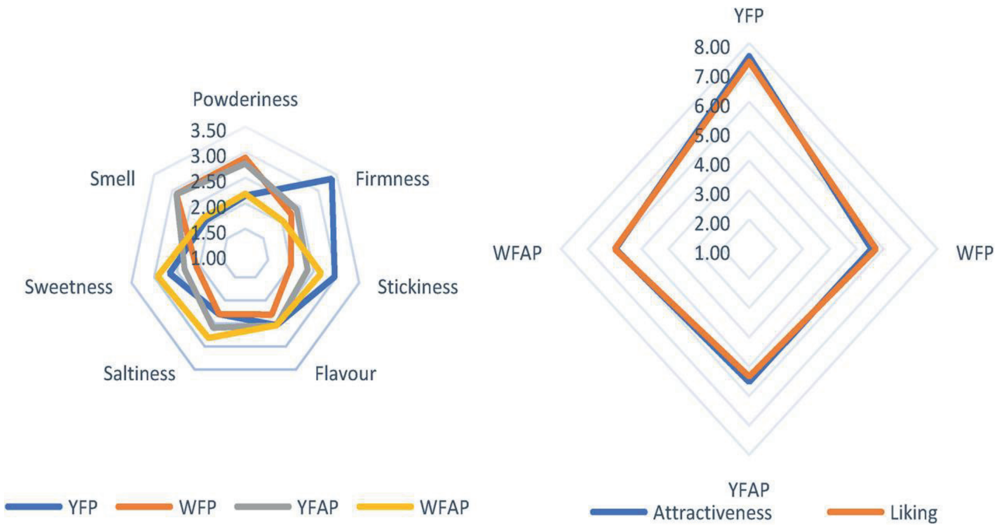

Figure 2.6. Results of sensory perception YFP (Yellow cassava pasta, WFAP (white cassava with amaranth vegetable pasta, YFAP (yellow cassava with amaranth vegetable pasta and WFP (white cassava pasta).

2.4 Conclusion

Our study, aimed at ascertaining consumer perception and attitude to consuming a new yellow cassava pasta complemented with a vegetable, confirmed modest acceptance for the novel food product. Generally, consumers eat vegetables more frequently than yellow cassava and preferred ugwu to other leafy vegetables. Interestingly, health considerations were the main driver in consuming yellow cassava foods. In contrast, the most prominent barrier to consumption was availability, as awareness is still low in Nigeria, almost a decade after the introduction of yellow cassava. Efforts at making yellow cassava more accessible to people thus need to be intensified by cassava sector stakeholders across the country. The health benefits of the novel food, used as the key selling point, should be mentioned on package labels and marketing materials to improve the acceptance of cassava– vegetable pasta. Yellow pasta was better accepted than cooked yellow pasta with vegetables, an indication that the sensory quality of the product needs to be improved. To our knowledge, this is the first time a vegetable-enriched yellow cassava pasta was introduced to the Nigerian population, and acceptability was higher than expected. There is thus a potential to develop more quality nutritional novel food products using yellow cassava, as consumers mentioned several other convenience food products that differ from the well-known, traditional products. The findings of this study highlight a need to conduct experimental studies on the nutritional composition of the novel pasta products to ascertain the nutritional value after processing and the bioavailability of vitamin A and minerals in the human system.

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Chapter 2

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APPENDIX A. Overview of methods used in the study

Methods	Variables	Scale
Focus group discussions	Attitudes and perception of consumers	Identify drivers of consumption
Consumer acceptance study	Socio-demographics variables of participants	Nominal
	Food neophobia attitudes (Neophobia level)	0=No, 1= Yes
	Predicted consumption by state, tribe and gender	From 1=never to 6= always
	Self-reported consumption frequency	From 1=once in 2 weeks to 5= everyday
	Food preference	Ordinal
	Perceived barriers to consumption	Ordinal
	Drivers of food choice	7-point Likert scale from 1= Totally disagree to 7= Totally agree
	Self-reported perception	7-point Likert scale from 1= Strongly disagree to 7= Strongly agree
Sensory Perception	Preference among samples of cooked biofortified cassava and leafy vegetable pasta.	Liking and attractiveness on a 9-point Hedonic scale (1=dislike extremely, 9= like extremely) Ranking of pasta samples from 1=least intense to 4=most intense

APPENDIX B Socio-demographic characteristics of yellow cassava and green leafy vegetable consumers

Variables		**Ondo** n=142 %	**Lagos** n=121 %	**Bayelsa** n=100 %	**Enugu** n=78 %	**Abuja** n=134 %	**Total** **N=575** **%**
Gender	Male	38.7	45.5	48.0	57.7	59.0	49.1
	Female	45.8	52.9	52.0	41.0	38.8	46.1
Age	≤20yrs (Gen.Z)	10.8	6.0	3.0	3.9	14.5	7.8
	21-35yrs (Gen.Y)	60.8	44.4	58.0	69.7	75.7	58.3
	36-55yrs (Gen. X)	20.9	41.0	27.0	23.7	8.4	22.5
	>55 yrs (Baby boomers)	7.5	8.5	12.0	2.6	1.5	6.1
Family status	Single	43.7	40.5	42.0	48.7	73.9	50.4
	Married	38.7	52.9	42.0	42.3	20.1	38.4
	Widowed	1.4	3.3	14.0	7.7	3.0	5.2
Children< 5yrs in household	No	49.3	66.9	53.0	52.6	55.2	55.5
	Yes	19.0	25.6	37.0	34.6	34.3	29.6
Education	No Secondary	13.4	14.0	27.0	7.7	0.7	12.2
	Secondary	21.1	27.3	21.0	24.4	26.9	24.2
	Post-Secondary	29.6	20.6	29.0	17.0	25.3	15.1
	University	18.3	25.6	19.0	42.3	43.3	29.0

	Post University	2.8	4.1	44.0	6.4	1.5	3.5
Employment	Manager & above	0.0	5.8	7.0	5.2	0.0	3.1
	Professional	9.9	10.7	7.0	11.5	9.0	9.6
	Skilled vocational	11.3	15.7	24.0	25.6	21.6	18.8
	Unskilled worker	7.0	29.8	13.0	10.3	3.7	12.5
	Clerical worker	2.8	4.1	7.0	6.4	3.7	4.5
	Between jobs	17.6	10.7	9.0	5.1	12.7	11.8
	Students	24.6	9.1	9.0	33.3	44.0	24.3
	Others	16.9	5.0	19.0	2.6	3.0	9.2
Willingness to try new food (neophobia)	No	39.2	38.3	42.3	18.7	21.7	32.5
	Yes	60.8	61.7	57.7	81.3	75.4	67.5
Always eat cassava with green leafy vegetables	No	18.5	18.4	28.6	14.5	9.5	17.7
	Yes	81.5	81.6	71.4	85.5	90.5	82.3

Questionnaire on Biofortified (Yellow) Cassava and Green Leafy Vegetables (GLV) consumption/utilization

DATE:
LOCATION:

Introduction

My name is ……………………………………………………………………………… from the Department of Food Science and Technology, Federal University of Technology, Akure. I am here to ask your opinion about biofortified cassava and green leafy vegetable food products. We are conducting a survey about biofortified(yellow) cassava and green leafy vegetables food products and are looking for people to take part in this research. If you are willing, I will ask you a few questions to establish whether you qualify and I will need about 10 minutes of your time to complete the survey.

Selection questions for inclusion :

INTERVIEWER: *Q1 and Q2 should be asked by you*

Q1. Have you ever eaten a yellow (biofortified) cassava food product?

[please pick one answer]

- ☐ Yes
- ☐ No –> INTERVIEWER says: I am sorry but you do not qualify because our research is on yellow (biofortified) cassava food products. Thank you for your time.

Q2. Do you eat green leafy vegetables?

[please pick one answer]

- ☐ Yes, I eat green leafy vegetables regularly
- ☐ Yes, I eat green leafy vegetables once in a while
- ☐ No, I do not eat green leafy vegetables –> INTERVIEWER say: I am sorry but you do not qualify since our research is also on green leafy vegetables. Thank you for your time.

INTERVIEWER: *If the participant meets the inclusion criteria, is willing to participate please ask for their name and telephone number or email address.*

____.

May I please have some background information about you?

APPENDIX C Sensory test – Cassava pasta

Welcome to this sensory evaluation, and thank you for joining us!

Location: ____

Gender:
- ☐ Male
- ☐ Female

Are you familiar with Cassava consumption?
- ☐ Yes
- ☐ No

Please read the instructions, sign the consent form and use the codes provided on the sheet.

<u>Part 1</u>

How much do you feel attracted to this food, knowing it is made of cassava?

	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Sample A	○	○	○	○	○	○	○	○	○
Sample B	○	○	○	○	○	○	○	○	○
Sample C	○	○	○	○	○	○	○	○	○
Sample D	○	○	○	○	○	○	○	○	○

How much is the product liked?

	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Sample A	○	○	○	○	○	○	○	○	○
Sample B	○	○	○	○	○	○	○	○	○
Sample C	○	○	○	○	○	○	○	○	○
Sample D	○	○	○	○	○	○	○	○	○

<u>Part 2</u>

Smell the sample

Please rank the sample based on **smell**, from less (1) to intense (4)

1. Sample: _________
2. Sample: _________
3. Sample: _________
4. Sample: _________

Taste the sample

Please rank the sample based on **sweetness**, from less (1) to intense (4). Sweetness can be associated with sugars.

1. Sample: _________
2. Sample: _________
3. Sample: _________

4. Sample: _________

Please rank the sample based on **saltiness**, from less (1) to intense (4)

1. Sample: _________
2. Sample: _________
3. Sample: _________
4. Sample: _________

Please rank the sample based on **flavour intensity**, from tasteless (1) to intense (4)

1. Sample: _________
2. Sample: _________
3. Sample: _________
4. Sample: _________

Feel the sample in your mouth

Please rank the sample based on **stickiness**, from less (1) to intense (4)

1. Sample: _________
2. Sample: _________
3. Sample: _________
4. Sample: _________

Please rank the sample based on **firmness**, from less (1) to intense (4)

1. Sample: _________
2. Sample: _________
3. Sample: _________
4. Sample: _________

Please rank the sample based on the **granular mouthfeel**, from less (1) to intense (4)

1. Sample: _________
2. Sample: _________
3. Sample: _________
4. Sample: ________

Focus group discussion file

Introduction to the study

Welcome, everyone! Thank you for taking the time to participate in this discussion. I am ___________ and I will be moderating this discussion. With me, is ________________________ who shall be taking notes for us. Today we shall be discussing cassava and leafy vegetables. We expect this session to last for about 2 hours, so we shall take a break after the first hour. I will ask some specific questions to keep the conversation flowing. Please indicate if you want to add anything or if you have any questions. Our co-moderator is ___________________ who may also ask some questions to clarify issues. We want to be sure to g*et al*l of the important things you say.

Your participation in today's discussion will help us understand better how to make better use of cassava and leafy vegetables. Please note that our discussion is being audio-taped to help us remember what we said. But you may ask me to turn it off at any time if you do not want to answer a question. Please feel free to speak openly as there are no right or wrong questions.

Consent Process

Before we start, we need you to officially consent in writing to participate in this research study

START RECORDING

Description of the participants of the three focus group sessions

Description of participants	Session 1 (n = 5)	Session 2 (n = 7)	Session 3 (n = 4)
Gender			
Male	2	0	4
Female	3	7	0
Age			
18-25	0	3	2
26-35	2	1	1
36-55	1	1	1
>55	1	2	0
Education			
Basic	3	1	0
Secondary	2	2	2
University	0	4	2
Post University	0	1	0
Employment			
Manager	0	0	1
Skilled worker	2	0	0
Clerical worker	1	1	0
Between jobs	1	2	1
Student	1	4	2

Focus group discussion questions guide

Do you like cassava food?

Where do you eat cassava, home or outside? How do you make cassava food in your home?

Which food made with cassava do you like most?

Have you eaten yellow cassava before? When did you eat yellow cassava for the first time?

What are the differences between white and yellow cassava?

What do you think about yellow cassava?

How do you prepare yellow cassava?

Do you like yellow cassava? If yes, what do you like most about it?

What do you dislike about it?

What do you think about leafy vegetables? Which ones do you know?

How do you prepare leafy vegetables?

What ways do you combine cassava with vegetables?

What do you think about pasta products?

Which pasta product do you like?

What do you think of pasta made with cassava?

How about when pasta is made with yellow cassava?

What do you think of pasta with vegetables already included?

Any comments about the future of yellow cassava as food?

Perceptions of the participants of the three focus group discussions regarding yellow cassava and leafy vegetable food products

Topics	Verbatim statements	Discussions
Consumer perception about yellow cassava	*'It is sweeter than white cassava'* *'I like the yellow colour'* *'I think it has more nutrients than the white one'.* *The yellow cassava is better for our health.* *'I always feel full after eating cassava'* *'It is more costly than the white'* *'Yellow cassava is still scarce in the market here.'* *'Gari and Fufu from yellow cassava is most liked by my family'*	The most frequently expressed opinions about yellow cassava concerned the sweet taste and health benefits. Scarcity and a higher price than for the conventional white variety were major concerns.
Consumer perception about leafy vegetables	*'I think leafy vegetables are very good for my body.'* *'It is more tasty to eat cassava products with vegetable stew.'* *'Vegetables like Ugwu is very good for the blood'.* *'Vegetables are cheap here* *'I only eat the ones we are used to'.* *'I prefer cooking the vegetables, I don't like eating them raw.'* *'I prefer growing my vegetables to avoid fertilizer which is bad for my health'.* *'I don't eat vegetables because I don't know how to prepare them'*	Leafy vegetables were generally perceived as healthy and a complement to cassava dishes. About 15 types of vegetables were mentioned as the most preferred. Participants are aware of the existence of other vegetables but prefer the ones they are familiar with.
Consumer perception about yellow cassava and leafy vegetable combination	*'I always eat yellow-fleshed cassava with green leafy vegetables to balance the meal'* *'Cassava meals are not complete without vegetables'* *'I grew up to eat cassava with vegetable soup'* *'A breakfast product will be very good for me'*	Overall, participants agree that it is acceptable to combine leafy vegetables in cassava dishes.
Perception of yellow cassava and leafy vegetable pasta products	*'If it is like spaghetti it will be good for my kids. We eat spaghetti 2 or 3 times a week'.* *'If the noodles are already combined with vegetable, it will be very good for the body'* *'It may be too costly'* *'A pasta like this already combined with vegetables will be good for my family, hope it won't be too dear'*	Generally, there were positive comments about cassava pasta but some of the more elderly participants believe that pasta is for the younger generation and may be too expensive for poor people to afford.
Willingness to consume yellow cassava and leafy vegetables pasta products	*'Yellow cassava pasta is not available in our markets, if it was I would like to buy.'* *'I am not familiar with this yellow cassava pasta.'* *'It has the faint smell of cassava, I like it.'* *'The one in green colour, children may not like it.'* *'Pasta is for children, I think the students may like this more.'* *'I don't like the green colour pasta'.*	Most participants were willing to consume the pasta products, provided they would be available in the markets. Some expressed dissatisfaction with the green colour.
Additional information	*'I believe I eat enough vegetables because I take vegetable soup with cassava almost*	With cassava meals, participants eat cooked leafy

every day'. *'Vegetable soups with cassava is better for the body than ordinary stew'*	vegetables, referred to as 'soup', which they perceive as more nutritious than 'stew' due to the inclusion of leafy vegetables.
'Government workers from the ministry told us to eat vegetables every day' *Announcements by the Ministry of Health on importance of leafy vegetables now on the radio.*	Leafy vegetables consumption awareness may have increased due to advocacy programs of the local authorities.

APPENDIX D Most preferred choice of leafy vegetable across the states

Leafy vegetables	Enugu (%)	Ondo (%)	Bayelsa (%)	Abuja (%)	Lagos (%)	Total % (N)
Ugwu/ Fluted pumpkin (*Telfairia occidentalis*)	33.3	20.4	22.0	27.6	43.0	28.9 (166)
Bitter leaf/Onugbo (*Vernonia amygdalina*)	25.7	10.7	24.0	22.4	7.4	14.6 (84)
Tete/Amaranth(*Amarantus cruentus/hybridus*)	2.6	28.9	3.0	7.5	2.5	10.1 (58)
Water leaf/Gbure (*Talinum triangulare*)	5.1	7.0	8.0	4.5	4.1	5.7 (33)
Ukazi *(Gnetum Africanum*)	1.0	0.0	2.0	1.0	0.8	5.4 (31)
Bush buck/Utazi (*Gongronema latifolium*)	2.6	0.7	15.0	6.0	2.5	5.0 (29)
Ewedu/Jute mallow/Saluyot (*Corchorus*)	0.0	4.2	2.0	3.0	9.9	4.2 (24)
Sokoyokoto/Spinach (*Celosia argentea* L.)	0.0	2.1	0.0	1.0	8.6	3.3 (19)
African eggplant leaf *(Solanum macrocarpon*)	3.8	1.6	2.0	2.2	0.8	2.4 (14)
Basil African/Effirin (*Ocimum Gratissimum* L.)	2.6	1.4	5.0	0.0	0.8	1.7(10)
Worowo (*Solanecio biafrae*)	0.0	9.2	0.0	0.7	1.7	1.7 (10)
Wild lettuce/Yanrin (*Taraxacum officinale*)	0.0	7.0	1.0	3.0	0.8	1.2 (7)
Ebolo (*Crassocephalum rubens*)	0.0	0.7	1.0	0.7	1.7	1.0 (6)
Igbo ((*Detarium microcarpum*)	0.0	0.1	5.0	0.0	0.0	0.9 (5)
Oha/Ora (*Pterocarpus mildbraedi*i)	1.0	0.0	1.0	1.0	0.1	0.5 (3)
Amunututu (*Basella alba*)	0.0	0.1	0.0	0.5	0.5	0.5 (3)
Uziza (*Piper guineense*)	1.0	0.0	2.0	0.0	0.8	0.5 (3)
Odu (*Solanum nigrum*)	0.0	0.1	1.0	0.0	0.0	0.3 (2)
Roselle/Isapa *(Hibiscus sabdariffa*)	0.0	0.0	1.0	0.7	0.0	0.3 (2)
Afang (*Gnetum africanum*)	1.0	0.0	0.0	0.7	0.8	0.3(2)
Marugbo (*Clerodendrum volubile*)	0.0	0.1	0.0	0.7	0.0	0.2 (1)
Others (Moringa, Cassava leaves etc)	20.3	5.7	6.0	16.3	13.2	11.4 (63)
						100.0(575

APPENDIX E Frequency of consumption of food outside the home by gender (Across states)

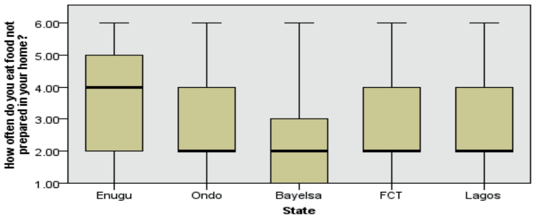
How often do you eat food not prepared in your home? *Gender*

	Never	*Once/2ce per week*	*3-5 times per week*	*Once daily*	*> than once daily*	*Always*	*Total*
Male(n)	42	91	26	47	42	32	280
(%)	15.0%	32.5%	9.3%	16.8%	15.0%	11.4%	100.0%
Female(n)	78	94	22	30	8	27	259
(%)	30.1%	36.3%	8.5%	11.6%	3.1%	10.4%	100.0%
Total (n)	120	185	48	77	50	59	539
%	22.3%	34.3%	8.9%	14.3%	9.3%	10.9%	100.0%

APPENDIX G Frequency of consumption of food outside the home (Across states)

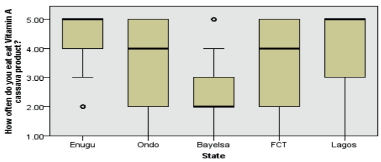
and frequency of green leafy vegetables and yellow cassava foods across the states

Independent-Samples Kruskal-Wallis Test



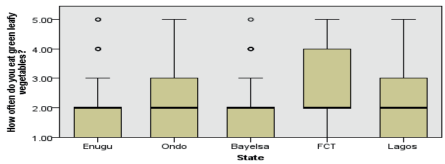
Total N	544
Test Statistic	30.505
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.000

Independent-Samples Kruskal-Wallis Test



Total N	461
Test Statistic	74.029
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.000

Independent-Samples Kruskal-Wallis Test



Total N	513
Test Statistic	21.040
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.000

APPENDIX H Consumer's perception about yellow cassava and green leafy vegetables (GLV) among the study

	Components of perception		
	1	2	3
Yellow (Vitamin A) cassava foods should be eaten with GLV at least weekly	.728		
Cassava meals are not complete without vegetables	.699		
Yellow (Vitamin A) cassava is more healthy than white cassava	.688		
I would like to try new yellow (Vitamin A) cassava food products	.681		
I like yellow (Vitamin A) cassava combined only with my favourite green leafy vegetable	.648		
I like all foods made from Vitamin A cassava	.627	.314	-.348
Fast food can be healthy if GLVs are added	.609		.315
I like food made with yellow (Vitamin A) cassava to be combined with any GLV.	.558		
I like snacks made from yellow (Vitamin A) cassava	.534	.469	-.319
I do believe that vegetables are essential for my health.		.721	
I am too busy to make meals with vegetables		.646	
It is difficult to prepare and cook GLV for a meal		.536	.388
It takes too long to prepare a balanced meal with GLVs			.743
I cannot pay more money to buy yellow cassava, it is the same as the white cassava			.667

Extraction Method: Principal Component Analysis. Rotation Method: Oblimin with Kaiser Normalization

a. Rotation converged in 19 iterations.

APPENDIX I: Preferred new food products from yellow (Vitamin A) cassava

	Frequency	Percentage	Mean	SD
Pasta (Macaroni/Noodles/Spaghetti)	117	25.0	3.58	1.09
Breakfast Cereal (e.g. Flakes, Custard)	112	19.5	3.18	1.49
Flour	96	16.7	3.06	1.53
Others	46	8.0	3.00	0.47
Chips	38	6.6	2.89	1.47
Baked Snacks	37	6.4	3.19	1.54
Baked foods	21	3.7	2.77	1.64
Total	467	100.0	3.19	1.39

P <0.0001

CHAPTER THREE

TECHNOLOGICAL AND NUTRITIONAL PROPERTIES OF AMARANTH-FORTIFIED YELLOW CASSAVA PASTA

This chapter has been published as:

Lawal, O. M., Stuijvenberg, L., Boon, N., Awolu, O., Fogliano, V., & Linnemann, A. R. (2021). Technological and nutritional properties of amaranth-fortified yellow cassava pasta. *Journal of Food Science*, 1750-3841.15975. https://doi.org/10.1111/1750-3841.15975

Abstract

Yellow cassava is an affordable starting material to design a healthy food, having high β-carotene content. White and yellow cassava functional pasta were fortified with 50 g/kg (w/w) amaranth dry leaf powder and analyzed to evaluate the impact of cultivar difference, processing, and addition of amaranth leaf powder on the physicochemical, functional, pasting, antioxidant, and cooking properties of the white and yellow cassava pasta samples. Significant differences were observed among the cassava pasta samples. Leaf powder addition significantly enhanced the dietary fibre (7.6–9.1 g/100 g) and protein (1.41–4.69 g/100 g) contents of formulated cassava pasta. Yellow cassava-amaranth pasta had higher β-carotene (2.07 μg/g), iron (59 mg/kg), and zinc (9 mg/kg) contents than the white cassava-amaranth pasta. The addition of amaranth leaf powder also enhanced the antioxidant capacities of pasta products. Cooking time and gruel solid loss were reduced upon the addition of amaranth leaf powder, which is beneficial to the consumers. Data showed the potential of amaranth-fortified yellow cassava pasta in contributing to a healthy diet in low- and middle-income countries by combining a biofortified crop with leafy vegetables via food-to-food fortification.

Keywords

Yellow cassava, amaranth, pasta, micronutrient deficiencies, fortification

Practical Application

This work demonstrates the feasibility of a cassava-based pasta fortified with amaranth vegetables as an affordable and nutritious food to benefit micronutrient deficient consumers in countries with high cassava consumption but low vegetable intake. The inclusion of amaranth leaf powder enhanced the developed pasta's nutritional and technological properties, thus presenting a healthy food choice with the potential for scaling up commercially.

3.1 Introduction

Consumption of a nutritious diet contributes to good health and the prevention of diseases (WHO, 2013). Accordingly, micronutrient-rich diets can prevent vitamin A, iron, and zinc deficiencies, which are public health challenges, especially in low-and middle-income countries. The nutritional potential of biofortified cassava with provitamin A carotenoids (also known as yellow cassava) as a sustainable food strategy to ease micronutrient deficiency is therefore of interest. Yellow cassava contains up to 15 µg/g carotenoids on a fresh weight basis and is low in cyanogenic glucosides (HCN
Consumption of a nutritious diet contributes to good health and the prevention of diseases (WHO, 2013). Accordingly, micronutrient-rich diets can prevent vitamin A, iron, and zinc deficiencies, which are public health challenges, especially in low-and middle-income countries. The nutritional potential of biofortified cassava with provitamin A carotenoids (also known as yellow cassava) as a sustainable food strategy to ease micronutrient deficiency is therefore of interest. Yellow cassava contains up to 15 µg/g carotenoids on a fresh weight basis and is low in cyanogenic glucosides (HCN
<10 mg/kg WHO recommended level). Yellow cassava potentially meets about 25 % of the daily recommended intake of vitamin A. However, carotenoid degradation during various processing (Ilona *et al*., 2017), and the full impact of food processing and storage are still largely unknown (Alamu *et al*., 2017).

In sub-Saharan Africa, cassava is popular for its versatility and affordability. It is the principal staple food of over 500 million people with an average cassava consumption of up to 940 g per adult per day on a fresh weight basis (De-Moura *et al*., 2015; FAO, 2019). Cassava can be processed into many commonly consumed calorie-rich food products, as both flour and starch have wide food applications. Previous studies examined the production of traditional foods such as gari, fufu, and chikwangue with provitamin A enriched yellow cassava (Bechoff *et al*., 2018; Taleon *et al*., 2019), as vehicles for increased intake of vitamin A, as well as snacks such as cookies and cakes (Maziya-Dixon *et al*., 2015). These studies revealed that the micronutrient content of these traditional food products is usually inadequate to meet recommended levels of nutrient intake needed to maintain a healthy diet, as processing degraded the micronutrients in yellow cassava (Eyinla *et al*., 2019; Maziya-Dixon *et al*., 2016). This is not surprising as β-carotene, the most abundant provitamin A in yellow cassava is susceptible to degradation due to heat, light, and oxygen (Lawal *et al*., 2020; Taleon *et al*., 2019). These researchers reported provitamin A carotenoid losses of up to 75% in food products such as fufu, chikwangue, and lafun. The direct consequence of this loss during food processing is that the vitamin A content of yellow cassava is insufficient to meet the daily recommended daily intake allowance (RDI) for vitamin A, which has been estimated at 600 μg for adults per day (Wolfe, 2001). Therefore, vitamin A contents of yellow cassava food products need to be enhanced with other food sources. This can be achieved by food-to-food fortification using readily available materials such as leafy vegetables to enhance the profile of proteins, vitamins, and minerals.

Leafy vegetables contain substantial amounts of carotenoids, minerals, and vitamins, and are abundant, affordable, and accessible to the populace (Lawal *et al*., 2018; Moyo *et al*., 2020). A popular leafy vegetable in Nigeria is amaranth, which is liked for its taste and ease of cultivation (Achigan-Dako *et al*., 2014). Amaranth leaves are rich in protein (17.9 g/kg DW), calcium (44.2 mg/100 g DW), iron (13.6 mg/100 g DW), zinc (3.8 mg/100 g DW), vitamin A (3.3 mg/100 g DW), and vitamin C (25.4 mg/100 g DW; Lawal *et al*., 2018). They are also high in antioxidant activity because of the presence of carotenoids, flavonoids, and polyphenols (Achigan-Dako *et al*., 2014).

According to Staatz and Hollinger (2016), changes in consumer preference and taste witnessed in Africa have led to increasing consumption of processed cassava foods and a higher demand for healthy convenience (easy-to-prepare) food products. A typical example of such an easy-to-prepare food is pasta, which is the basis of a large number of diverse, affordable, convenient meals and it has a long shelf life (Nilusha *et al*., 2019). In Nigeria, about 2 billion servings of noodles were recorded in 2019, according to the World Instant Noodles Association (WINA, 2019). Bustos *et al*. (2013), reported that among the convenient foods, pasta is an ideal vehicle for the intake of nutrients for its low cost and high worldwide consumption. The development of wheat pasta enriched with vegetables is a well-studied strategy for boosting the intake of vegetables (Nilusha *et al*., 2019; Oliviero & Fogliano, 2016). The World Health Organization (WHO) and the Food and Drug Administration (FDA) consider pasta as appropriate food for incorporating nutrition supplements. To make the convenient choice also the healthy choice, pasta products are now increasingly fortified with ingredients such as vegetable powders as the large volume of pasta consumption enhances its suitability as a carrier of bioactive substances (Michalak-Majewska *et al*., 2020). In an earlier study, the authors, Lawal *et al*. (2021), evaluated the sensory properties and consumer acceptability of pasta made from yellow cassava and leafy vegetables and reported an appreciable acceptance for yellow cassava pasta among the consumers. Thus, as a follow-up, this study evaluated the nutritional and technological properties of the newly developed cassava-amaranth pasta to provide a better understanding of the nutritional value and functional properties.

3.2 Materials and Methods

3.2.1. Materials

3.2.1.1. Chemicals

Butylated hydroxytoluene, 2,6-di-tert-butyl-4-methyl phenol, 2-dichloroethane, hexane, ethanol, methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were bought from Sigma Co. (St Louis, MO, USA). Folin–Ciocalteu's reagent was purchased from Merck Kenilworth, NJ, U.S.A. All solvents and chemicals were of analytical grade.

3.2.1.2. Sample collection

The International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria, provided yellow cassava roots and flour (variety TMS07/0593). Conventional white cassava root tubers were bought from Zamzam store, Wageningen, and processed into flour. The white cassava roots were peeled with a knife, washed and grated with the excess water removed by using a muslin cloth for easy handling and to enhance the drying of the wet mash. The wet cake was air-dried for 4 h to a moisture content below 12% and dry milled with the cryo-miller. The flour was stored at −20◦C until needed for analysis. Amaranth was grown in the Unifarm greenhouse of Wageningen University and Research, The Netherlands, at 25◦C day and 16◦C night under 12−18 h of natural daylight for 1 month. After harvest, the vegetable was de-stalked, washed, freeze-dried, and milled under liquid nitrogen to get leaf powder, which was stored at −20◦C until further use.

3.2.2 Experimental design

The influence of cassava variety and the addition of amaranth leaf powder (both independent variables) on the quality of cassava pasta (dependent variable) were assessed through a full-factorial central composite design (CCD). Table 3.1 presents the samples analysed in this study.

3.2.3 Preparation of cassava pasta samples

Boiling water (100 ml) was gradually added to the yellow/white cassava flour (95 g) and amaranth leaf powder (5 g) to make a dough. Initially, water was added under constant stirring, after which the dough was shaped. A stainless-steel manual pasta machine (Gusta RVS, Italy) was used to make the pasta strands, followed by drying in an incubator at 65◦C for 12 h. Pictures of developed pasta samples are presented in Figure 3.1.

Table 3.1 Overview of samples used in the study

Ingredient-based samples	**1. WHITE CASSAVA FLOUR (WF)**
	2. YELLOW CASSAVA FLOUR (YF)
	3. WHITE CASSAVA FLOUR + 5% AMARANTH (WFA)
	4. YELLOW CASSAVA FLOUR + 5% AMARANTH (YFA)
	5. AMARANTH (A)
Pasta-based samples	6. WHITE CASSAVA PASTA (FLOUR-BASED) (WFP)
	7. YELLOW CASSAVA PASTA (FLOUR-BASED) (YFP)
	8. WHITE CASSAVA PASTA WITH 5% AMARANTH (WFA P)
	9. YELLOW CASSAVA PASTA WITH 5% AMARANTH (YFAP)

3.2.4 Chemical analysis

3.2.4.1 Chemical properties

The moisture, ash, protein, and fat of flour and pasta were determined using standard methods of the Association of Official Analytical Chemists (AOAC, 2019). Specifically, the protein content was measured according to the Dumas method, whereas fat content was determined by the Soxhlet extraction method using petroleum ether. The total dietary fibre content was analysed using the Official Method 991.43 (AOAC 2005). The amount of total carbohydrate was determined by difference. The sugar content was determined using AOAC (2019) method by weighing about 0.2 g of samples into a centrifuge tube with 1 ml of ethanol (0.789 g/ml), 2 ml of distilled water and 10 ml of hot ethanol. The mixture was vortexed and centrifuged at 2,500 rpm for 10 min after which the supernatant was decanted into another centrifuge tube and used for sugar determination. Atwater factors were used to calculate the total energy content per 100 g of sample (FAO, 2019). All analyses were done in triplicates.

3.2.4.2 Mineral analysis

Iron and zinc contents were determined by AOAC (2019) method 999.10 and measured by Inductively Coupled Plasma Atomic Emission Spectrometry (Thermo iCAP6500 DV; Thermo Fisher Scientific, Waltham, MA, USA) following the validated protocol of the Chemisch Biologisch Laboratorium Bodem (CBLB), Wageningen, the Netherland.

Figure 3.1 *White cassava pasta (upper left), yellow cassava pasta (upper right), white cassava pasta with 5% amaranth leaf powder (lower left), and yellow cassava pasta with 5% amaranth leaf powder*

3.2.5 Functional properties

Water solubility and swelling power were determined at 60 and 90 °C using the method by Chinma (2013), with slight modifications. The method of Abbey and Ibeh (1988), was used to determine the water-holding (WHC) and oil absorption capacity (OAC). Gelation capacity was determined according to the method of Coffman and Garcia (1977). Suspensions of 2–18 g sample per 100 ml in distilled water were prepared. Ten millilitres each of dispersion was transferred into a test tube and heated in a boiling water bath for 1 h, followed by rapid cooling in a cold-water bath. The tubes were further cooled at 4 °C for 2 h. The least gelation concentration (LGC) was determined as the concentration at which the sample from the inverted test tube did not slip or fall. The gravimetric method, as described by Amandikwa *et al*. (2015), was used to determine bulk densities (loose and packed) of samples. The particle size distribution of cassava starch and flour samples was determined in a particle size analyser (Mastersizer 3000; Malvern Panalytics, Malvern, UK) by laser scattering following the description of Han *et al*. (2019).

3.2.6 Pasting and thermal properties

The pasting properties were determined according to Chinma *et al*. (2013), using a Rapid-Visco Analyzer (RVA: Perten Co., Glen Waverley, Melbourne, Australia). The viscosity curve and corresponding parameters, namely pasting temperature, peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown (BD), and setback (SB), were plotted. The unit of viscosity was expressed as mPas. Powder samples were tested for thermal properties following the method by Huerta Abrego *et al*. (2010), using differential scanning calorimetry (DSC; Perkin Elmer DSC 8000, USA) to determine the onset temperature (To), peak temperature (TP), and the cease temperature (TC) using Pyrus Software.

3.2.7 Cooking properties of pasta

The method described by Rathod and Annapure (2017), was used at intervals of 30 s to determine the optimum cooking time by compressing the cooked product between two glass slides until the central white axis disappeared. Five grams of pasta strands were cooked following the ideal cooking time (20 min) and the cooked strands were weighed to determine the cooking gain. The pasta was put in a pre-weighed dish and placed in the incubator for 12 h at 110 °C. The residual pasta was then weighed to determine the gruel solid loss.

3.2.8 Extraction and determination of total phenolics, flavonoids, and antioxidant activity

Extraction of phenolics and flavonoids was performed using the method of Li *et al*. (2009), with slight modifications. Two grams of finely ground pasta samples were extracted with 80 % acidified (0.1 %) methanol by refluxing for 2 h twice in a shaking Water Bath SW 23 JULABO: Seelbach, Germany at 40 °C and centrifuged at 2,000 × g for 10 min in a centrifuge (Thermo Scientific Multifuge X3R Refrigerated Centrifuge; Marshall Scientific, Hampton, NH, USA). The methanolic extract was stored at 4°C until needed for further analysis. DPPH radical scavenging activity was determined using the method by Brand-Williams *et al*. (1995). Reaction mixtures containing 0.1 ml of sample and 3.9 ml of 50 µM DPPH (prepared in methanol) were incubated in a water bath at 37°C for 30 min. After incubation, an aliquot of the sample was placed into a cuvette, and absorbance was measured at 515 nm using the Varian Cary 50 UV-Vis Spectrophotometer. The percentage inhibition was calculated against the control, compared to an ascorbic acid standard curve (0–1,000 µm) and expressed as Trolox equivalent (mmol/kg). The total phenolic content (TPC) was determined by the Folin–Ciocalteu spectrophotometric method (Sharma & Gujral, 2010). Absorbance at 525 nm was read and TPC was expressed as milligrams of gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

3.2.9 Determination of β-carotene

β-Carotene extraction and analysis were performed by HPLC according to Bechoff *et al.* (2015), with some modifications. About 2 g of sample was added to a 15 ml centrifuge tube with 10 ml of hexane, vortexed for 10 min, and centrifuged 5 min at 3,000 revolutions per min (rpm). Next, the supernatant was discarded in a 50-ml centrifuge tube and the procedure was repeated twice with tetrahydrofuran (THF) until the sample became colourless. Water confined within the collected supernatant was eliminated by freezing at −20 °C. The upper coloured part (hexane, THF, and extracted carotenes) was collected in a new tube and the extract was evaporated using a Büchi vacuum evaporator at 40 °C and 270 mbar vacuum. Dry extracts were reconstituted in sample buffer (1:1 methanol and THF + 0.01 % BHT), after which 1.5 ml was filtered into amber vials for analysis. β-Carotene was analysed using a YMC C30 column, 3 μm, 150 mm × 4.6 mm column (YMC Europe GMBH, Dinslaken, Germany) in an Alliance 2695 HPLC system (Waters, Milford, MA, USA).

3.2.10 Statistical analysis

The study was conducted using a completely randomised design with three replications of the experiments and the analysis done in triplicate. Data were analysed using an SPSS statistical package (SPSS, version 12.0; Chicago, IL, USA). To determine the difference in significance in means, analysis of variance and Duncan's multiple range tests were performed at a 95 % significance level.

3.3 RESULTS AND DISCUSSION

3.3.1 Physicochemical properties of cassava-amaranth flour and pasta

The chemical composition of yellow and white cassava flour and pasta, with and without amaranth leaf powder is presented in Table 3.2. Low moisture content (< 12 %) was observed in all the samples, indicating a proper shelf life when stored in dry conditions and in line with Codex Alimentarius standards. Energy contents of all samples ranged from 303 - 484 kcal kg^{-1}. Protein, fat and ash contents were significantly higher in samples with amaranth leaf powder while the carbohydrate content remained above 80 g 100 g^{-1} DW in all pasta samples. The characteristic low protein profile of cassava (1- 3 g 100 g^{-1} DW) was improved, as higher protein contents were recorded with the addition of amaranth leaf powder in both the white and yellow cassava-based samples. The total dietary fibre content of the white and yellow cassava flour products was also improved up to 19 % higher levels with amaranth leaf powder addition. This shows the beneficial effect of amaranth as a plant protein source with the leaf powder having protein and fibre contents of 33.5 g 100 g^{-1} and 17.20 g 100 g^{-1} (dry weight basis) respectively. Previous studies reported similar appreciable protein and fibre contents of amaranth (Ngugi *et al.*, 2017) while the fibre content of the cassava-amaranth flour and pasta was comparable to wheat flour (Nirmala & Joye, 2020). Iron and zinc contents of yellow cassava were higher than for the white cassava samples (9.6 mg kg^{-1} and 6.4 mg kg^{-1} respectively), similar to Maziya-Dixon *et al*. (2015). Iron is essential for humans, due to its involvement in multiple processes of cell energy metabolism, in which its presence is vital while zinc plays a key role in human metabolic, immunological and many other biological processes (Allen *et al*., 2006). Our results confirmed that amaranth leaf powder is a valuable source of mineral and contain a high amount of iron and zinc to meet at least 50 % of the daily recommended intake, its addition thus enhanced the nutritional quality of the cassava-amaranth pasta.

Sucrose was found in the white cassava products but none was detected in the yellow cassava flour, which agrees with Ayetigbo *et al*. (2018). Maltose at a range of 1.21- 2.13 mg g^{-1} was also present in the white cassava flour while fructose and glucose were present in similar quantities, with slightly higher values for glucose. The yellow cassava samples had appreciably lower sugar contents than the white cassava samples.

3.3.2 TPC and TFC of the pasta

TPC and TFC of cassava samples were significantly enhanced with the addition of amaranth (4-fold increase in TPC and 5-fold increase in TFC). As shown in Table 3.3, amaranth dry leaf powder samples had the highest phenolic and flavonoid contents among the samples with a TPC of 8,620 µg GAE/g and a TFC of 111.5 mg RE/g, respectively. This result agrees with Obeng *et al*. (2020), who reported high levels of flavonoids and phenolic acids in amaranth leaves. Cultivar differences influenced the antioxidant activity of the cassava flour as the yellow flour had higher levels than the white. Processing also affected the TPC and TFC as marginal loss in antioxidant activity occurred when the flour was made into pasta (6– 50 %). Jiménez-Monreal *et al*. (2009), similarly reported losses in TPC and TFC ranging from 5 % to 50 % when vegetables were processed using different cooking techniques. Fortification of pasta with vegetables is increasingly being used as a food strategy to enhance the antioxidant activity of pasta products (Oliveiro & Fogliano, 2016), thus the modest loss in the antioxidant activity must be put into consideration when designing vegetable-fortified pasta products.

Table 3.2 Chemical and mineral composition of amaranth, cassava flour and pasta

Parameter	White Flour	Yellow Flour	White Flour + Amaranth	Yellow Flour + Amaranth	White Flour Pasta	Yellow Flour Pasta	White Flour + Amaranth Pasta	Yellow Flour + Amaranth Pasta	Amaranth dry powder
Moisture Content ($g100g^{-1}$)	4.18 ± 0.66^{d}	3.42 ± 0.17^{d}	4.24 ± 0.09^{c}	4.20 ± 0.44^{cd}	2.15 ± 0.21^{e}	2.71 ± 0.70^{e}	2.85 ± 0.13^{e}	2.57 ± 0.54^{e}	11.97 ± 0.09^{ab}
Protein ($g100g^{-1}$)	4.23 ± 0.57^{b}	1.41 ± 1.11^{c}	4.69 ± 0.47^{b}	3.30 ± 0.61^{b}	3.48 ± 0.35^{ab}	0.99 ± 0.02^{c}	4.50 ± 0.04^{b}	2.48 ± 0.21^{b}	33.49 ± 1.44^{a}
Fat ($g100g^{-1}$)	0.24 ± 0.02^{b}	0.20 ± 0.02^{b}	0.34 ± 0.02^{b}	0.32 ± 0.06^{b}	0.24 ± 0.02^{b}	0.20 ± 0.02^{b}	0.34 ± 0.02^{b}	0.32 ± 0.06^{b}	2.53 ± 0.18^{a}
Ash ($g100g^{-1}$)	2.01 ± 0.00^{b}	1.55 ± 0.00^{b}	2.07 ± 0.09^{b}	2.23 ± 0.07^{b}	2.16 ± 0.03^{b}	1.55 ± 0.04^{b}	2.75 ± 0.06^{b}	2.26 ± 0.00^{b}	15.41 ± 0.03^{a}
CHO* ($g100g^{-1}$)	89.3	93.4	78.7	90	92	94.6	89.6	92.4	36.6
Energy content ($kcal100g^{-1}$)	376.5	381.1	336.5	375.8	384	384	379.3	382.3	303.1
Dietary fibre	7.6±0.01^{d}	9.0±0.03^{bc}	7.8±0.09^{c}	9.1±0.02^{b}	ND	ND	ND	ND	17.20±0.15^{a}
Fe ($mgkg^{-1}$) DW	2.9	7.6	16	69	1	16	6	59	97
Zn ($mgkg^{-1}$) DW	5.6	8.1	3	8	6	4	8	9	49
Fructose (mgg^{-1})	8.15 ± 0.33	n.d	7.41 ± 0.04	n.d	8.38 ± 0.12	0.49 ± 0.01	17.54 ± 1.12	0.90 ± 0.12	n.d
Glucose (mgg^{-1})	11.13 ± 0.27	n.d	10.67 ± 0.16	n.d	11.81 ± 0.06	0.54 ± 0.09	22.20 ± 1.45	n.d.	n.d
Sucrose (mgg^{-1})	69.07 ± 0.09	n.d	62.75 ± 1.14	n.d	69.21 ± 0.68	n.d.	47.12 ± 2.59	n.d.	n.d
Maltose (mgg^{-1})	1.59 ± 0.08	n.d	2.13 ± 0.04	n.d	1.21 ± 0.16	n.d.	1.29 ± 0.12	n.d.	n.d

Values are means ± standard deviation. means having different superscripts within the same row differ significantly ($p < 0.05$). n.d. means not detected or quantifiable MC(Moisture content)
**CHO (Carbohydrate) calculated by difference n.d. means not detected or quantifiable ND: Not determined*

Chapter 3

3.3.3 DPPH radical scavenging

The addition of amaranth leaf powder significantly impacted the DPPH radical scavenging activity of cassava-amaranth vegetable pasta samples (Table 3.3). The DPPH free-radical scavenging activity of white and yellow cassava pasta samples was doubled from less than 1.0 Trolox Equivalents (mmol/kg) due to the addition of amaranth vegetable powder. Obeng *et al*. (2020), reported appreciably high scavenging activities in amaranth vegetables but was lower than other African leafy vegetables in their study. This indicates that leafy vegetables with higher antioxidant capacities could be explored to determine how much they can contribute to the free radical scavenging activity of vegetable-fortified cassava pasta. Retention of phenolics and flavonoids after cooking was however higher in the white (93 %) than the yellow cassava pasta (56 %) while DPPH retention after cooking was also higher for the white cassava pasta products (96 %).

3.3.4 β-Carotene concentration

The addition of amaranth significantly increased the β-carotene content of the cassava-amaranth products (Table 3.2). β-Carotene was not detected in white cassava but was expectedly present in the white cassava pasta samples with amaranth (1.2–2.4 µg/g DW). La Frano *et al*. (2014), reported between 3 and 4 µg/g DW trans-β-carotene in gari, a traditional cassava food product, which was higher than in the yellow cassava-amaranth pasta in this study. Taleon *et al*. (2019), found provitamin A carotenoids in fresh yellow cassava samples between 4.0 and 25.0 µg/g DW. Negi and Roy (2000), also reported β-carotene contents of 9.8 ± 0.5 µg/g DW in fresh amaranth leaf samples. Variations in β-carotene content are also due to varietal differences. However, processing resulted in about 11 % loss of the β-carotene contents of the amaranth-cassava pasta (Table 3.2).

3.3.5 Techno-functional properties of cassava flour and pasta

The water holding capacity (WHC) of flour is dependent on its starch fraction having hydrophilic groups and indicates the ability of the flour to bind and hold water thus forming a gel at elevated temperatures. WHC is important in pasta production as it provides volume, bulk, and good texture to the final product (Sharma *et al*., 2021). The WHC of the yellow and white cassava samples differed significantly ($P \leq 0.05$) at two temperatures, 60°C and 90°C, ranging from 3.0 to 5.7 g/g at 60°C and increased from 7.0 to 17.9 g/g at 90°C. The addition of amaranth resulted in a lower WHC for the yellow variety but an increase in the white due mainly to the varietal difference (Table 3.4).

Table 3.3 ***TPC,TFC, DPPH free radical scavenging activity and beta-carotene contents of cassava products.***

Sample	DPPH (Trolox Equivalents (mmolkg^{-1})	TPC {GAE (µgg^{-1})}	TFC {RE (mgg^{-1})}	Beta-carotene (µgg^{-1})
White Flour	0.17 ± 0.09^{e}	277.9 ± 31.1^{c}	5.56 ± 0.48^{d}	Not detected
White Flour + Amaranth	2.50 ± 0.38^{b}	813.6 ± 44.2^{a}	10.95 ± 0.81^{ab}	1.16 ± 0.38^{c}
White Flour + Amaranth Pasta	2.41 ± 0.42^{ab}	765.6 ± 51.8^{a}	9.21 ± 0.51^{b}	1.03 ± 0.16^{c}
Yellow Flour	0.44 ± 0.04^{d}	220.0 ± 31.7^{b}	2.29 ± 0.50^{e}	0.72 ± 0.02^{d}
Yellow Flour + Amaranth	2.77 ± 0.29^{a}	674.9 ± 57.4^{ab}	11.76 ± 2.23^{a}	2.34 ± 0.17^{ab}
Yellow Flour + Amaranth Pasta	0.98 ± 0.12^{c}	450.0 ± 21.2^{b}	5.80 ± 0.07^{c}	2.07± 0.13^{b}
Amaranth	16.42 ± 0.37*	8620.2 ± 45.2*	111.46 ± 1.09*	5.48 ± 0.08^{a}

*Values are means ± standard deviation. means having different superscripts within the same column differ significantly ($p < 0.05$).*separately analysed*
TPC: Total phenolic content, TFC: Total flavonoid content and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

Chapter 3

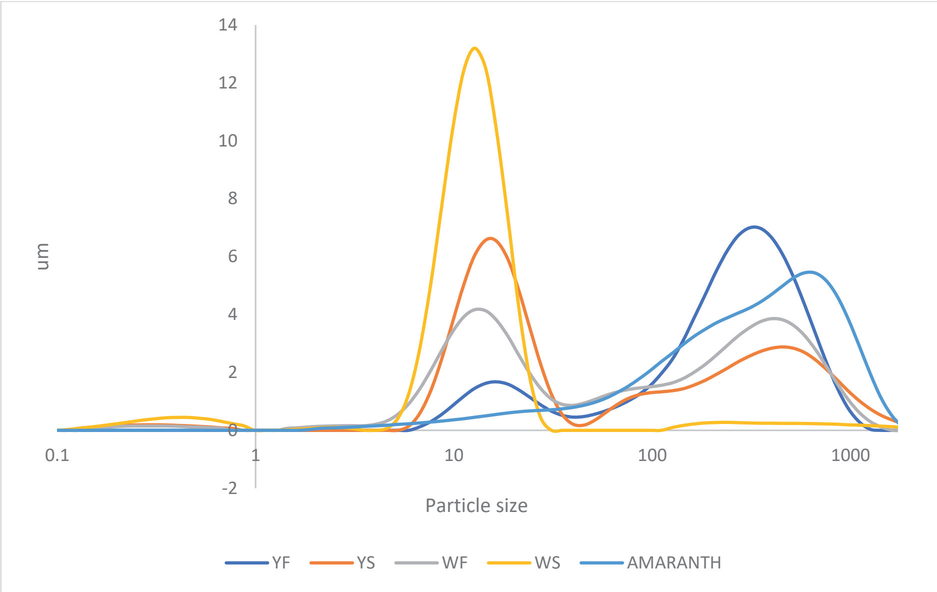


Fig. 3.2. Particle size distribution of all ingredient samples

YF: Yellow flour, YS: Yellow starch, WF: White flour, WS: White starch

Table 3.4 Functional properties of amaranth and cassava flour

Functional property	**WF**	**WFA**	**YF**	**YFA**	***Amaranth***
Solubility @ 60 °C	10.00 ±0.00 ^{c}	15.00 ±7.07^{b}	30.00 ±0.00^{a}	10.00 ±14.14^{c}	n.a
Solubility @ 90 °C	60.00 ±14.41^{a}	29.51±14.41^{d}	39.36 ±0.07^{bc}	34.25 ±7.15^{c}	n.a
Swelling Power @ 60 °C	4.50 ±1.68^{d}	6.93 ±0.45^{ab}	6.77 ±0.09^{b}	5.57 ±1.36^{c}	n.a
Swelling Power @ 90 °C	19.18 ±2.85^{d}	20.92 ±4.40^{c}	29.45 ±0.08^{a}	25.43 ±3.49^{b}	n.a
Water Absorption Capacity @ 60 °C (g water/g sample)	4.05 ±1.52^{bc}	4.85 ±0.08^{ab}	5.74 ±0.07^{a}	4.91 ±0.45^{ab}	n.a
Water Absorption Capacity @ 90 °C (g water/g sample)	7.88 ±3.85^{d}	14.43 ±0.08^{c}	17.86 ±0.07^{a}	16.59 ±0.48^{b}	n.a
Oil Absorption Capacity (g/g oil)	1.91 ±0.00^{b}	1.96 ±0.12^{b}	2.42 ±0.0^{a}	1.79 ±0.01^{c}	n.a
Least Gelation Capacity (%)	0.70	1.20	0.80	0.80	n.a
Loose Bulk Density (g/mL)	0.53 ±0.00 ^{a}	0.53 ±0.00 ^{a}	0.52 ±0.01^{a}	0.51 ±0.01^{a}	0.21
Packed Bulk Density (g/mL)	0.80 ±0.00 ^{a}	0.76 ±0.01^{a}	0.59 ±0.01^{bc}	0.58 ±0.01^{c}	0.34
Total surface area	11.15 ±0.0	-	82.35 ±0.64	-	88.75
Volume distribution	228.50 ±0.00	-	313.50 ±2.12	-	451
Result range (0.1, 0.2) um	0.24 ±0.00	-	0.00 ±0.00	-	0
Result above (0.2) um	99.76 ±.0.00	-	100.00 ±0.00	-	100

Values are means ± standard deviation. means having different superscripts within the same row differ significantly ($p < 0.05$).
WF (White cassava flour), WFA (White cassava flour plus amaranth), YF (Yellow cassava flour), YFA(Yellow cassava flour plus amaranth)

Table 3.5 Pasting properties of cassava pasta products

	Peak viscosity (RVU)	Holding viscosity (RVU)	Final viscosity (RVU)	Set back (RVU)	Gel forming	Hardness (N)
WF	2119.5 ±37.48^{b}	1089.5 ±13.44^{e}	1671.0 ±25.46^{c}	581.5 ±12.02^{d}	Good	0.20 ±0.03^{d}
WFA	1956.0 ±80.61^{c}	926.0 ±16.97^{e}	1328.5 ±30.41^{e}	402.5 ±13.44^{e}	No gel	0.56 ±0.69^{cd}
WFP	2265.0 ±130.11^{b}	1052.0 ±35.36^{c}	1617.5 ±57.28^{cd}	565.5 ±21.92^{d}	-	12.70 ±3.54^{b}
WFPA	2123.5 ±198.70^{b}	1159.5 ±77.08^{d}	1726.0 ±114.55^{b}	566.5 ±37.48^{d}	-	14.74 ±0.00^{a}
YF	2913.0 ±86.27^{b}	1707.5 ±30.41^{a}	2539.5 ±27.58^{a}	832.0 ±2.83^{a}	Good	0.14 ±0.05^{d}
YFA	2789.5 ±207.18^{b}	1571.0 ±65.05^{ab}	2340.0 ±84.85^{a}	769.0 ±19.80^{b}	Partial gel	0.17 ±0.09^{d}
YFP	2208.5 ±152.03^{b}	1550.0 ±80.61^{ab}	2209.0 ±103.24^{a}	659.0 ±22.63^{cd}	-	13.34 ±2.42^{a}
YFPA	2335.0 ±189.51^{ab}	1672.5 ±91.22^{a}	2233.5 ±105.36^{a}	561.0 ±14.14^{d}	-	14.74 ±0.00^{a}

Values are means ± standard deviation. means having different superscripts within the same column differ significantly ($p < 0.05$) WF (White cassava flour), WFA (White cassava flour plus Amaranth), YF (Yellow cassava flour), YFA(Yellow cassava flour plus amaranth powder)

The solubility of the cassava samples ranged from 10 % to 45 % at 60 °C and from 10 % to 60 % at 90°C, while the swelling power of the cassava flours and starches increased significantly (3.4–29.5 g/g) with the addition of amaranth leaf powder for both the yellow and white cassava ingredients. The swelling power of cassava products indicates the absorption of water as a result of starch gelatinization and is functionally beneficial in the food industry (Ayetigbo *et al*., 2018). Awoyale *et al*. (2015), reported lower swelling power values for yellow cassava (7.5 g/g at a solubility of 1.7 % at 60 °C). The yellow cassava pasta also had higher swelling power than the white cassava pasta, but the amaranth addition resulted in a slight decrease of swelling power in yellow cassava pasta, whereas white cassava pasta remained unchanged (Table 3.3). The swelling power of the yellow cassava pasta was comparable to commercial wheat pasta (2.4 g/g) (Odey & Lee, 2020).

Least gelation capacity, which is the smallest amount of starch required to form a stable gel, is important for the food industry as a better gelling activity is achieved with a lower least gelation capacity and is also preferred for energy efficiency. The ability of the cassava flour to form a gel as measured by the least gelation capacity was found to be at appreciable levels in this study and may have a favourable economic impact on use since this implies that less material is required to make food gels. The gelation capacity is an important quality factor considered for flours used in pasta production. The least gelation capacity of the cassava samples was similar to those reported by Awoyale *et al*. (2015), for gel formation in three biofortified yellow cassava starches, and an LGC of 4.01–4.06 was recorded. The yellow and white cassava samples were similar in LGC and could be interchangeably utilized for gel formation purposes. The yellow cassava samples also had lower bulk density than white cassava because of the difference in particle size. A low bulk density in food is beneficial for packaging purposes, as fewer materials are used in low bulk density foods. The bulk density of the cassava flours was also lower than those of wheat (0.76) and rice flours (0.91). The packed bulk density (PBD) has also been shown to be positively related to peak time for pasting of the starches and this report is similar to the findings of Agunbiade and Ighodaro (2010).

3.3.6 Pasting properties of cassava flour and pasta

Pasting properties are critical to the activities of cassava starch or flour suspension during regulated heating, holding, and cooling temperature regimes. Some pasting properties of cassava can differ significantly depending on the variety, location, and cultivation conditions. The peak viscosity shows the ability of starch to freely expand before their physical breakdown, thus a high peak viscosity is desirable as it enhances the texture of the paste (Alamu *et al*., 2017). The white and yellow cassava flours in this study had significantly high peak viscosities and as depicted in Table 3.5, peak viscosities reduced slightly with the addition of amaranth. The holding viscosity also reduced with the addition of amaranth but increased with processing into pasta. Yellow cassava also had a significantly higher holding viscosity than white. The final viscosity varied from 1,329 to 2,010 RVU and 1,409 to 2,540 RVU for the white and yellow cassava samples, respectively. Ayetigbo *et al*. (2018), reported a higher final viscosity in yellow compared to white cassava starch, which contrasts with the result in this study which may be due to varietal differences. According to Mandge *et al*. (2014), final viscosity is the thickness after cooling of cooked starch to at least 50°C and reveals the ability of starch to form a viscous gel or paste following cooking and cooling. The setback results ranged from 402 to 832 RVU with the addition of amaranth, resulting in a decrease in the setback viscosity of the samples. Varietal differences had an impact on the cassava pasting properties, as the yellow varieties had higher peaks than the white. This could be a result of the different alignment of amylose molecules. This is corroborated by the findings of Ayetigbo *et al*. (2018). Longer peak times may cause extra production costs for the food industry, but are functionally beneficial, as starches resistant to rapid peak time can be valuable in maintaining the structural integrity of foods (Cheng *et al*., 2020).

Table 3.6 Thermal properties of cassava flour and pasta

Cassava products	***Onset temp (°C)***	***Peak temp (°C)***	***Cease temp (°C)***	***Enthalpy (jg^{-1})***
White cassava flour	68.70	78.40	91.34	12.66
White flour pasta	61.66	77.89	91.34	12.66
White flour amaranth pasta	62.43	78.49	93.06	13.88
Yellow cassava flour	63.76	75.44	90.96	13.86
Yellow flour pasta	62.56	75.85	91.26	10.52
Yellow flour amaranth pasta	62.41	77.04	92.60	12.14

3.3.7 Particle size distribution (PSD) of the ingredients

Particle size categorization of powder materials is a requirement for food product design and specification purposes. PSD may have a significant effect on the final product performance in terms of content uniformity, dissolution, and stability. The yellow cassava flour had a significantly higher surface area and volume distribution than the white, but no significant difference was observed with the addition of amaranth (Figure 3.2). Volume distribution was 225.5 for white flour and 313.5 for yellow flour. Overall, almost all results were in a range above 0.2 µm with yellow cassava flour having the highest percentage in the range above 0.2 µm. The average sizes of the flours used in this study are similar to those mentioned in the report of Ayetigbo *et al*. (2018).

3.3.8 Thermal properties of cassava-amaranth pasta

The application of heat to starch in the presence of water results in an irreversible endothermic reaction known as gelatinisation. Thermal properties are essential factors in food design as lower thermal properties and gelatinisation temperatures of flours, as observed among the samples in this study, may be advantageous, requiring less energy for gelatinisation, thus reducing energy costs (Ayetigbo *et al*., 2018). The effect of processing on thermal properties of cassava flour and pasta samples are shown in Table 3.6 with yellow cassava having a lower onset temperature (To), peak temperature (Tp), and cease temperature (Tc) than the white probably as a result of the larger particle size distribution. Enthalpy ranged from 10.5 to 16.4 J/kg. The pasting temperatures were lower than wheat's in agreement with similar studies of Ubwa *et al*. (2012).

3.3.9 Cooking properties

Cassava cannot be eaten raw but must undergo some form of processing, usually cooking, to enhance palatability and reduce cyanogenic glucosides. In this study, the cassava pasta samples became firmer and stronger internally when cooked. The cooking properties are reported in Table 3.7. Cooking time ranged from 14 to 22 min, which was longer than for commercial wheat pasta (8–10 min; Reddy *et al*., 2019). The longer cooking time is probably due to the differences in starch structure. The addition of amaranth leaf powder however significantly decreased the cooking time. The cooking weight gain ranged from 6.27 % to 9.11 %, whereas the gruel solid loss ranged between 1.38 % and 2.5 %, which was lower than the values reported by Brennan *et al*. (2004), who found a cooking loss of 7.93 % for commercial wheat pasta. Pasta firmness relates to the hydration of the starch granules during the cooking process and the subsequent embedding of gelatinizing starch granules in a matrix of partially denatured protein (Pellegrini, Vittadini, & Fogliano, 2020). The observed decrease in firmness and swelling index may be associated with a reduction in starch gelatinization in the pasta as cooking time increases with a decrease in the starch content.

Table 3.7 Functional and cooking properties of yellow and white cassava pasta products

	Solubility (%)	*Swelling capacity (%)*	*Water absorption (g^{-1} sample)*	*Oil absorption (mLg^{-1})*	*Cooking time (min)*	*Weight gain(%)*	*Gruel solid loss (%)*
WFP	20.87 ± 2.00^{a}	3.10 ± 0.06^{c}	2.45 ± 0.00^{b}	1.96 ± 0.04^{a}	22.00 ± 0.00^{a}	8.37 ± 0.12^{b}	1.74 ± 0.25^{a}
WFPA	19.55 ± 0.16^{a}	3.21 ± 0.06^{c}	2.58 ± 0.04^{b}	2.19 ± 0.04^{a}	14.00 ± 0.00^{c}	6.27 ± 1.44^{d}	2.50 ± 0.37^{a}
YFP	3.97 ± 1.31^{c}	4.09 ± 0.07^{a}	3.93 ± 0.03^{a}	1.79 ± 0.04^{b}	22.00 ± 3.71^{a}	6.79 ± 0.60^{c}	1.58 ± 0.01^{a}
YFAP	6.16 ± 0.08^{b}	3.53 ± 0.04^{b}	3.32 ± 0.04^{a}	1.89 ± 0.04^{b}	15.50 ± 0.00^{b}	9.11 ± 0.17^{a}	1.38 ± 0.35^{b}

Values are means ± standard deviation. means having different superscripts within the same column differ significantly ($p < 0.05$).WFP (White cassava pasta), WFAP (White cassava amaranth pasta),YF (Yellow cassava pasta), YFAP(Yellow cassava flour plus amaranth pasta)

3.4 CONCLUSION

This study provides the first in-depth evaluation of the nutritional and technological profile of vegetable-fortified pasta made from yellow and white cassava and amaranth leaf powder. The observed higher protein, dietary fibre, iron, zinc, total phenolics, flavonoids, and β-carotene contents in pasta formulated with the addition of amaranth leafy vegetables can improve the nutritional profile of the novel cassava pasta. This is important for addressing the global health issue of vitamin A, iron, and zinc deficiencies, especially in low- and middle-income countries of sub-Saharan Africa where cassava is widely consumed. The technological properties evaluated also gave positive indications of the feasibility of vegetable inclusion in a cassava pasta product and its applicability for the food industry and as a more affordable, gluten-free alternative to wheat. Cooking, pasting, and thermal properties were considerably improved with the addition of amaranth into the cassava pasta formulation. The yellow cassava also showed significantly higher nutritional value than the white cassava. However, the DPPH free radical scavenging activity of the developed amaranth-cassava vegetable pasta was lower than reported in previous studies for other vegetables. There is thus a need to explore the use of other vegetables as well as the factors that may hinder the digestibility. Further studies are required to determine the bioaccessibility of the nutrients of the developed pasta.

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CHAPTER FOUR

THE ADDITION OF FLUTED PUMPKIN LEAF POWDER IMPROVES THE TECHNO-FUNCTIONAL PROPERTIES OF CASSAVA PASTA

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Lawal, O. M., Sanni, O., Oluwamukomi, M., Fogliano, V., & Linnemann, A. R. (2021). The addition of fluted pumpkin (Telfairia occidentalis) leaf powder improves the techno-functional properties of cassava pasta. *Food Structure*, *30*, 100241. https://doi.org/10.1016/j.foostr.2021.100241

Abstract

In this study, techno-functional and sensorial properties of cassava pasta, as influenced by incorporating fluted pumpkin leaf ("ugwu") powder and the cultivar variation effect were evaluated. Fortification of cassava pasta with the leaf powder at incorporation levels of 5% and 10% decreased the particle sizes of the products while yellow cassava flour showed larger particle size distribution than the white cultivar. Pasta colour was significantly impacted, as lightness values reduced with an increase in leaf powder. Fluted pumpkin inclusion decreased the gelation capacity of the flour blends but enhanced the water solubility, swelling power and oil absorption capacities of the products. Interestingly, the cooking time and gruel solid loss were reduced while the weight gain increased in the formulated pasta improved with the addition of fluted pumpkin leaf powder. The textural properties of the cassava pasta were enhanced as the hardness and pasting viscosities of the gel and pasta decreased by 12% with leaf powder inclusion. Pasting temperatures were also lower in the fluted pumpkin-fortified pasta than the gluten-laden wheat pasta. Furthermore, the yellow cassava products had significantly higher pasting viscosities than the white cassava products and cultivar variation had significant effects on the thermal properties of the food products. Overall acceptability and the likelihood of purchase of the novel pasta were modest among the consumers. Our results showed that incorporating fluted pumpkin leaf powder in cassava pasta led to some improvements in the techno-functional and altered sensorial attributes.

4.1. Introduction

Micronutrient deficiencies, also known as hidden hunger, are public health issues affecting resource-poor populations in sub-Saharan Africa. The low-income earners are mostly affected because of the high dependence on starchy crops and a poor-quality diet low in sources of other food components such as vitamins and minerals (Bouis & Saltzman, 2017). In Nigeria, for example, average cassava consumption is up to 940 g/adult/day fresh weight (De Moura *et al.*, 2015). The conventional white cassava is low in micronutrients; hence, its frequent consumption contributes to hidden hunger (Odoemelam *et al.*, 2020). Recent plant breeding research aiming at alleviating hidden hunger in developing countries provided the provitamin A carotenoid biofortified cassava varieties, otherwise known as yellow cassava. With vitamin A, iron and zinc contents of up to 15, 4 and 3 μg/g respectively, yellow cassava can contribute about 40 %, 30 % and 40 % of the estimated average requirement (EAR) for vitamin A, iron and zinc (Eyinla *et al.*, 2019; Gegios *et al.*, 2010). It has thus become a veritable game-changer in the quest for nutritious alternatives to the more expensive imported wheat flour to produce staples, snacks and convenient food products such as pasta (Ilona, Bouis, Palenberg, Moursi, & Oparinde, 2017).

An increase in the consumption frequency of pasta products in sub-Saharan countries has been observed within the last decade (International Pasta Organization, 2020). Pasta products, valued for their convenience, affordability, and long shelf life, are progressively being fortified with vegetable powders to improve their nutritional profile (Simonato *et al.*, 2020; Mercier *et al.*, 2016). The high volume of pasta consumption enhances its suitability as a bioactive substance carrier (Michalak Majewska *et al.*, 2020). The food industry recently introduced newer functional pasta products enriched with nutrients and bioactive compounds such as pseudo-cereals, legume flours and vegetable or fruits powders (Gao *et al.*, 2018), but none have been made with yellow cassava. Thus, in the sub-Saharan Africa region, the available, affordable, and nutrient-dense materials can be explored to provide a nutritionally superior pasta product that improves consumers' vitamin A, iron, and zinc status.

Fluted pumpkin (*Telfairia occidentalis*), known as "ugwu" in Nigeria, is the most preferred, widely cultivated leafy vegetable in Nigeria (Lawal *et al.*, 2021). The leaves are good sources of vitamins (Vit. A 6120 IU), minerals (iron 3.6 mg/100 g, iron 4.2

mg/100 g) and proteins (22–39 g/100 g DW), while the seeds are rich in oil and protein (Aworh, 2015; Omimakinde *et al*., 2018). Including a leafy vegetable such as fluted pumpkin in yellow cassava, pasta can improve the pasta's nutritional composition and alter pasta quality attributes such as texture, colour and functional properties (Oliviero & Fogliano, 2016; Michalak-Majewska *et al*., 2020).

Previous studies have focused on the physico-functional properties of white cassava but reports on the new yellow-fleshed cassava cultivars are still few (Chisenga *et al*., 2019; De Moura *et al*., 2015). More precisely, reports on the impact of leaf powder addition on the techno-functional and sensory properties of yellow cassava flour and pasta are not yet available, despite several studies on wheat-based pasta. We hypothesised that leaf addition modifies the structure of the formulated pasta. Thus, this study aimed at evaluating, for the first time, the influence of fluted pumpkin leaf powder addition on the functional, textural, pasting, thermal, cooking and sensory properties of yellow-and white-fleshed cassava flours and pasta. The technological feasibility of leaf fortification on yellow cassava pasta structure was also ascertained. Thus, these outcomes provided knowledge into the possibilities of using yellow cassava flour and its fluted pumpkin-fortified equivalent as a wheat substitute to benefit the health status of its consumers.

4.2. Materials and methods

4.2.1. Materials

Yellow cassava flour was obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, packaged in food-safe polyethylene pouches, shipped on dry ice and stored at − 20 °C until further analysis. Conventional white cassava flour was purchased from Toko Indrani, Wageningen, Netherlands.

4.2.1.1. Preparation of leaf powder

Fluted pumpkin vegetables were cultivated at the Unifarm of Wageningen University and Research, Netherlands. After harvest, they were freeze-dried, milled into powder with a cryogenic grinder (6875D Freezer/Mill®, SPEX SamplePrep, New Jersey, USA) and stored at − 20 °C until further analysis.

4.2.1.2. Preparation of pasta

The sample formulations are shown in Table 4.1. The pasta samples were produced as described by Sakurai *et al*. (2019) with some modifications. The cassava flour samples with 0 g, 5 g into 95 g and 10 g into 90 g of fluted pumpkin leaf powder were weighed and mixed manually to achieve a homogenous mixture. The dry formulation was mixed with boiling water in a ratio of 1:1 to form a dough which was manually kneaded and allowed to rest for 20 min. The dough was fed through a laboratory-scale pasta machine (Pasta machine RVS, Gusta, Zeeland, Netherlands) to produce the desired pasta strands. The pasta strands were dried at 65 °C, packaged with aluminium foil and stored at -18 °C until further analyses (Fig 4.1).

Table 4.1. Blend formulations of cassava samples

Code	Formulation
WFU0	100 % white cassava flour
WFU5	95 % white cassava flour + 5 % fluted pumpkin powder
WFU10	90 % white cassava flour + 10 % fluted pumpkin powder
YFU0	100 % yellow cassava flour + 5 % fluted pumpkin powder
YFU5	95 % yellow cassava flour + 5 % fluted pumpkin powder
YFU10	90 % yellow cassava flour + 5 % fluted pumpkin powder
WPU0	100 % white cassava pasta
WPU5	95 % white cassava flour + 5 % fluted pumpkin pasta
WPU10	90 % white cassava flour + 10 % fluted pumpkin pasta
YPU0	100 % yellow cassava flour + 5 % fluted pumpkin pasta
YPU5	95 % yellow cassava flour + 5 % fluted pumpkin pasta
YPU10	90 % yellow cassava flour + 5 % fluted pumpkin pasta

4.2.2 Physical properties determination

4.2.2.1 Particle size distribution

The particle size distribution of the flour samples was measured by laser scattering technique using a particle size analyser (Mastersizer 3000, Malvern panalytics, UK) following standard operating procedures.

4.2.2.2 Colour measurements

The colour measurements were carried out using a Hunter Lab flex colourimeter (Elscolab, Kruibeke, Belgium). Results were expressed according to the CIE L*a*b* system. The colourimeter was calibrated using standard black and green tiles. Samples were homogenised, placed into the spectrophotometer plastic cuvettes and measured.

4.2.3 Determination of functional properties

4.2.3.1 Bulk density and gelation capacity

Bulk densities of the flour samples were determined according to Ajibola and Olapade (2017), while the least gel concentration (LGC) of the flour samples was determined as described by Chinma *et al*. (2013).

4.2.3.2 Water solubility, swelling power, water holding and oil absorption capacities

Water solubility and swelling power were determined using a modified version by Chinma *et al*. (2013), using a shaking water bath (SW23, JULABO, Seelbach, Germany) at 60 and 90 °C for 30 min. The weight of dry solids was noted to determine water solubility and swelling power. The water113 holding capacities (WHC) and oil absorption capacities (OAC) were also determined at 60 and 90 ºC, as reported by Lu *et al*. (2020), with slight modification.

4.2.3.3 Cooking properties

The cooking properties of the pasta samples were determined as described by Rathod and Annapure (2017). Five grams (5 g) of pasta samples were cooked until the optimal cooking time (OCT), which was noted as when the starchy white core of the pasta disappeared. The cooked pasta samples were rinsed with distilled water (50 ml), drained for 30 secs and weighed to determine the cooking weight gain (WG). The cooking water was collected in an aluminium dish and dried at 110 °C for 12 h. The residue was weighed, and gruel solid loss (GSL) was reported as a percentage of starting material.

4.2.4. Pasting and textural properties

A Rapid Visco Analyzer (RVA-4, Newport Scientific, Inc., Maryland, USA) was used to determine the pasting properties of the flour and pasta samples were measured in terms of peak viscosity, peak time, pasting temperature, breakdown and setback viscosities and final viscosity as described by Alake *et al*. (2016). The hardness of the gel formed from the flour samples was investigated according to the method of Baraheng and Karrila (2019) using a texture analyser (TA.XT.plus, Stable Micro Systems, Surrey, UK), while the hardness of gel for pasta samples was as described by Simonato *et al*. (2019).

4.2.5 Thermal properties

Thermal properties of the flours and ground pasta samples were investigated with a differential scanning calorimeter (DSC-Q200, TA Instruments, Delaware, USA) as described by Duta *et al*. (2019). The samples were analysed in the DSC by first equilibrating at 20 °C for 5 min and then heated up to 140 °C at 5 °C/min.

4.2.6 Sensory analysis

Fifty-four participants (ages 18–27 years, 55% women, 44% men) received samples of the fluted pumpkin fortified and commercial cassava pasta in plastic plates (3 g) at 40 ^{o}C and coded with three-digit random numbers. The participants were recruited through social media and/or personal contact and individuals who habitually eat cassava products were selected. Half of the participants were provided with nutritional information while the other half received none. The samples were evaluated on the overall acceptability, likability and intensity of colour, aroma, appearance, flavour, taste, firmness, stickiness and sliminess using a 9-point hedonic scale (Michalak-Majewska *et al*., 2020). The likelihoods of purchase and consumption were also evaluated. The number of participants is adequate for early sensory acceptance evaluations in the development of new products and is commonly used in consumer sensory profiling tests (Costa *et al*., 2020).

4.2.7 Statistical analysis

All analyses were carried out in triplicate and the results were expressed as mean ± standard deviation. The data were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, Version 26.0, Armonk, New York, USA). ANOVA and Duncan's multiple range tests were performed at a 95 % confidence interval ($P < 0.05$) level to determine the significant difference between means.

4.3. Results and Discussion

4.3.1. Physical properties

4.3.1.1 Particle size distribution (PSD) of flour samples

The particle size distribution of the investigated flour formulations (Table 4.2) revealed a significant difference ($p < 0.05$) between the white and yellow cassava flours. The mean volume distribution of the white and yellow cassava flour samples ranged from 135.7-149.7 µm and 326.0-342.7 µm, respectively. The mean surface area of the yellow cassava flour samples was thus larger than the white samples with about a difference of 89 µm due to variation in the cultivar. The range of Dx10, Dx50 and Dx90 of the flour samples showed that the cassava flours have a combination of varying particle sizes which impacted the processing performance of the flours. This was in line with the findings of Chisenga *et al*. (2019) and Oladunmoye *et al*. (2014), which suggested that a decrease in particle sizes enhances hydration, thus increasing the solubility of flours.

Fluted pumpkin leaf addition decreased the mean volume distribution (4 – 9 %) and surface area (12 -26 %) of the flour samples. The results also confirmed that yellow-fleshed cassava flours had a larger volume distribution than the white-fleshed cultivar, in line with the reports of Ayetigbo *et al*. (2018). The particle size influences starch behaviour; while lower onset gelatinisation temperatures, peak, final and hold viscosities with a decrease in particle sizes have also been reported (Chisenga *et al*., 2019; Ahmed *et al*., 2018). However, in a separate study, the protein content of the yellow cassava flours was found lower than wheat flour at 0.99-2.95 g/100g while the total dietary fibre was comparable at between 9.0-10.0 g/100 g dry weight (Lawal *et al.*, 2021).

4.3.1.2 Colour measurement

Colour is a critical quality attribute of pasta as it directly influences the consumers' willingness to purchase (Zen *et al*., 2020). The colour profile of the flour and pasta samples is shown in Table 4.3, while the pictures of the samples are shown in Fig.4.1. Cassava cultivar variation significantly influenced L*, a* and b* values. L* values of the whole white cassava flour and pasta samples were 90.6 and 81.2, respectively, while the plain yellow cassava flour and pasta samples had L* values of 89.7 and 70, respectively. These results were similar to previously reported lightness values of white and yellow cassava flours ranging from 83 to 96 (Rachman *et al*., 2019; Chisenga *et al*., 2019; Ayetigbo *et al*., 2018). The higher lightness in the white cassava products compared to those from the yellow cassava variety may be due to the difference in particle sizes (Table 4.2).

L* values increase with decreasing particle size, as smaller particles have a larger surface area, enhancing light reflection (Oladunmoye *et al.*, 2010). L* values of cassava-fluted pumpkin flour and pasta samples ranged from 66.2 to 77.7 and 52.0 to 61.7. As expected, lightness decreased significantly with the inclusion of fluted pumpkin leaf powder in the blends, suggesting that leaf powder addition impacts both cassava varieties' products' lightness. Previous authors similarly recorded a decrease in lightness values of wheat pasta on green leaf powder inclusion because of the darkness of the enriching materials (Simonato *et al.*, 2020; Zen *et al.*, 2020). The decreased lightness from 66 to 90 in flour samples to 52-81 in pasta shows that pasta production steps may modify the initial flour colours.

The a* (red-green) values of the yellow cassava-fluted pumpkin flour blends were higher for the white cassava blends (Table 4.2). The yellow cassava flour and pasta samples also had higher b* values due to the yellow pigment in the biofortified cassava variety. The b* values of the fortified cassava-flour blends and pasta were high (above 11.0). The a* and b* values of white and yellow cassava products were similar to previously reported values (Falade *et al.*, 2019; Rachman *et al.*, 2019; Ayetigbo *et al.*, 2018). The addition of fluted pumpkin leaf powder resulted in products with negative a* and positive b* , thus more pronounced greenness due to the chlorophyll present in the vegetable powder. The pasta greenness values are comparable with commercial spinach lasagne (a* of − 5 to − 6.5), as Suman *et al.* (2008), reported. The increased yellowness could be due to the presence of carotenoid pigments, such as β-carotene and xanthophyll (Zen *et al.*, 2020). Increased pasta yellowness is perceived as a positive quality attribute as consumers prefer pasta with golden yellow colour (Sharma *et al.*, 2021; Mercier *et al.*, 2016). Yellow cassava was also preferred to the white variety among consumers in Nigeria (Bechoff *et al.*, 2018). Lawal, *et al.* (2021), similarly reported consumer acceptance of cassava pasta made with leafy vegetables and reported a more appreciable acceptance for yellow cassava pasta among the consumers.

4.3.2 Functional properties

4.3.2.1 Bulk densities of cassava flours

The bulk density of flour is a critical quality factor as it provides information for storage, processing, and packaging (Chisenga *et al.*, 2019). The values of the Loose bulk density (LBD) and Packed bulk density (PBD) of white cassava flour were lower than the yellow cassava flour (0.41 g/ml and 0.59 g/ml vs 0.47 g/ml and 0.55 g/ml, respectively), likely due to varietal differences and processing conditions. These figures are similar to previously reported results (Falade *et al.*, 2019; Ajibola & Olapade, 2017) for the white and yellow cultivars of cassava. A decrease in bulk density was observed with fluted pumpkin enrichment as the LBD and PBD of the cassava-fluted pumpkin flour blends ranged from 0.36 to 0.46 g/ml and 0.56–0.62 g/ml, respectively (Table 4.2). The lower LBD on fluted pumpkin leaf enrichment could be due to lower carbohydrate content in the flour blends (Ocheme *et al.*, 2018; Alake *et al.*, 2016). Chisenga *et al.* (2019) attributed the higher PBD compared to LBD to changes in geometry, size and surface properties of the flour which are influenced by the compacting forces. Previous authors similarly reported that, as particle size increased, bulk density decreased (Chisenga *et al.*, 2019). The lower bulk densities of the investigated cassava flour blends in contrast to wheat flour could be seen as an advantage since it allows for high nutrient density and easy transportation (Ocheme *et al.*, 2018; Alake *et al.*, 2016).

Table 4.2. Particle size distribution, bulk density and gelation capacity of cassava flour samples

Parameters	WFU0	WFU5	WFU10	YFU0	YFU5	YFU10
Mean VD (μm)	149.7^{a}±3.2	149.0^{a}±9.2	135.7^{a}±0.3	338.3^{b}±8.2	326.0^{b}±2.7	342.7^{b}±6.5
Dx10 VD(μm)	10.1^{a}±0.0	9.5^{a}±0.0	8.7^{a}±0.0	33.8^{c}±4.3	24.6^{b}±0.6	23.6^{b}±1.0
Dx50 VD(μm)	52.7^{b}±0.1	47.3^{ab}±1.3	39.2^{a}±0.7	304.3cd±7.7	292.0^{c}±2.1	309.7^{d}±5.9
Dx90 VD(μm)	430.3^{a}±7.4	429.3^{a}±2.6	399.0^{a}±2.7	654.3^{b}±3.5	647.0^{b}±3.8	677.3^{b}±12.8
Mean SA(μm)	10.4^{a}±0.2	10.0^{a}±0.1	9.2^{a}±0.1	99.4^{d}±3.1	79.6^{c}±1.1	73.5^{b}±1.0
Dx10 SA(μm)	0.2^{a}±0.0	0.2^{a}±0.0	0.2^{a}±0.0	10.1^{d}±0.0	5.9^{c}±0.0	3.5^{b}±0.0
Dx50 SA(μm)	0.5^{a}±0.0	0.5^{a} ±0.0	0.6^{a}±0.0	20.2^{d}±0.2	17.2^{c}±0.1	15.1^{b}±0.1
Dx90 SA(μm)	18.9^{a}±0.2	18.5^{a}±0.2	17.9^{a}±0.1	315.0^{c}±8.7	268.3^{b}±3.3	259.0^{b}±3.8
LBD (g/ml)	0.41^{b} ±0.0	0.39^{b} ±0.0	0.36^{a} ± 0.0	0.47^{c} ± 0.0	0.45^{c} ± 0.0	0.46^{c} ± 0.0
PBD (g/ml)	0.59^{ab} ±0.0	0.62^{b} ±0.0	0.59^{ab} ±0.0	0.55^{a} ± 0.0	0.56^{a} ± 0.0	0.56^{a} ± 0.0
LGC (%)	12.67^{a} ±0.2	12.00^{a} ±0.0	15.33^{c} ± 0.2	12.00^{a} ± 0.0	14.00^{b} ± 0.0	16.00^{c} ± 0.0

Results are presented as means ± standard deviations (n = 3). Rows with the same superscript are not significantly different ($p < 0.05$).

VD: Volume distribution; SA: Surface area; Dx 10: maximum particle diameter below which 10% of the sample volume exist; Dx 50: maximum particle diameter below which 50% of the sample volume exist; Dx 90: maximum particle diameter below which 90% of the sample volume exist. LBD: Loose bulk density; PBD: Packed bulk density; LGC: Least gelation concentration.

WFU0- White cassava flour, WFU5- White cassava flour with 5 % fluted pumpkin leaf powder, WFU10- White cassava flour with 10 % fluted pumpkin leaf powder, YFU0- Yellow cassava flour, YFU5- Yellow cassava flour with 5 % fluted pumpkin leaf powder, YFU10- Yellow cassava flour with 10 % fluted pumpkin leaf powder.

4.3.2 Functional properties

4.3.2.1 Bulk densities of cassava flours

The bulk density of flour is a critical quality factor as it provides information for storage, processing, and packaging (Chisenga *et al.*, 2019). The values of the Loose bulk density (LBD) and Packed bulk density (PBD) of white cassava flour were lower than the yellow cassava flour (0.41g/ml and 0.59 g/ml vs 0.47 g/ml and 0.55 g/ml, respectively), likely due to varietal differences and processing conditions. These figures are similar to previously reported results (Falade *et al.*, 2019; Ajibola and Olapade, 2017) for the white and yellow cultivars of cassava. A decrease in bulk density was observed with fluted pumpkin enrichment as the LBD and PBD of the cassava-fluted pumpkin flour blends ranged from 0.36-0.46 g/ml and 0.56-0.62 g/ml, respectively (Table 4.2). The lower LBD on fluted pumpkin leaf enrichment could be due to lower carbohydrate content in the flour blends (Ocheme *et al.*, 2018; Alake *et al.*, 2016). Chisenga *et al.* (2019) attributed the higher PBD compared to LBD to changes in geometry, size and surface properties of the flour which are influenced by the compacting forces. Previous authors similarly reported that, as particle size increased, bulk density decreased (Chisenga *et al.*, 2019). The lower bulk densities of the investigated cassava flour blends in contrast to wheat flour could be seen as an advantage since it allows for high nutrient density and easy transportation (Ocheme *et al.*, 2018; Alake *et al.*, 2016).

4.3.2.2 Gelation capacity

The samples' least gelation concentration (LGC) ranged from 12% to 15% in the white cassava flours and 12–16% in the yellow cassava flour samples (Table 4.2). The least gelation concentration (LGC) indicates the minimum amount of flour needed to form a stable gel, and it serves as a measure of the flour's gelation capacity (Alake *et al.*, 2016). Comparable cassava flour LGC of 12–14% was previously reported (Ojo *et al.*, 2017; Alake *et al.*, 2016), while Chandra (2013), reported a lower LGC of 8 for wheat flour. The variation in the flour samples' gelation capacities could be due to interaction between their structural components such as carbohydrates, proteins, lipids, and amylose- amylopectin ratio (Alake *et al.*, 2016; Chinma *et al.*, 2013). Gelation capacity decreased significantly with the addition of fluted pumpkin leaf since the higher the LGC, the lower the gelation capacity of the flour (Chandra *et al.*, 2015; Chinma *et al.*, 2013). The higher LGC in these blends suggests that they may have better capability to hold in food ingredients during preparation than pulse or legume flours whose surrounding protein matrix, as well as soluble and insoluble fibres, restrict starch gelatinisation (Noordraven *et al.*, 2021).

Table 4.3. Colour measurement of flour and pasta samples

Sample	L*	a*	b*
WFU0	90.6^{l} ± 0.1	0.7^{i} ± 0.0	7.8^{a} ± 0.1
WFU5	76.2^{h} ± 0.3	-4.4^{f} ± 0.1	15.2^{de} ± 0.4
WFU10	71.2^{g} ± 0.0	-5.8^{e} ± 0.0	16.8^{f} ± 0.0
YFU0	89.7^{k} ± 0.0	0.3^{h} ± 0.0	11.4^{b} ± 0.0
YFU5	77.7^{i} ± 0.4	-7.8^{b} ± 0.0	11.7^{b} ± 0.1
YFU10	66.2^{e} ± 0.0	-7.9^{a} ± 0.0	16.6^{f} ± 0.2
WPU0	81.2^{j} ± 0.2	1.5^{k} ± 0.1	11.4^{b} ± 0.1
WPU5	61.7^{d} ± 0.1	-0.1^{g} ± 0.0	14.2^{c} ± 0.1
WPU10	53.4^{b} ± 0.0	-0.2^{g} ± 0.0	15.5^{e} ± 0.1
YPU0	70.0^{f} ± 0.7	1.2^{j} ± 0.1	15.1^{d} ± 0.3
YPU5	58.5^{c} ± 0.1	-6.4^{d} ± 0.1	26.2^{g} ± 0.1
YPU10	52.0^{a} ± 0.0	-6.7^{c} ± 0.0	27.1^{h} ± 0.1

The values are expressed as mean ± standard deviation, n = 3 Columns with the same superscript are not significantly different (p<0.05)

L scale: 0-50 (dark); 51-100 (light) a* scale: +ve value (red); -ve value (green) b* scale: +ve value (yellow); -ve value (blue)*

WFU0- White cassava flour, WFU5- White cassava flour with 5 % fluted pumpkin leaf powder, WFU10- White cassava flour with 10 % fluted pumpkin leaf powder, YFU0- Yellow cassava flour, YFU5- Yellow cassava flour with 5 % fluted pumpkin leaf powder, YFU10- Yellow cassava flour with 10 % fluted pumpkin leaf powder, WPU0- White cassava pasta, WPU5- White cassava pasta fortified with 5 % fluted pumpkin leaf powder, WPU10- White cassava pasta with fortified with 10 % fluted pumpkin leaf powder, YPU0- Yellow cassava pasta, YPU5- Yellow cassava pasta fortified with 5% fluted pumpkin leaf powder, YPU10- Yellow cassava pasta fortified with 10 % fluted pumpkin leaf powder

4.3.2.3 Water solubility, swelling power, water and oil absorption capacities

The water solubility (WS) of the yellow cassava flour and pasta samples was higher than the white variety's at 60 °C (4.0-12.7%), but the reverse occurred at 90 °C with the white cassava flour and pasta samples' solubility ranging from 10.7-26.7% while the yellow cassava flour and pasta samples had solubilities ranging from 3.0 - 8.0% (Table 4.4). Similar WS values of 4 – 32 % and 2 – 24 % were reported for white and yellow cassava flours, respectively (Ojo *et al*., 2017; Alake *et al*., 2016; Chinma *et al*., 2013; Oladunmoye *et al*., 2010). The water solubility of the flours is expected to increase at higher temperatures hence the evaluation at two temperatures 60 and 90 ℃. The WS of the samples significantly increased with fluted pumpkin inclusion and increased temperature, similar to the results of Ojo *et al*. (2017). The decrease in yellow-cassava pasta samples' water solubility is desirable, suggesting lower cooking loss (Sharma *et al*., 2021). Swelling power (SP) decreased significantly on fluted pumpkin powder addition due to reduced starch content, higher fibre and weak internal forces between the starch granules (Gallo *et al*., 2020). This is likely because of the lack of formation of a network between proteins and starch, an indication of higher water absorption due to starch gelatinisation in the flour and pasta samples on heating. Swelling power is an important quality parameter in pasta products because it influences the volume and yield (Ibrahim and Ani, 2018; Zen *et al*., 2020). Simonato *et al*. (2020), similarly reported a decrease in the swelling power of wheat pasta on the addition of moringa leaf powder. However, at 90 °C, fluted pumpkin inclusion had no significant effect on the investigated samples' swelling power, an indication of the effect of the insoluble fibres, suggesting that the added fluted pumpkin did not significantly interfere with starch gelatinisation. Comparable SP of 7-14% in white and yellow cassava flours was previously reported (Ayetigbo *et al*., 2018; Alake *et al*., 2016). The variation in swelling power between cassava cultivars may be due to their particle sizes, amylose and amylopectin contents, the interaction between non-starch components and starch molecules during gelatinisation (Awuchi *et al*., 2019).

Water holding capacity (WHC) influences the dough handling, volume and texture of the flour and pasta products (Sharma *et al*., 2021). WHC of the flours could be viewed as a measure of the optimum amount of water to be added before the dough becomes exceptionally sticky to handle (Awuchi *et al*., 2019). At 60 °C, the WHC of the white cassava flour and pasta samples ranged from 6.2 to 7.0 g/g, while the yellow cassava flour and pasta samples had lower WHC ranging from 4.7 to 7.0 g/g. At 90 ℃, the WHC of the white cassava flour and pasta samples ranged from 5.2 to 5.8 g/g, while the yellow cassava flour and pasta samples had higher WHC ranging from 6.0 to 6.5 g/g. The higher WHC in the yellow cassava variety may be due to its higher amorphous amylose content (Ayetigbo *et al*., 2018), which may have more water binding sites (Ojo *et al*., 2017; Chandra *et al*., 2015). WHC of the pasta products studied was similar to that of wheat (7.33 g/g), as reported by Ibrahim and Ani, (2018). WHC decreased

Table 4.4. Water solubility, swelling power, water holding capacity and oil absorption capacity *of cassava*-fluted pumpkin *formulations*

Sample	WS 60°C (%)	WS 90°C (%)	SP 60°C (%)	SP 90°C (%)	WHC 60°C(g water/g sample)	WHC 90°C (g water/g sample)	OAC g oil/g sample
WFU0	$3.7^{a}\pm0.6$	$20.3^{d}\pm0.1$	$6.4^{c}\pm0.2$	$7.2^{c}\pm0.3$	$6.2^{c}\pm0.2$	$5.8^{b}\pm0.2$	$1.9^{b}\pm0.0$
WFU5	$5.0^{bc}\pm0.0$	$26.7^{e}\pm0.1$	$6.8^{d}\pm0.3$	$7.2^{c}\pm0.2$	$6.5^{d}\pm0.3$	$5.3^{a}\pm0.3$	$1.8^{b}\pm0.1$
WFU10	$6.0^{c}\pm1.0$	$22.7^{d}\pm0.2$	$6.9^{d}\pm0.3$	$7.8^{c}\pm0.1$	$6.5^{d}\pm0.3$	$6.1^{c}\pm0.0$	$2.3^{b}\pm0.6$
YFU0	$4.0^{a}\pm0.0$	$6.6^{b}\pm0.5$	$7.3^{e}\pm0.1$	$6.5^{b}\pm0.3$	$7.0^{e}\pm0.1$	$6.1^{c}\pm0.3$	$2.0^{b}\pm0.1$
YFU5	$6.7^{cd}\pm0.6$	$6.9^{b}\pm0.8$	$6.4^{c}\pm0.1$	$6.6^{b}\pm0.5$	$6.0^{b}\pm0.1$	$6.2^{c}\pm0.4$	$2.0^{b}\pm0.1$
YFU10	$12.7^{e}\pm0.5$	$8.0^{b}\pm1.0$	$5.7^{b}\pm0.2$	$6.7^{b}\pm0.6$	$5.0^{a}\pm0.2$	$6.1^{c}\pm0.5$	$2.1^{b}\pm0.0$
WPU0	$2.3^{a}\pm0.7$	$10.7^{c}\pm0.9$	$7.1^{de}\pm0.1$	$6.0^{a}\pm0.2$	$7.0^{e}\pm0.0$	$5.4^{ab}\pm0.2$	$1.9^{b}\pm0.4$
WPU5	$4.3^{b}\pm0.6$	$14.7^{c}\pm0.0$	$6.6^{c}\pm0.1$	$6.2^{a}\pm0.3$	$6.3^{c}\pm0.1$	$5.3^{ab}\pm0.4$	$1.5^{a}\pm0.1$
WPU10	$4.7^{b}\pm0.7$	$15.0^{c}\pm0.5$	$6.5^{c}\pm0.1$	$6.2^{a}\pm0.3$	$6.2^{c}\pm0.0$	$5.2^{a}\pm0.2$	$1.9^{b}\pm0.5$
YPU0	$4.3^{b}\pm0.6$	$3.0^{a}\pm0.6$	$5.9^{b}\pm0.4$	$6.7^{b}\pm0.2$	$5.7^{b}\pm0.3$	$6.5^{d}\pm0.2$	$1.9^{b}\pm0.2$
YPU5	$5.7^{c}\pm0.6$	$4.0^{a}\pm0.6$	$5.4^{a}\pm0.2$	$6.2^{a}\pm0.2$	$5.1^{a}\pm0.2$	$6.0^{c}\pm0.1$	$1.6^{a}\pm0.1$
YPU10	$7.0^{d}\pm0.6$	$3.7^{a}\pm0.3$	$5.1^{a}\pm0.3$	$6.3^{a}\pm0.2$	$4.7^{a}\pm0.3$	$6.1^{c}\pm0.2$	$1.9^{b}\pm0.2$

*The values are expressed as mean ± standard deviation, n = 3 Columns with the same superscript are not significantly different (p=0.05) WS: Water solubility; SP: Swelling power; WAC: Water holding capacity; OAC: Oil absorption capacity **WFU0**- White cassava flour, **WFU5**- White cassava flour with 5 % fluted pumpkin leaf powder, **WFU10**- White cassava flour with 10 % fluted pumpkin leaf powder, **YFU0**- Yellow cassava flour, **YFU5**- Yellow cassava flour with 5 % fluted pumpkin leaf powder, **YFU10**- Yellow cassava flour with 10 % fluted pumpkin leaf powder, **WPU0**- White cassava pasta, **WPU5**- White cassava pasta fortified with 5 % fluted pumpkin leaf powder, **WPU10**- White cassava pasta with fortified with 10 % fluted pumpkin leaf powder, **YPU0**- Yellow cassava pasta, **YPU5**- Yellow cassava pasta fortified with 5% fluted pumpkin leaf powder, **YPU10**- Yellow cassava pasta fortified with 10 % fluted pumpkin leaf powder* significantly on the addition of fluted pumpkin leaf, probably due to competition between entrapped soluble fibre particles and starch molecules for water. An unexpected decrease in WHC in some of the samples on the increase in temperature was observed. The higher temperature could result in higher kinetic energy, allowing increased interaction between the solutes and water. However, the temperature increase could have weakened the molecular bonds and starch network beyond a certain point, leading to leakage of the absorbed water; consequently, the observed lower WHC. The reduced WHC could be advantageous since an excess could lead to undesirably soft and brittle pasta (Ibrahim and Ani, 2018).

The oil absorption capacity of the white cassava flour and pasta samples ranged from 1.5-2.3 g/g, while the yellow cassava flour and pasta samples had higher OAC ranging from 1.6-2.1 g/g. Previous authors similarly reported OAC ranging from 1 to 3 g/g in white and yellow cassava flours (Falade *et al*., 2019; Ajibola and Olapade, 2017).

Due to the protein content in fluted pumpkin leaf (Omimakinde *et al*., 2018), the OAC was expected to increase since proteins contain both hydrophilic and hydrophobic side chains; the non-polar side chains can interact with lipid chains, enhancing the oil absorption (Awuchi *et al*., 2019; Tharise *et al*., 2014). An interesting observation was made in the pasta samples as the OAC decreased significantly on 5 % fluted pumpkin inclusion, followed by an increase at 10 % fluted pumpkin leaf inclusion. The initial decrease at 5 % leaf powder inclusion could be due to competition between the protein and other components.

Subsequently, at 10 % inclusion, it could be that there was sufficient protein to allow for the non-polar chains to bind to the oil, thus increasing the oil absorption capacity. The higher OAC occurrence, at 10 %, could be due to the ease of oil penetration under this condition, where more lipophilic groups (more dry leaf powder) were available. The OAC of the formulated samples is advantageous because oil is commonly employed as an anti-stick agent during pasta preparation by many sub-African consumers (Ayetigbo *et al*., 2018). Additionally, oil enhances flavour retention and mouthfeel (Awuchi *et al*., 2019).

4.3.2.4 Cooking properties (Optimum cooking time, weight gain and gruel solid loss)

The optimum cooking time of the investigated cassava pasta samples was 5-11 min while cooking times decreased significantly with fluted pumpkin addition (Fig. 4.2).

1. Yellow cassava pasta

2. White cassava pasta

3. Yellow cassava-fluted pumpkin (5%) pasta

4. White cassava-fluted pumpkin (5%) pasta

5. Yellow cassava-fluted pumpkin (10 %) pasta

6. White cassava-fluted pumpkin (10 %) pasta

Fig 4.1. ***Cassava pasta made with 0, 5 and 10 g 100 g^{-1} fluted pumpkin (Telfairia occidentalis) leaf powder***

Comparable cooking times of 5-9 min and 7-9 min have been reported for wheat and cassava-based pasta products, respectively (Odey and Lee, 2020; Kaur *et al.*, 2016). Previous authors have reported a similar trend of decreased cooking time of pasta on enrichment (Michalak-Majewska *et al.*, 2020; Simonato *et al.*, 2020; Sobota *et al.*, 2020).

A decrease in OCT could be due to the fibres in fluted pumpkin, which create interference in the structural starch network, thus facilitating water penetration and consequently, faster gelatinisation of the starch core (Simonato *et al.*, 2020; Sobota *et al.*, 2020). The lower starch content of the fortified pasta could also have influenced the OCT since gelatinisation of a lower amount of starch would have been needed. The shorter OCT in the fortified pasta is favourable since longer cooking times result in higher nutrient losses and increased energy use.

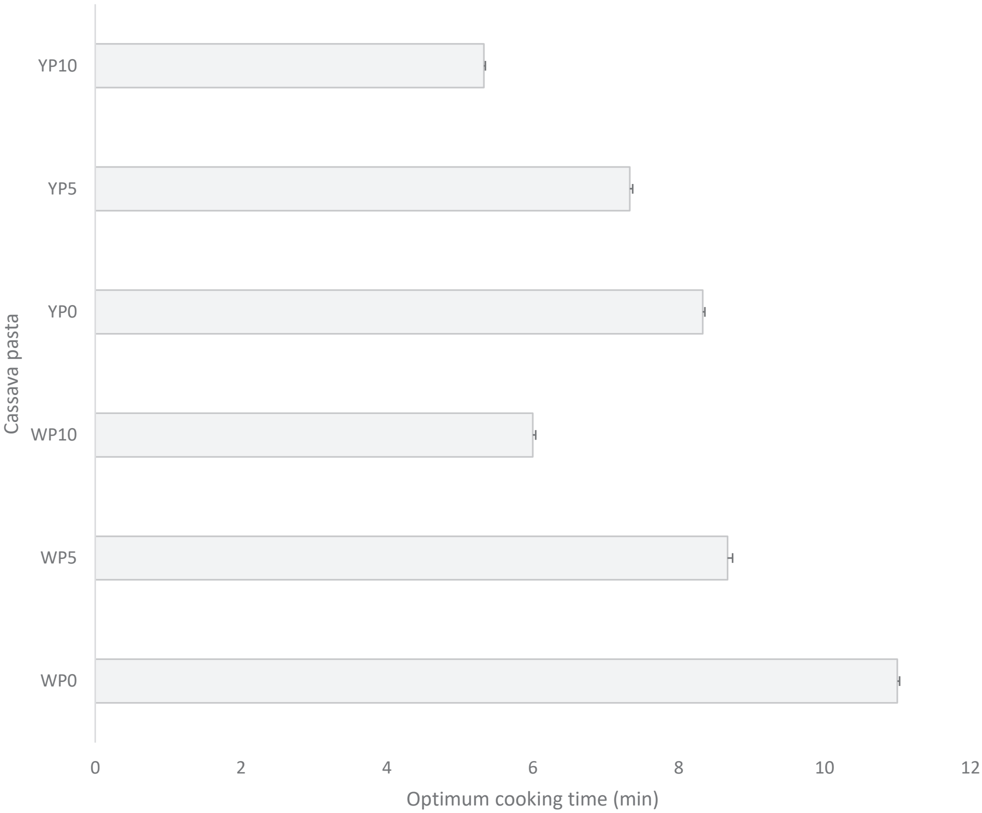


Fig. 4.2. Optimum cooking time of cooked cassava pasta samples

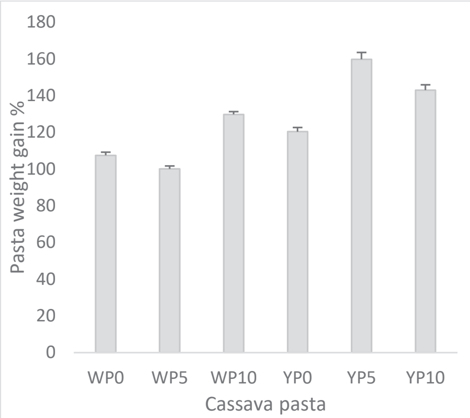
WPU0- White cassava pasta, WPU5- White cassava pasta fortified with 5 % fluted pumpkin leaf powder, WPU10- White cassava pasta with fortified with 10 % fluted pumpkin leaf powder, YPU0- Yellow cassava pasta, YPU5- Yellow cassava pasta fortified with 5% fluted pumpkin leaf powder, YPU10- Yellow cassava pasta fortified with 10 % fluted pumpkin leaf powder

4.3.2.4.1.Weight gain

Weight gain (WG) of the cooked pasta samples, a measure of the water absorbed by the pasta during cooking ranged from 100 to 160 % (Fig. 4.3). Inadequate water absorption results in harder pasta, while excess water absorption may lead to overly soft and sticky pasta. The cooking weight gain in pasta products from wheat (100-195 %) and cassava (170 – 200 %) has been reported (Odey and Lee, 2020; Gao *et al*., 2018). Yellow pasta had a higher WG than white pasta, similar to the WAC trend. An increase in cooking mass on the addition of fluted pumpkin powder may result from the interaction between the present fibres and starch matrix. The fibres could have weakened the starch network, resulting in better water diffusion and more water absorption (Panghal *et al*., 2019). The increase in weight gain was accompanied by a concomitant decrease in gruel solid loss, signifying the pasta products' excellent cooking quality. According to Kruger *et al*. (1996), a minimum weight gain of 100 % is expected in high-quality pasta products. Thus, all formulated pasta products in the present study showed high quality in this parameter. The increased weight gain due to water absorption indicates that the pasta could be more effective in providing a feeling of satiety to consumers and making the pasta easier to chew (Michalak-Majewska *et al*. 2020).

4.3.2.4.2. Gruel solid loss

Gruel solid loss (GSL) of the cooked pasta samples ranged between 0.8 % and 2.6 % (Fig. 4.3). Gruel solid loss (GSL) is associated with the leakage of soluble starch and non-starch components into the cooking water, which results in an unpleasant sticky texture (Zen *et al*., 2020). It is a measure of the pasta’s resistance to structural disintegration during cooking, thus an important quality attribute of cooked pasta. Odey and Lee (2020) and Gao *et al*. (2018), similarly reported GSL of 0.6 - 1.2 % in cassava pasta products. Although no significant difference between the white and yellow cassava varieties was observed, yellow cassava pasta samples showed a tendency to have lower gruel solid losses than the white variety. The cooking losses of the formulated pasta samples were lower than GSL of 2.7-7% reported for wheat pasta products (Sharma *et al*., 2021; Kaur *et al*., 2016). Maximum acceptable cooking loss has been defined as ≤ 8 % (Kruger *et al*., 1996); thus, the formulated pasta in this study showed acceptable cooking qualities.



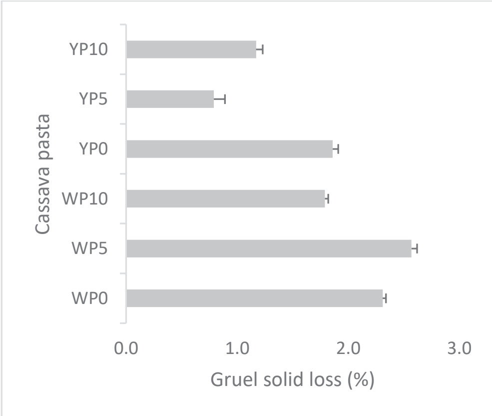


Fig. 4.3. Cooking properties (weight gain and gruel solid loss) of cooked cassava pasta samples

WPU0- White cassava pasta, WPU5- White cassava pasta fortified with 5 % fluted pumpkin leaf powder, WPU10- White cassava pasta with fortified with 10 % fluted pumpkin leaf powder, YPU0- Yellow cassava pasta, YPU5- Yellow cassava pasta fortified with 5% fluted pumpkin leaf powder, YPU10- Yellow cassava pasta fortified with 10 % fluted pumpkin leaf powder

4.3.3 Hardness of gel and pasta

The effect of leaf powder addition on the textural properties of the pasta samples is indicated by the hardness of the flour gels and pasta samples, as shown in Table 4.5. Hardness ranged from 0.8-1.8 N in the gel samples and 10.4-13.2 N in the cooked pasta samples. Gel and pasta hardness decreased on the inclusion of fluted pumpkin leaf due to the lower starch content, thus less retrogradation during gel formation and the emulsifying effect of the protein and fibre on the dough. Fluted pumpkin leaves have been reported to act as an emulsifier in food due to their high protein and fibre content which aids the reduction of hardness (Gbadamosi & Famuwagun, 2018; Sobowale *et al.*, 2011). This observation was confirmed by the weight gain of cooked pasta due to water absorption, which also increased with fluted pumpkin leaf inclusion (Fig 4.3). The samples' hardness indicates the amount of compression force that the product can withstand before mechanical disintegration (Ayetigbo *et al.*, 2018). Simonato *et al.* (2020), also reported a decrease in pasta hardness on the addition of moringa leaf powder to wheat pasta. Cassava-fluted pumpkin blends contained a higher proportion of fibre, so it holds a large amount of water and interrupts the starch matrix, resulting in a weak gel and pasta network. The reported firmness of wheat (43 N) and potato noodles (29-31 N) by Kaur *et al.* (2016), were higher than the investigated pasta. The firmness of cooked gluten-free pasta is lower than high-gluten pasta due to weaker dough matrix, starch leaching and water absorption (Zen *et al.*, 2020).

4.3.4 Impact of leaf powder addition on pasting properties of cassava pasta

The influence of fluted pumpkin leaf powder inclusion on the pasting properties: peak viscosity, trough, breakdown, final viscosity, setback, peak time and pasting temperature of the cassava pasta is shown in Table 4.5. Results showed that the peak viscosity of white and yellow cassava samples decreased with increasing fluted pumpkin leaf addition from 1373-1858 cP and 2386-2899 cP, respectively. The decrease in peak viscosity on fluted pumpkin inclusion could be due to lower starch content and dilution of the starch network by fibre particles (Panghal *et al.*, 2019). Peak viscosity (PV) indicates the thickening power of the flour and potential viscous load encountered during mixing (Alake *et al.*, 2016; Chinma *et al.*, 2013). The Hold viscosity (HV) of white and yellow cassava samples ranged from 747-1414 cP and 1368-1865 cP, respectively. Yellow cassava flour and pasta samples had significantly higher HV than the white variety. The higher peak, final and hold viscosities of the yellow-cassava flour could result from its larger particle size and higher starch content (Ahmed *et al.*, 2018). The lower HV on the addition of fluted pumpkin leaf was probably due to the reduced starch content (Alake *et al.*, 2016). HV thus represents the ability of the sample to withstand breakdown during cooling (Ayetigbo *et al.*, 2018), an indication of the better stability of the fortified cassava pasta.

The breakdown viscosity (BV) of white and yellow cassava samples was 833-1309 cP and 1017-1187 cP. BV measures the flour and pasta's disintegration on heating (Panghal *et al.*, 2019). As expected, the yellow cassava products' breakdown viscosities were higher since the breakdown viscosity is related to the peak viscosity. Low breakdown viscosities of the fluted pumpkin-fortified samples indicate the products' increased capacity to withstand breakdown during cooking. Previous authors confirmed that the higher the breakdown viscosity, the lower the starch stability during heating and mechanical stress (Ocheme *et al.*, 2018; Alake *et al.*, 2016). Increased thermal stability in the fluted pumpkin-fortified products could be due to fibres' hydrophilic nature, facilitating interaction between starch-protein-water molecules (Panghal *et al.*, 2019).

The final viscosity (FV) of the yellow cassava samples were significantly higher than the white (2000-2782 cP vs 1006-1867 cP, respectively) with the yellow unfortified pasta (YPU0) having the highest value. FV indicates the sample's ability to form a gel network after cooking and cooling due to the re-association of the starch molecules (Sharma *et* *al*., 2021). High final viscosity is desirable in pasta since it requires an increase in cooking volume (Falade *et al*., 2019).

Setback viscosity (SV) is a measure of the retrogradation tendency of the flour and pasta samples (Falade *et al*., 2019). White cassava flour and pasta samples' setback viscosities ranged from 258-453 cP, while yellow cassava flour and pasta samples had SV ranging from 632-917 cP. Similar to the previously described viscosities, yellow cassava flour and pasta samples had significantly higher SV than the white variety. SV also decreased significantly on the addition of fluted pumpkin leaf, which indicates a higher tendency of retrogradation of the starch (Ojo *et al*., 2017; Tharise *et al*., 2014). The peak time indicates the cooking time of the products. The flour and pasta samples' peak time ranged from 4 - 5 min (Table 5). Peak times of 4 - 6 min in cassava flour blends have been reported (Ajibola and Olapade, 2017; Ojo *et al*., 2017). Peak time decreased significantly on 5% fluted pumpkin leaf inclusion, which is favourable since longer peak times could indicate additional production costs. The pasting temperature of the cassava samples ranged from 73.1-74.9 ℃. Similar pasting temperatures of 70 - 76 °C in white and yellow cassava products have been recorded (Odey and Lee, 2020; Ayetigbo *et al*., 2018). Pasting temperature represents the minimum temperature required to gelatinise or cook the flour and pasta samples (Alamu *et al*., 2017). Similar to gelatinisation temperatures, the pasting temperatures increased with fluted pumpkin inclusion. A previous report noted that pasting and gelatinisation temperatures increased with a decrease in starch content (Chandra *et al*., 2015). Considering that wheat had a higher pasting temperature of 77 °C (Tharise *et al*., 2014), the production of the fortified cassava products may require lower energy costs. Pasting properties reflect the flour and pasta behaviour under varying shear stress and temperatures (Ayetigbo *et al*., 2018). Wheat flour has been reported to have lower viscosities and higher pasting temperature (Oladunmoye *et al*., 2014; Tharise *et al*., 2014) than the examined cassava flours. Increased pasting properties in the pasta samples indicate that the starch granules retained their structure during extrusion and heating (Leonel *et al*., 2011).

4.3.5 Thermal properties

Thermal properties, otherwise known as gelatinisation of starch (Tonset, Tpeak, Tend and enthalpy) were evaluated for all the samples (Fig. 4.4). The onset temperature (Tonset) ranged from 65.8-67.7℃, Peak temperature (Tpeak) from 68.3-70 °C, while cease/end temperature Tend ranged from 79.9-82.4 °C. The gelatinisation enthalpy ranged from 8.7-12.7 J/g. Similar Tonset (63-66 °C), Tpeak (68.3-70 °C), Tend (74.4 °C) and enthalpy (9-14 J/g) of white and yellow cassava have been reported by other

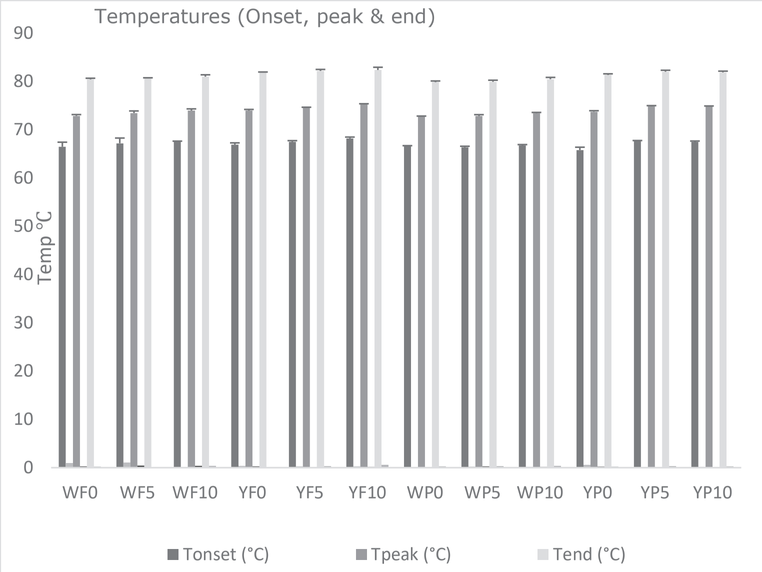
Chapter 4

Table 4.5. Pasting properties of cassava flour and pasta

Sample	Peak viscosity (cP)	Hold viscosity(cP)	Breakdown viscosity (cP)	Final viscosity(cP)	Setback viscosity (cP)	Peak Time(min)	Pasting Temp.(°C)	Hardness (N)
WFU0	1858^{d}±57.8	1025^{c}±28.1	833^{b}±42.1	1354^{c}±26.6	329^{c}±10.4	4.69^{c}±0.1	74.3^{b}±0.1	1.7^{a} ± 0.7
WFU5	1757^{cd}±2.3	823^{a}±8.7	934^{c}±7.0	1091^{a}±1.3	268^{a}±3.7	4.41^{a}±0.0	74.9^{b}±0.4	1.7^{a} ± 1.0
WFU10	1672^{bc}±12.4	747^{a}±7.5	924^{c}±10.2	1006^{a}±3.8	258^{a}±4.1	4.21^{a}±0.0	74.9^{b}±0.5	0.8^{a} ± 0.2
YFU0	2899^{g}±63.5	1752^{g}±6.5	1148^{d}±25.1	2530^{h}±43.0	778^{h}±4.6	4.62^{bc}±0.1	74.9^{b}±0.5	1.7^{a} ± 0.6
YFU5	2631^{f}±30.1	1508^{e}±14.5	1124^{d}±21.9	2194^{f}±14.8	687^{f}±8.7	4.47^{ab}±0.1	74.6^{b}±0.5	1.8^{a} ± 0.4
YFU10	2386^{e}±4.7	1368^{d}±24.4	1017^{c}±17.1	2000^{e}±9.1	632^{e}±11.1	4.36^{a}±0.0	74.9^{b}±0.5	1.4^{a} ± 1.0
WPU0	2722^{f}±32.5	1414^{d}±7.7	1309^{e}±25.0	1867^{d}±8.2	453^{d}±9.3	4.56^{b}±0.1	73.8^{a}±0.5	12.2^{a} ± 0.7
WPU5	1610^{b}±17.5	933^{b}±6.1	677^{a}±11.8	1230^{b}±11.0	297^{b}±4.9	4.36^{a}±0.0	74.0^{b}±0.5	11.6^{a} ± 1.0
WPU10	1373^{a}±15.3	769^{a}±7.8	604^{a}±9.5	1030^{a}±13.8	261^{a}±6.3	4.43^{a}±0.0	74.8^{b}±0.5	11.6^{a} ± 0.5
YPU0	2841^{g}±19.4	1865^{h}±17.5	976^{c}±7.3	2782^{i}±22.7	917^{i}±8.5	4.63^{bc}±0.0	73.1^{a}±0.4	13.2^{a} ± 0.8
YPU5	2844^{g}±59.8	1658^{f}±27.0	1187^{d}±33.8	2383^{g}±31.2	725^{g}±7.0	4.58^{b}±0.1	74.0^{b}±0.5	11.8^{a} ± 1.1
YPU10	2674^{f}±37.1	1547^{e}±21.3	1127^{d}±15.9	2223^{f}±24.5	676^{f}±3.2	4.54^{b}±0.0	74.0^{b}±0.4	10.4^{a} ± 0.9

The values are expressed as mean ± standard deviation, n = 3 Columns with the same superscript are not significantly different (p=0.05)

WFU0- White cassava flour, WFU5- White cassava flour with 5 % fluted pumpkin leaf powder, WFU10- White cassava flour with 10 % fluted pumpkin leaf powder, YFU0- Yellow cassava flour, YFU5- Yellow cassava flour with 5 % fluted pumpkin leaf powder, YFU10- Yellow cassava flour with 10 % fluted pumpkin leaf powder, WPU0- White cassava pasta, WPU5- White cassava pasta fortified with 5 % fluted pumpkin leaf powder, WPU10- White cassava pasta with fortified with 10 % fluted pumpkin leaf powder, YPU0- Yellow cassava pasta, YPU5- Yellow cassava pasta fortified with 5% fluted pumpkin leaf powder, YPU10- Yellow cassava pasta fortified with 10 % fluted pumpkin leaf powder



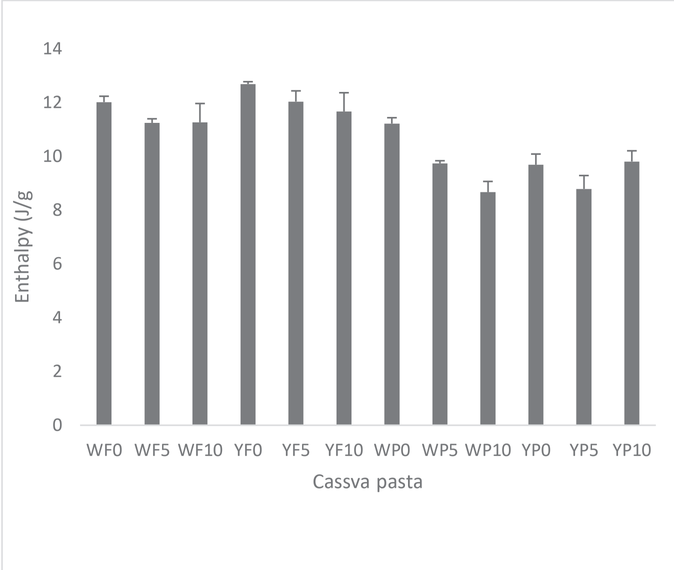


Fig. 4.4. Thermal properties of cassava flour and pasta.

Tonset- Onset temperature, Tpeak- Peak temperature, Tend-Cease temperature. WPU0- White cassava pasta, WPU5- White cassava pasta fortified with 5% fluted pumpkin leaf powder, WPU10- White cassava pasta with fortified with 10% fluted pumpkin leaf powder, YPU0- Yellow cassava pasta, YPU5- Yellow cassava pasta fortified with 5% fluted pumpkin leaf powder, YPU10- Yellow cassava pasta fortified with 10% fluted pumpkin leaf powder.

authors (Ayetigbo *et al*., 2018; Leonel *et al*., 2011). Yellow cassava flour and pasta samples had higher gelatinisation temperatures than white cassava-based samples, probably due to their larger particle size distribution (Ahmed *et al*., 2018). The increase in gelatinisation transition temperatures on fluted pumpkin leaf addition may be due to the samples' lower starch content (Chandra *et al*., 2015). Fluted pumpkin leaf-fortified formulations expectedly had lower starch contents; consequently, the lower enthalpies indicate that less energy was required to break down the starch structures. Literature also agrees that enthalpy decreases with a longer cooking time (Lu *et al*., 2020; Lionel *et al*., 2011). In comparison, wheat flour reportedly had Tonset, Tpeak, Tend and enthalpies in the range of 57.4 - 64.2 °C, 60.9 - 67.1°C, 64.3-70.1°C and 3.2 - 8.4 J/g, respectively (Kaur *et al*., 2016). The lower gelatinisation characteristics of wheat than cassava may be due to its lower starch content, proteins and lipids, as well as particle sizes (Ahmed *et al*., 2019; Kaur *et al*., 2016).

4.3.6 Sensory perception of pasta

In most of the organoleptic attributes studied as shown in Fig. 4.5, the yellow cassava pasta was preferred to the white cassava pasta (score 6.6). The colour likability and firmness scores of the pasta samples however decreased significantly on fluted pumpkin leaf addition. A similar decrease in acceptability of pasta fortified with vegetables was reported by Simonato *et al*. (2020), for wheat pasta fortified with *Moringa oleifera* L. leaf powder and Sato *et al* (2019), for wheat pasta fortified with dried leaves of *Pereskia aculeata Miller*. Ayetigbo *et al*. (2018), also observed that yellow-flesh cassava was perceived as more attractive than white-flesh cassava. Yellow pasta without leaf addition (YPU0) was thus rated best in overall acceptability. Furthermore, the provision of nutritional information about the samples also boosted the overall acceptability and likability scores of the pasta, an indication that consumers may be more willing to purchase unfamiliar products perceived as healthier. Similarly, Sato *et al*. (2019), suggested that although there is an increased market for healthier food products, information on new ingredients and healthy products should be provided to the consumers to enhance acceptance.

4.4. Conclusion and recommendation

The fortification of cassava pasta with fluted pumpkin leaf powder provided some beneficial effects to the techno-functional properties but also downsides particularly in sensory perception, compared to traditional wheat pasta. Fluted pumpkin-fortified pasta had lower pasting temperatures (73.1 - 74.9 °C) and may require lower energy costs than the wheat pasta with a pasting temperature of 77 °C. Interestingly, the fluted pumpkin leaf addition improved the cooking qualities of the cassava pasta, which was not the case observed for vegetable addition to wheat pasta. This is likely due to the absence of a gluten protein network, making the cassava matrix more suitable to incorporate vegetable materials without changing its properties. The addition of fluted pumpkin leaf powder expectedly impacted the colour parameters of developed pasta significantly. Yellow cassava flour had higher pasting viscosities and larger particle size distribution, enabling its suitability in pasta production. The addition of fluted pumpkin also resulted in a decrease in the hardness and cooking quality of pasta samples. To improve the texture of fluted pumpkin-fortified pasta, process modifications such as pre-gelatinisation, use of hydrocolloids, hot extrusion or extrusion with smaller dies are recommended. In future studies, we will extend this novel work by evaluating the physicochemical attributes and digestibility of the newly developed cassava-fluted pumpkin leaf pasta.

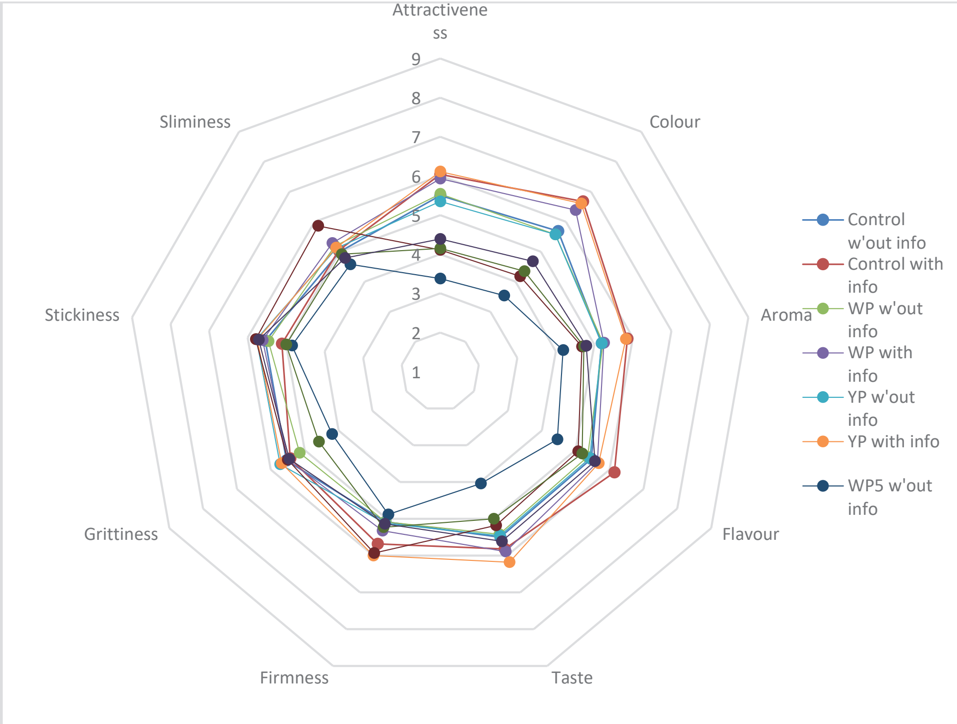


Fig. 4.5. Sensory perception of cassava pasta.

Control- Commercial white cassava pasta, WPU0- White cassava pasta, WPU5- White cassava pasta fortified with 5% fluted pumpkin leaf powder, YPU0- Yellow cassava pasta, YPU5- Yellow cassava pasta fortified with 5% fluted pumpkin leaf powder.

pumpkin leaf addition improved the cooking qualities of the cassava pasta, which was not the case observed for vegetable addition to wheat pasta. This is likely due to the absence of a gluten protein network, making the cassava matrix more suitable to incorporate vegetable materials without changing its properties. The addition of fluted pumpkin leaf powder expectedly impacted the colour parameters of developed pasta significantly. Yellow cassava flour had higher pasting viscosities and larger particle size distribution, enabling its suitability in pasta production. The addition of fluted pumpkin also resulted in a decrease in the hardness and cooking quality of pasta samples. To improve the texture of fluted pumpkin-fortified pasta, process modifications such as pre-gelatinisation, use of hydrocolloids, hot extrusion or extrusion with smaller dies are recommended. In future studies, we will extend this novel work by evaluating the physicochemical attributes and digestibility of the newly developed cassava-fluted pumpkin leaf pasta.

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CHAPTER FIVE

LEAFY VEGETABLE-FORTIFIED YELLOW CASSAVA PASTA SHOWED REDUCED GLYCEMIC INDEX AND INCREASED ZINC BIOACCESSIBILITY

This chapter is under review as:

Lawal, O.M., Fogliano, V., Rotte, I., Fagbemi, T.N., Dekker, M. & Linnemann, A.R. (2021). Leafy vegetable-fortified yellow cassava pasta had a decreased glycemic index and increased zinc bioaccessibility *Food & Function*

Abstract

Food-to-food fortification of yellow cassava with leafy vegetables (amaranth and fluted pumpkin leaves) was employed in this study to develop cassava-vegetable pasta products to enhance the nutritional quality of yellow cassava pasta. The incorporation of leafy vegetable powder resulted in increases in protein (up to 3-fold) in fortified yellow cassava pasta, increased the fibre (11%) ash, beta-carotene, iron and zinc. The phenolic content of fluted pumpkin leaf-fortified pasta with 10% leaf powder inclusion (YPU10) was 1100 µg GAE/g, almost four times higher than that of the unfortified yellow cassava pasta. Leaf powders in the cassava pasta also favoured the retention of micronutrients during cooking and slowed down starch digestibility. The retention during cooking was up to 91% in YPU10 for beta-carotene with no loss in iron, while the estimated glycemic index was reduced by 19% and 15% in YPU10 and YPA10, respectively. Data showed that the inclusion of vegetables reduced the glycemic index of the fortified yellow cassava pasta while zinc content and bioaccessibility were improved with leaf addition and cooking. Thus, adding leafy vegetable powder up to 10% into yellow cassava pasta is a promising approach to both valorise yellow provitamin A biofortified cassava and adding further nutritional benefits.

5.1. Introduction

Cassava (*Manihot esculenta* Crantz) is an important African staple crop. However, the conventional, white-fleshed variety is deficient in protein and several micronutrients, of which vitamin A, iron, and zinc are especially lacking in cassava-based diets. Thus, yellow-fleshed cassava varieties, biofortified with provitamin A carotenoids and low in cyanide, were developed in large-scale breeding programmes.[1] These varieties provide nutritional benefits over the white-fleshed cultivars.[2,3] Thus, since 2011, provitamin A biofortified cassava varieties are being promoted and have received appreciable consumer acceptance in sub-Saharan African (SSA) countries.[4] Yellow cassava shows excellent potential to alleviate vitamin A deficiency but is still poor in iron and zinc. Moreover, studies revealed post-processing losses of beta-carotene up to 70 % in yellow cassava products.[5,6]. In SSA, leafy vegetables are abundant, affordable and good sources of essential amino acids, fibre, vitamins and minerals, including iron and zinc.[7] Leafy vegetables are commonly prepared by boiling, stewing, frying and blanching, and eaten as a sauce or so-called soup with starchy staples such as cassava. Despite the health benefits, the current consumption of vegetables is insufficient to meet the daily requirements of people living on cassava-based diets.[7] Food-to-food fortification is thus an emerging approach, mostly implemented in the developing world, to complement other strategies in combating micronutrient deficiencies.[8,9] To date, this approach has not been used with biofortified yellow cassava. Based on compositional data, a food product combining leafy vegetables and yellow cassava into a convenient and popular food, such as pasta, seems a way to address the still prevailing nutritional deficiencies. Leafy vegetables can be dried and made into leaf powder, simplifying the possibilities for food-to-food fortification of staple foods with leafy vegetables.[10] Fluted pumpkin(*Telfairia occidentalis) and* Amaranth *(Amaranthus cruentus)*are the two most preferred leafy vegetables in Nigeria.[19] The superior nutritional profile and the blood-glucose-lowering effect of these two vegetables have been reported by various authors [47,48] thus their hypoglycemic activities could be utilised to produce functional food products.

This food design strategy meets the demand for functional foods with added health benefits which is increasing worldwide due to growing consumer awareness of their role in preventing chronic diseases.[11] Inclusion of dried leafy vegetables could also promote the development of low-glycemic-index (below 50) foods resulting in a slower rise in blood glucose and insulin level.[12] In this respect, wheat-based products have been widely investigated in the last 50 years and research is still ongoing about the impact of the quality of gluten network on the glycemic index (GI). The research was boosted by the rising popularity of gluten-free products, not only for celiac people but for all consumers wishing to reduce gluten in their diet. This is an interesting opportunity for cassava-based products particularly gluten-free pasta whose market share is steadily growing.[13] Pasta products are increasingly used as a carrier of functional ingredients in food fortification to enhance nutritional quality, improve health and reduce the risk of diseases.[14]

The unique combination between increasing consumption of cassava pasta in countries where this crop is a staple food and the interest in gluten-free alternatives in Western countries prompted us to study the techno-functional characteristics of yellow cassava pasta fortified with leafy vegetable powders.[15,16] Yellow cassava, having no gluten network to entrap the starch granules, has a high GI.[17,18] However, we hypothesized that the insoluble dietary fibre of the leafy vegetables can also create a network to delay starch hydrolysis while the polyphenols in the vegetables can also reduce amylase activity. In principle, the ideal vegetable-fortified pasta product should have a low GI in combination with a high bioaccessibility of the micronutrients from the food matrix during digestion.

Consequently, this study aimed at 1) evaluating the effects of the addition of leaf powder (amaranth and fluted pumpkin leaves) on the retention and bioaccessibility of beta-carotene, iron and zinc in vegetable-fortified yellow cassava pasta, and 2) assessing the impact of the addition of leaf powder on the starch digestibility and in-vitro GI of the leafy vegetable-fortified yellow cassava pasta.

5.2. Materials & methods

5.2.1 Materials

Yellow cassava flour (TMS 07/0593) was supplied by the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria. The leafy vegetables, amaranth (*Amaranthus cruentus*) and fluted pumpkin (*Telfairia occidentalis*) leaves were cultivated at the Unifarm of Wageningen University and Research, The Netherlands. The leaves were washed, freeze-dried and milled (6875D Freezer/Mill®, SPEX SamplePrep, UK) after harvest. All the materials were packaged in amber bottles and stored at -20 ºC until they were required for analysis. The chemicals used in the analyses were of analytical grade.

5.2.2 Methods

5.2.2.1 Pasta preparation and processing

We prepared pasta samples from yellow cassava flour by incorporating amaranth and fluted pumpkin dry leaf powders at diverse levels [0 %, 5 % (5 g leaf powder into 95 g yellow cassava flour) and 10 % (10 g leaf powder into 90 g yellow cassava flour)], in the composition as described in Table 1. The yellow cassava flour and leaf powders were weighed and mixed manually to achieve homogenous mixtures. The dry formulation was then mixed with boiling water in the ratio of 1:1 to form a dough which was manually kneaded and allowed to rest for 20 min. Long pasta strands (spaghetti-like) were produced with a small-scale manual pasta extruder (CuisinU RVS Compact Pasta machine, Roelofsarendsveen, the Netherlands) and laid out on an aluminium foil. The pasta was then dried in an incubator at 60 °C for 5-6 h. The pasta samples were stored in plastic amber bottles at -20 °C until needed for analysis.

We prepared pasta samples from yellow cassava flour by incorporating amaranth and fluted pumpkin dry leaf powders at diverse levels [0 %, 5 % (5 g leaf powder into 95 g yellow cassava flour) and 10 % (10 g leaf powder into 90 g yellow cassava flour)], in the composition as described in Table 1. The yellow cassava flour and leaf powders were weighed and mixed manually to achieve homogenous mixtures. The dry formulation was then mixed with boiling water in the ratio of 1:1 to form a dough which was manually kneaded and allowed to rest for 20 min. Long pasta strands (spaghetti-like) were produced with a small-scale manual pasta extruder (CuisinU RVS Compact Pasta machine, Roelofsarendsveen, the Netherlands) and laid out on an aluminium foil. The pasta was then dried in an incubator at 60 °C for 5-6 h. The pasta samples were stored in plastic amber bottles at -20 °C until needed for analysis.

5.2.2.2 Determination of the chemical, mineral and physicochemical properties

The moisture content (MC) of the pasta samples was determined by oven-drying at 105 °C, and ash content was estimated by burning organic matter in a muffle furnace at 550 °C overnight according to the method 925.09.[21]The protein content (PC) was measured using the Dumas combustion method (EA 1112 NC, Thermo fisher scientific Inc., Waltman, USA) to estimate the nitrogen content with a protein conversion factor of 6.25. D-methionine (ACROS Organics) was used as standard.[21]The Soxhlet-petroleum ether extraction system extracted fat from 5 g of the sample according to an AOAC method 925.07. Total carbohydrate content was calculated by the subtraction method, i.e., the fraction retained after deduction of other proximate compositions on a dry weight basis. The total dietary fibre (TDF) was determined using the Official Method 991.43[22] while the total energy value was calculated per 100 grams of the sample using the Atwater conversion values. The sugar content was measured by HPLC based on AOAC 977.20 with minor modifications.[23]The Apparent amylose content (AAC) of the flour was determined following a modified method based on an iodine colourimetry method described by Man *et al.*[24]The iron and zinc contents were determined using inductively coupled plasma mass spectrometry ICP MS NEN-ISO 17053 according to AOAC 999.10.[25]

The colour of the pasta samples was measured using a Hunter Lab flex colourimeter (Elscolab, USA). The parameters were recorded as L*(lightness: L* = 0 black and L* = 100 white), a*(redness–greenness: -a* = greenness and +a* = redness) and b*(yellowness–blueness: -b* = blueness and +b* = yellowness) values. The colourimeter was standardised with a white plate supplied with the equipment, and three readings were done for each sample.

5.2.2.3. Determination of total phenolic, flavonoid and antioxidant activity

Extraction of phenolics and flavonoids was performed as described by Li *et al.*[26] with few modifications. 2.0 g of finely ground pasta samples were extracted with 80 % acidified (0.1 %) methanol by refluxing twice in a shaking water bath (SW23 JULABO GmbH, Germany) at 40 °C for 2 h and centrifuged at 2000 × g for 10 min in a centrifuge (Thermo Scientific Multifuge X3R Refrigerated Centrifuge, Marshall Scientific, USA). The methanolic extract was stored at 4 °C until needed for further analysis. The total phenolic content of the samples was determined by the Folin–Ciocalteau colourimetric method as described in AOAC 2017.13. The result was expressed as µg of gallic acid equivalent (GAE) per g of the sample. Total flavonoid was determined as Li *et al.*[26] described and results expressed as mg Rutin equivalent (RE) per g of sample. Antioxidant activity of pasta was assessed in terms of DPPH (2,2-Diphenyl-2-picrylhydrazyl) radical scavenging activity using the method of Plank *et al.*[27], with an adaptation of the Quencher method[28]. 0.1 mL of methanolic extracts were added to freshly prepared 3.9 mL of 0.2 mM DPPH solution followed by incubation of 30 min in the dark, and absorbance was noted at 515 nm. The result was expressed as the percentage inhibition of the DPPH radical. The percentage inhibition of the DPPH radical was calculated according to the following equation:

% Inhibition of DPPH = (Abs control − Abs sample/ Abs control) × 100   (1)

where: Abs control is the absorbance of the DPPH solution without the extract.

5.2.2.4. Beta carotene content determination

The extraction and instrumentation were carried out using Sadler, Davis & Dezman[29] method with slight modifications. Beta carotene was analysed using an HPLC system (Water Corporation, Milford, MA, USA) consisting of a guard column, C30 YMC Carotenoid column (4.6 × 150 mm, 3 μm) supplied by YMC Europe (GMBH, Dinslaken, Germany). A 2996 photodiode array detector (PDA) was used for beta-carotene quantification. Chromatograms were generated at 450 nm, and subsequent identification of cis and trans isomers of beta-carotene was made.

5.2.2.5. Percentage retention of beta carotene, iron and zinc

The beta-carotene, iron and zinc retention percentages in the yellow cassava pasta samples were calculated by the method as described by Lee *et al.*[30], using the following formula:

$$True\ Retention\ (\%) = \frac{(Nutrient\ content\ of\ cooked\ pasta)\ X\ g\ of\ pasta\ after\ cooking}{Nutrient\ content\ of\ raw\ pasta\ X\ g\ of\ pasta\ before\ cooking} \times 100$$

5.2.2.6. Bioaccessibility of beta carotene, iron and zinc determination

The harmonised INFOGEST in-vitro static digestion protocol, as described by Minekus *et al.*[31], was used in this study to estimate the bioaccessible fraction of the beta carotene and minerals. In this protocol, the *in-vitro* bioaccessibility was determined after samples had passed through a simulated gastrointestinal model, which included oral, gastric, and intestinal phases.[32] In the oral digestion stage, 3.5 mL of simulated salivary fluid (SSF) electrolyte stock solution was added to 5 g each of sample, followed by 25 μL of 0.3 M $CaCl_2$ and 1475 μL of Milli Q water. The mixture was shaken for 2 min at 37 °C. In the gastric digestion conditions, the above mixture (10 mL) was mixed with 7.5 mL of simulated gastric fluid (SGF) electrolyte stock solution, 1.6 mL porcine pepsin stock solution (25000 U mL−1), 5 μL of 0.3 M $CaCl_2$, then HCl (1 M) was added to lower the pH to 3.0 and water up to 20 mL. The reaction vessel was placed into a shaking water bath at 37 °C for 2 h. During the intestinal phase, the gastric mixture was mixed with 11 mL of simulated intestinal fluid (SIF) electrolyte stock solution, 5.0 mL of pancreatin solution (800 U mL−1), 2.5 mL of bile salt solution (160 mM) and 40 mL of $CaCl_2$ (0.3 M). NaOH (1 M) was then added to neutralise the mixture to pH 7.0 and Milli Q water up to 40 mL. Additionally, we also prepared samples containing 2 mL of sunflower oil to determine the impact of oil on beta-carotene, iron and zinc bioaccessibility. The bioaccessibility was calculated as the percentage of micronutrients present in the supernatant after in-vitro digestion and centrifugation (IVD), based on the concentration and volumes in the cooked samples (adapted from Oomen *et al.*[33]).

$$Bioaccessibility\ (\%) = \frac{Concentration\ in\ IVD\ supernatant}{Concentration\ in\ the\ cooked\ sample} \times 100 \qquad (3)$$

5.2.2.7. Determination of *in-vitro* starch digestibility

The in-vitro starch digestibility of the yellow cassava pasta products was measured using the method developed by Englyst *et al.*[34] Digestion of pasta samples was achieved by incubating with saturating levels of purified pancreatic alpha-amylase and amyloglucosidase at 37 °C for 4 h while stirring continuously. The solution's aliquots (1.0 mL) were removed while stirring at 20 min to measure the RDS and at 120 min to measure the SDS. Next, the aliquots were transferred to 50 mM acetic acid solution to stop the reaction. Finally, at 240 min, 4.0 mL of aliquot was removed, added to 4.0 mL of ethanol, and centrifuged. The pellets were washed with aqueous ethanol to remove free glucose and suspended in NaOH to dissolve RS and measure the TDS and RS. D-Glucose was measured separately with glucose oxidase/peroxidase (GOPOD) reagent. The total starch was determined separately using the Total starch assay procedure (Megazyme International, Ireland) as described by Englyst *et al.*[35] while the SDS and RS were determined with the Digestible & Resistant Starch Assay kit (K-DSTRS, Megazyme International, Ireland). RAG values were reported to help predict the glycemic responses of food.[36] The G20, G120, and TS values were used to calculate RDS, SDS, and RS amounts using the following equations (4-7):

Rapidly Digestible Starch (RDS) = (G20×F×0.9×100)/W (4)

Slowly Digestible Starch (SDS) = ((G120 - G20) ×F×0.9×100)/W (5)

Total Starch (TS) = (GTS×F×0.9×100)/W (6)

Resistant Starch (RS) = TS− (RDS + SDS) (7)

Where G_{TS} *= Absorbance value of total starch, F = 100/GOPOD absorbance, W= Sample weight (mg)*

5.2.2.7.1. Estimated glycemic index calculation

A non-linear model following the equation described by Goni *et al.*[37] with slight modification was applied to extrapolate the estimated GI: $C = C\infty\ (1 - e^{-kt})$ where C represented the percentage of starch hydrolysed at time t (min), C∞ is the maximum hydrolysis extent, and k is the kinetic constant.[38]The parameters, C and k, were estimated for each product based on the *in-vitro* starch digestion data. The hydrolysis index (HI) was then calculated by dividing the Area Under Curve (AUC) of each starch hydrolysis by the AUC of the reference food (white bread). The HI is expressed as a percentage representing the rate of starch digestion. The estimated GI indicated the digestibility of the starch with the digestibility of starch in the reference material white bread. The estimated glycemic index of yellow cassava pasta was calculated from Equation (8):

$$\text{Estimated GI} = 39.71 + 0.54HI. \qquad (8)$$

5.2.2.7. Statistical analysis

The experiments were performed in triplicates. First, a one-way analysis of variance (ANOVA) was used to analyse the data, followed by Duncan's multiple range test for mean comparisons using SPSS (version 25.0). Results were expressed as the mean ± standard deviation, and a p-value of < 0.05 was considered statistically significant.

5.3. Results and discussion

5.3.1. Proximate composition and physicochemical properties of yellow cassava pasta

5.3.1.1 Proximate composition

The proximate compositions were compared among the fortified and unfortified yellow cassava pasta and the dry leaf powders, as shown in Table 5.2. The moisture content of the pasta samples was < 12.0 g/100 g, in line with the regulatory standards for dried pasta.[39] Low moisture content is needed in pasta products because it limits microbial growth, off flavour and rancidity, as pasta is commonly supplied in the dry state to ensure storage stability and transportation.[40] Proximal composition of the amaranth and fluted pumpkin leaf powders confirmed the high content of, protein, ash, and total dietary fibre contents[41,42]. However, the protein values of amaranth and fluted pumpkin leaf in this study (31.5-34.7 g/100 g) were higher than those reported in previous literature, likely due to differences in the cultivar and planting conditions.[43] Data showed the addition of amaranth and fluted pumpkin leaf powders substantially enhanced the protein content of the developed pasta, and the fortified yellow cassava pasta had about double the protein content of unfortified yellow cassava pasta (Table 5.2). The fluted pumpkin leaf-fortified cassava pasta had a marginally higher protein level (2.9 - 3.0 g/100 g) versus the amaranth-fortified yellow cassava pasta (2.5 g/100 g - 2.7 g/100 g) due to the higher protein value of fluted pumpkin leaf. This higher level of protein in the yellow cassava pasta is particularly desirable. One of the weak nutritional features of cassava food products is the low level of protein, which is lower than in wheat-based pasta.

Besides proteins, this food-in-food strategy also improved the dietary fibre and mineral profile of the cassava pasta. The total dietary fibre of the yellow cassava pasta samples ranged between 9.0 - 10.0 g/100 g, mainly due to the yellow cassava flour's high fibre and resistant starch content rather than the high fibre in the leafy vegetables as the fortified pasta samples had marginally higher dietary fibre contents. The intake of dietary fibre rich products is desirable to meet nutritional recommendations as it helps in the proper control and management of diabetes and obesity. The structure provided by the dietary fibre network can control the kinetic of glucose release in the blood.[44] The ash content of fortified pasta ranged from 2.3 g/100 g - 5.1 g/100 g (dry weight), but not significantly different ($P > 0.05$) from the unfortified yellow pasta YP (1.6 g/100 g), a consequence of the high ash content of cassava.

The mineral content of the yellow cassava pasta was also enhanced by vegetable fortification (Table 5.2). A higher increase was found for iron (up to 72 % in YPU10) than zinc (10 % in YPA10). The mineral analysis results showed that fluted pumpkin leaf powder contained higher iron content but lower zinc than amaranth leaf powder. In comparison, the zinc content of amaranth was six-fold higher than found in fluted pumpkin leaf powder. This agrees with previous reports of amaranth vegetables being notably rich in zinc and fluted pumpkin leaf's superior iron content among other leafy vegetables.[45,46]

5.3.1.2 Colour attributes of yellow cassava pasta

The pasta's colour profile as presented in Table 5.3 showed that the addition of leaf powder significantly influenced the colour attributes of the fortified pasta. The quality of pasta could be estimated from its colour as it is a crucial factor impacting consumer preference for pasta and is known to change during processing.[49] Traditionally, semolina-based pasta exhibited light yellow colouration derived from beta carotene.[50] Thus, the yellow cassava pasta, YP, biofortified with beta carotene and bright yellow (Figure 5.1), could be a close gluten-free substitute to wheat pasta. The L* value, which is the most critical colour parameter related directly to the preferred brightness, decreased significantly among the pasta samples from 70.0 in YP to 52.0 in YPU10 ($p < 0.05$) due to the incorporation of the leaf powder. As expected, a pronounced greenness was observed in all the fortified pasta due to chlorophyll in the leaf powder. Colour losses were also observed after cooking the pasta due to the slight diffusion of the pigments into the cooking water, similar to the findings of Simonato *et al.*[51] with moringa leaf powder fortified wheat pasta. Furthermore, the b* value of the pasta samples increased with higher level fortification while those fortified with fluted pumpkin had significantly higher b* values than those fortified with amaranth leaf powder.

5.3.1.3 Starch profile of yellow cassava pasta

The starch profile analysis of yellow cassava pasta showed that the amylose content was significantly different among the samples ranging from 21.9 to 30.9, with yellow pasta fortified with 10 % fluted pumpkin leaves (YPU10) having the highest value (Table 5.2). The starch characteristics, specifically the amylose/amylopectin ratio, botanical source and the presence of other food components with their interactions during processing determine food starch digestibility.[52] Thus, starch types with a high amount of amylose are used as a source of resistant starch (RS) while high amylose in pasta samples is desirable. In addition, previous literature reported that starchy foods rich in amylose are associated with a drop in blood glucose levels and more gradual emptying of the human gastrointestinal tract versus those with low levels of amylose.[53] The amylose content of the yellow cassava pasta thus depend on the starch characteristics.

5.3.2. Total phenolics, flavonoids and DPPH of yellow cassava pasta

The phenolic contents of the yellow cassava pasta samples varied according to the presented food matrix and ranged from 226.6 to 1098.3 μg GAE/g (Figure 2), showing that leaf powder addition significantly ($p \leq 0.05$) increased the TPC of fortified yellow cassava pasta. Thus, the phenolic contents of fluted pumpkin leaf-fortified pasta YPU10 were almost four times higher than found in the unfortified yellow cassava pasta. Phenolic compounds have excellent antioxidant properties due to their ability to bind metal ions, reduce peroxides, and promote the potency of anti-oxidative enzymes.[54] They may also inhibit digestive enzymes (α-amylase and amyloglucosidase) through chemical interactions that lead to precipitation of the enzymes, thus limiting their activity on the digestion of carbohydrate foods. Moreover, starch-phenolics complexes have been reported to significantly slow down starch digestion.[55] Fluted pumpkin leaf and amaranth are particularly rich in flavonoids, the largest and most abundant group of secondary metabolites with marked antioxidant properties in leafy vegetables. In this study, fluted pumpkin leaves were found to contain higher phenolic content (9.31 μg $GAEg^{-1}$ *DW*) but fewer flavonoids (101.5 μg RE g^{-1} *DW*) than the amaranth leaves. This is similar to values reported by other authors for amaranth.[46] In addition, the highest flavonoid content was found in the fluted pumpkin-fortified yellow cassava pasta, YPU10 (80 % higher than in the unfortified sample). In comparison, the highest antioxidant activity as measured by the DPPH free radical scavenging activity was observed for the amaranth-fortified pasta, YPA10 (122 % higher than in unfortified yellow cassava pasta).

5.3.3. Retention and bioaccessibility of beta carotene, iron and zinc

The beta-carotene content of the vegetable-fortified yellow cassava pasta was six-fold higher (2.5-4.3 μg/g) than in the unfortified yellow cassava pasta (Figure 3). As previously reported, processing leads to losses in beta-carotene content of yellow cassava food products.[56,57] To mitigate these post-processing losses, the addition of leafy vegetables with high beta-carotene contents, amaranth (9.4 μg/g) and fluted pumpkin leaf (13.6 μg/g) was incorporated to make up for the lost carotenoids (Figure 2). The beta-carotene content of the cooked yellow cassava pasta was enhanced with increasing leaf powder fortification and retention was highest in YPU10 at 91 %. This is higher than beta-carotene retention values reported by Taleon *et al.*[57] for other processed yellow cassava products such as *fufu-fermented porridge* (21.6-35.7 %), *chickwangue-stiff dough* (1.5-5.6 %). As reported by Lawal *et al.*[6] *gari* and *pupuru* (traditional products of cassava in Nigeria), similarly had low beta-carotene retention (24.4 - 35.2 % and 34.7 - 39.9 %, respectively), while Eyinla *et al.*[5] reported an even lower β-carotene retention in yellow cassava chips (13.7 %), flour (11.7 %) and dough (5.5 %). Iron retention in the cooked vegetable-fortified yellow cassava pasta was 100 % in line with Chege *et al.*[58] who also reported high retention of iron (> 94 %) in their study. The zinc content of the fortified yellow cassava pasta YPU10 was substantially improved on cooking, as similarly reported by other authors, probably due to the leaching of the cooking pots.[59] On the other hand, iron bioaccessibility was found to be low in this study. Previous studies reported very low bioaccessibility of beta-carotene (<0.15 %) in leafy vegetables and

(0.6 % to 3.0 %) in orange-fleshed sweet potatoes.[60,61] In contrast, higher bioaccessibility of beta-carotene ranging from 8 % to 40 % was reported by Berni *et al.*[32] for orange-fleshed sweet potato. Icard-Vernière *et al.*[62] in their study on bioaccessibility of iron and zinc in leafy vegetables, however, reported up to 17 % bioaccessibility of iron in amaranth vegetable sauces while the study by Gautam *et al.*[63] showed up to 193 % bioaccessibility for zinc in cereal food products. Since the beneficial effects of bioactive compounds of food depend not only on their content and the amount consumed but also on their bioavailability/bioaccessibility, the nutritional content of food should be ultimately bioavailable/ bioaccessible.

5.3.4. In-vitro glycemic index of yellow cassava pasta

The rapidly available glucose content (RAG) varied significantly among the pasta samples from 21.9 - 27.6 with the highest in unfortified yellow cassava pasta (Table 4). Conversely, the slowly digestible starch (SDS) was found to be the lowest in the unfortified yellow pasta. The RS for the fortified cassava pasta samples was in the range of 1.78 - 2.45 % (Table 5.4), higher than Eyinla *et al.*[17] reported for traditional unfortified cassava products. RAG and RS content of starchy foods are significant determinants of their glycemic response.[34] Furthermore, several studies had reported the beneficial impact of RS in starch digestion and its consequent lower glycemic responses. The fractions of RDS varied among the yellow cassava pasta samples (40.9 - 47.11), with yellow cassava pasta fortified with the highest amount of fluted pumpkin (YPU10), having the lowest value. In this case, leaf addition is significant as the phenolic compounds in the leafy vegetables inhibit starch hydrolysis, slowing down starch digestion, thus lowering the glycemic response. The inclusion of fluted pumpkin leaves was also observed to have a higher impact than the amaranth leaves on the SDS in the pasta samples (107 % higher). Foster-Powell *et al.* [64] and Arvidsson-Lenner *et al.*[65] estimated the glycemic index of pasta products in the range between 40 and 78 depending on the processing method and plant material used. In addition, the glycemic index (GI) of a food is influenced by the relative presence of rapidly digested starch and slowly digested starch[66]. Foods with high SDS (42.8 - 47.1 %), such as the vegetable fortified cassava pasta, are ideal for diabetic patients. Its consumption could help manage diabetes due to its lower RDS and lower glycemic index (58.1 - 61.4) compared to unfortified yellow cassava pasta (71.7) or bread (100).

5.4 Conclusions

The fortification of yellow cassava pasta with amaranth and fluted pumpkin leaf powders fortified yellow cassava pasta enhanced the nutritional value concerning the unfortified yellow cassava pasta. Similar nutritious improvements of pasta in protein, dietary fibre, ash, minerals through the incorporation of leafy vegetables were reported by other authors.[51,67] The addition of amaranth and fluted pumpkin leaf powders delays the kinetic of glucose digestion as shown by the value of RDS, SDS, RS, TS and RAG. Consequently, it reduced the estimated glycemic index of yellow cassava pasta. In contrast, beta-carotene bioaccessibility was reduced by leaf powder addition to the fortified cassava pasta. Our results demonstrated that incorporating leaf powder in yellow cassava pasta is a feasible way to obtain pasta products with unique micronutrient profiles and improved glycemic response. Such functional food products are convenient and affordable and can help tackle deficiencies, especially in cassava consuming countries.

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Table 5.1 Composition of yellow cassava pasta.

Sample code	Composition
YP	100 % yellow cassava flour, 0 % leafy vegetables
YPA_5	95 % yellow cassava flour, 5 % amaranth vegetable
YPA_{10}	90 % yellow cassava flour, 10 % amaranth vegetable
YPU_5	95 % yellow cassava flour, 5 % *fluted pumpkin leaf* vegetable
YPU_{10}	90 % yellow cassava flour, 10 % *fluted pumpkin leaf* vegetable
YPA_5 O	95 % yellow cassava flour, 5 % amaranth vegetable

YP (Yellow cassava pasta), YPA_5 (Yellow cassava pasta 5 g of amaranth leaf powder/100 g of pasta), YPA_{10} (Yellow cassava pasta with 10 g of amaranth leaf powder/100 g of pasta), YPU_5(Yellow cassava pasta with 5 g of fluted pumpkin leaf powder/100 g of pasta), YPU_{10} (Yellow cassava pasta with 10 g of fluted pumpkin leaf powder /100 g of pasta), YPA_{5oil} (Yellow cassava pasta with 5 g amaranth leaf powder/100 g of pasta with the addition of one teaspoon sunflower oil during cooking)

Table 5.2 Chemical and mineral composition of yellow cassava pasta samples

Parameter	YP	YPA5	YPA10	YPU5	YPU10	YPA5oil	ALP	ULP
Moisture content	11.71 ± 0.70^{e}	11.91 ± 0.98^{d}	11.21 ± 0.08^{d}	11.02 ± 0.04^{d}	11.10 ± 2.32^{c}	11.61 ± 0.32^{b}	11.97 ± 0.09^{ab}	15.67 ± 0.11^{a}
Protein content	0.99 ± 0.02^{e}	2.48 ± 0.21^{cd}	2.65 ± 0.06^{cd}	2.88 ± 0.03^{c}	2.95 ± 0.01^{c}	2.43 ± 0.15^{cd}	31.49 ± 1.44^{b}	34.70 ± 0.02^{a}
Fat content	0.20 ± 0.02^{b}	0.32 ± 0.06^{b}	0.21 ± 0.04^{b}	0.31 ± 0.06^{b}	0.54 ± 0.11^{b}	1.43 ± 0.11^{b}	2.53 ± 0.18^{ab}	6.33 ± 0.11^{a}
Ash content	1.55 ± 0.04^{b}	2.26 ± 0.00^{b}	4.70 ± 0.30^{b}	5.10 ± 0.01^{b}	4.10 ± 0.11^{b}	4.77 ± 0.13^{b}	15.41 ± 0.03^{a}	15.78 ± 0.16^{a}
Carbohydrate content	94.60	92.40	92.40	91.10	90.40	91.33	36.60	27.31
Energy value	384.10	382.30	373.00	369.00	369.00	377.00	73.10	82.08
Total dietary fibre	9.00 ± 0.03^{bc}	9.10 ± 0.02^{bc}	9.15 ± 0.11^{bc}	9.30 ± 0.04^{bc}	10.00 ± 0.13^{b}	9.10 ± 0.15^{bc}	11.20 ± 0.11^{ab}	12.15 ± 0.22^{a}
Iron content	25.00	32.00	35.00	34.00	43.00	33.00	97.00	110.50
Zinc content	9.10	9.80	10.00	8.90	7.30	8.50	49.00	6.70
Fructose	0.49 ± 0.01^{ab}	0.90 ± 0.12^{a}	1.03 ± 0.04^{a}	0.92 ± 0.05^{a}	1.18 ± 0.01^{a}	1.11 ± 0.02^{a}	Nd	0.11 ± 0.05^{b}
Glucose	0.54 ± 0.09^{a}	0.70 ± 0.03^{a}	0.55 ± 0.01^{a}	0.86 ± 0.05^{a}	0.96 ± 0.02^{a}	0.58 ± 0.07^{a}	Nd*	0.21 ± 0.01^{a}
Sucrose	nd	0.12 ± 0.01^{c}	nd	1.53 ± 0.18^{a}	1.13 ± 0.04^{ab}	0.11 ± 0.01^{c}	Nd*	0.09 ± 0.04^{c}
pH	6.15 ± 0.05^{a}	6.17 ± 0.01^{a}	6.42 ± 0.02^{a}	6.16 ± 0.10^{a}	6.14 ± 0.04^{a}	6.11 ± 0.01^{a}	nd	nd
AAC	21.90 ± 0.04^{e}	25.01 ± 0.05^{de}	27.91 ± 0.11^{c}	29.37 ± 0.04^{b}	30.85 ± 0.08^{a}	25.43 ± 0.09^{d}	nd	nd

The data expressed as mean values ± standard deviation was of three independent experiments on a dry weight basis (n = 3). Different superscript letters on a row indicate significant differences among samples ($p \leq 0.05$), nd- not detected, Nd - not determined*

Table 5.3 Colour of the yellow cassava pasta

	L*	a*	b*
YP	70.0 ± 0.7^{d}	1.2^{e} ± 0.1	15.1^{b} ± 0.3
YPA_5	79.7^{e} ± 0.4	-7.3^{b} ± 0.1	11.2^{a} ± 0.1
YPA_{10}	66.2^{c} ± 0.0	-7.9^{a} ± 0.2	16.6^{c} ± 0.2
YPU_5	58.5^{b} ± 0.1	-6.4^{d} ± 0.1	26.2^{d} ± 0.1
YPU_{10}	52.0^{a} ± 0.0	-6.7^{c} ± 0.0	27.1^{e} ± 0.1

Mean ± SD, n = 3, Columns with different superscripts are significantly different ($p<0.05$)
L scale: 0-50 (dark); 51-100 (light); a* scale: +ve value (red); -ve value (green); b* scale: +ve value (yellow); -ve value (blue)*
YP (Yellow cassava pasta), YPA_5 (Yellow cassava pasta with 5 g of amaranth leaf powder/100 g of pasta), YPA_{10} (Yellow cassava pasta with 10 g of amaranth leaf powder/100 g of pasta), YPU_5(Yellow cassava pasta with 5 g of fluted pumpkin leaf powder/100 g of pasta), YPU_{10} (Yellow cassava pasta with 10 g of fluted pumpkin leaf powder /100 g of pasta), YPA_{5oil} (Yellow cassava pasta with 5 g amaranth leaf powder/100 g of pasta with the addition of one teaspoon sunflower oil during cooking)

Table 5.4 Estimated glycemic index and starch digestibility of yellow cassava pasta (% dw)

	RAG	RDS	SDS	RS	Estimated GI
YP	$27.60{\pm}1.16^{f}$	$42.23{\pm}0.17^{f}$	$40.90{\pm}0.15^{c}$	$1.12{\pm}0.03^{d}$	$71.72{\pm}1.12^{d}$
YPA_5	$25.48{\pm}0.35^{e}$	$38.98{\pm}1.02^{e}$	$42.78{\pm}1.08^{b}$	$1.78{\pm}0.01^{c}$	$61.39{\pm}0.07^{c}$
YPA_{10}	$22.80{\pm}0.25^{ab}$	$34.81{\pm}0.15^{b}$	$43.99{\pm}0.91^{ab}$	$2.05{\pm}0.08^{c}$	$61.11{\pm}0.14^{b}$
YPU_5	$24.11{\pm}0.23^{c}$	$36.88{\pm}0.18^{d}$	$45.06{\pm}0.75^{a}$	$2.31{\pm}0.11^{b}$	$59.68{\pm}0.33^{ab}$
YPU_{10}	$21.88{\pm}1.30^{a}$	$33.47{\pm}0.03^{a}$	$47.11{\pm}0.61^{a}$	$2.45{\pm}0.07^{a}$	$58.14{\pm}0.15^{a}$
YPA_{5oil}	$23.38{\pm}0.58^{d}$	$35.77{\pm}0.19^{c}$	$43.01{\pm}1.10^{b}$	$2.23{\pm}0.05^{c}$	$60.19{\pm}0.03^{cd}$

Mean ± SD, n = 3, Columns with different superscripts are significantly different ($p<0.05$)
RAG (Rapidly available glucose), RDS (Rapidly digestible starch), SDS (Slowly digestible starch), RS (Resistant starch)
YP (Yellow cassava pasta), YPA_5 (Yellow cassava pasta 5 g of amaranth leaf powder/100 g of pasta), YPA_{10} (Yellow cassava pasta with 10 g of amaranth leaf powder/100 g of pasta), YPU_5(Yellow cassava pasta with 5 g of fluted pumpkin leaf powder/100 g of pasta), YPU_{10} (Yellow cassava pasta with 10 g of fluted pumpkin leaf powder /100 g of pasta)

Fig 5.1. Yellow cassava pasta samples

Above (samples after extrusion), below (samples after drying)L-R: YP (Yellow cassava pasta), YPA_5 (Yellow cassava pasta 5 g of amaranth leaf powder/100 g of pasta), YPA_{10} (Yellow cassava pasta with 10 g of amaranth leaf powder/100 g of pasta), YPU_5(Yellow cassava pasta with 5 g of fluted pumpkin leaf powder/100 g of pasta), YPU_{10} (Yellow cassava pasta with 10 g of fluted pumpkin leaf powder/100g of pasta)

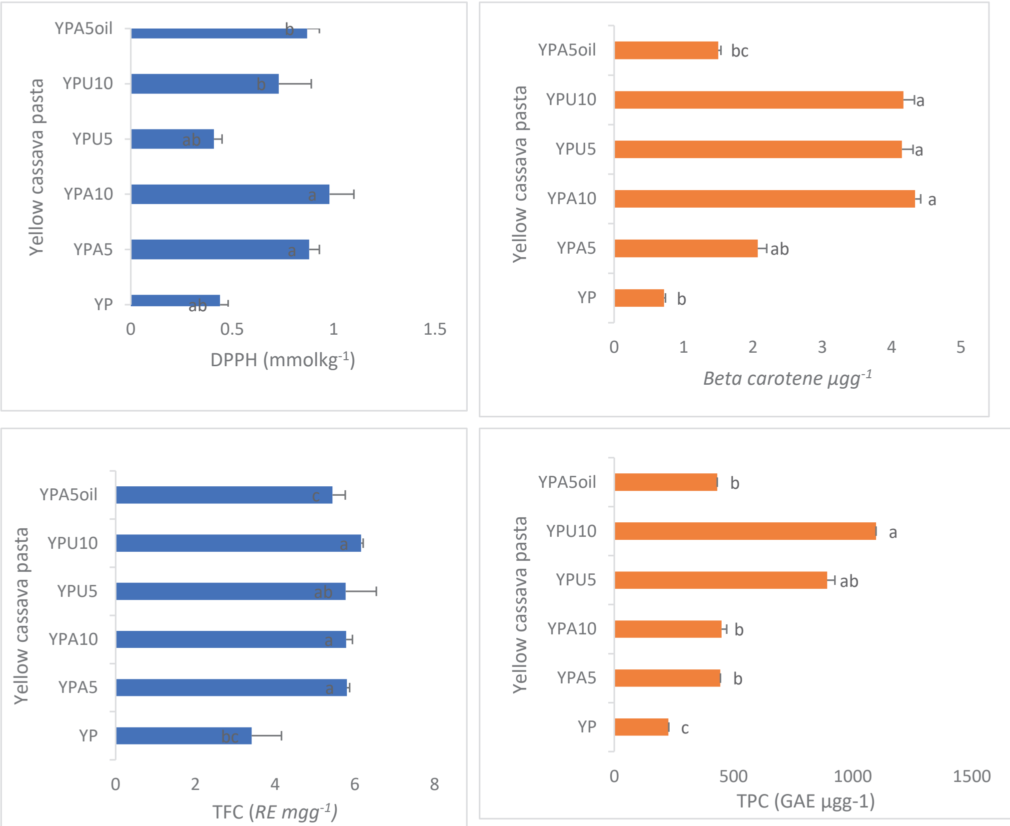

Fig 5.2. Total phenolic, flavonoid, antioxidant and beta-carotene contents of yellow cassava pasta

YP (Yellow cassava pasta), YPA_5 (Yellow cassava pasta 5 g of amaranth leaf powder/100 g of pasta), YPA_{10} (Yellow cassava pasta with 10 g of amaranth leaf powder/100 g of pasta), YPU_5(Yellow cassava pasta with 5 g of fluted pumpkin leaf powder/100 g of pasta), YPU_{10} (Yellow cassava pasta with 10 g of fluted pumpkin leaf powder /100 g of pasta), YPA_{5oil} (Yellow cassava pasta with 5 g amaranth leaf powder/100 g of pasta with oil added during cooking)

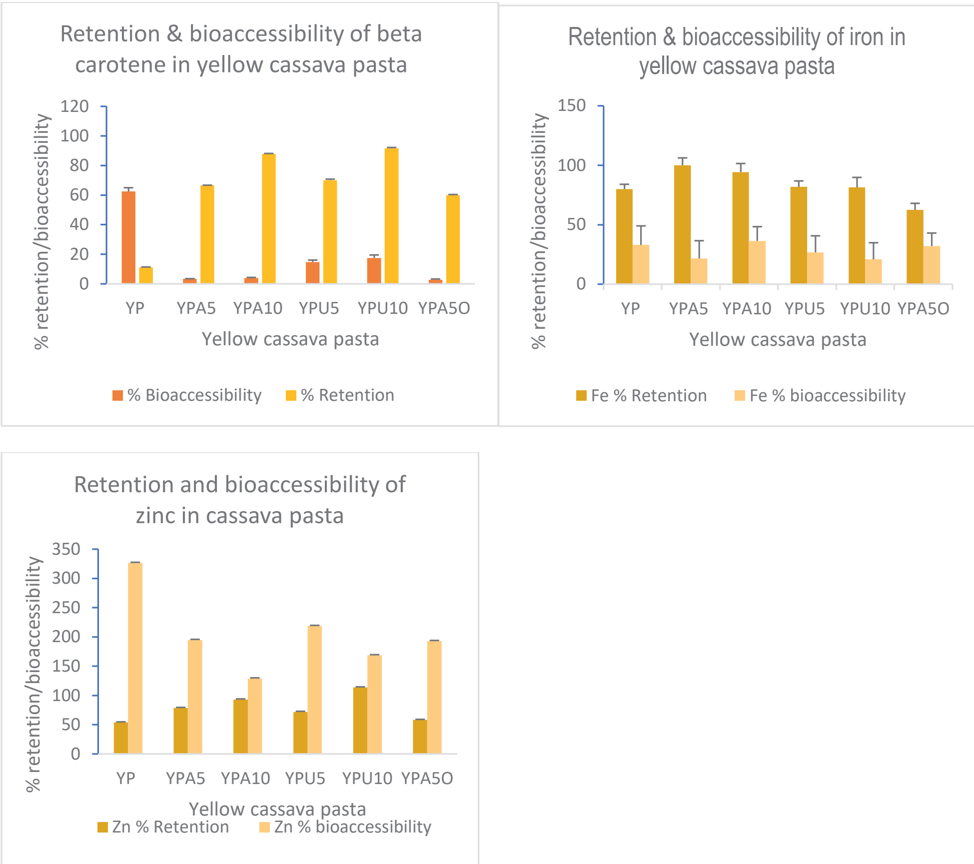

Fig 5.3. Retention and bioaccessibility of beta-carotene, iron and zinc in yellow cassava pasta

YP (Yellow cassava pasta), YPA_5 (Yellow cassava pasta 5 g of amaranth leaf powder/100 g of pasta), YPA_{10} (Yellow cassava pasta with 10 g of amaranth leaf powder/100 g of pasta), YPU_5(Yellow cassava pasta with 5 g of fluted pumpkin leaf powder/100 g of pasta), YPU_{10} (Yellow cassava pasta with 10 g of fluted pumpkin leaf powder /100 g of pasta), YPA_{5oil} (Yellow cassava pasta with 5 g of amaranth leaf powder/100g of pasta with a teaspoon of sunflower oil added).

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Chapter 5

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CHAPTER SIX

GENERAL DISCUSSION

6.1 Introduction

The modest gains of a global increase in agricultural productivity and food production are being undermined by the public health problem of micronutrient deficiencies (especially vitamin A, iron and zinc), also known as hidden hunger, which persists especially in low- and middle-income countries. Hidden hunger is stealthy and affects more than 2 billion people worldwide who may appear to be consuming an adequate amount of food (FAO, 2013). Yet, the calories of many staple crops disguise an invisible hunger that affects the health and wellbeing of, particularly, low-income earners. Thus, a shift from an increase in food production to the delivery of an adequate amount of nutritious food is necessary to ensure healthy living for all people (Bouis *et al*. 2011).

In this thesis, we evaluated the nutritional strategy of food-to-food fortification to valorise and enhance the value of yellow-fleshed provitamin A biofortified cassava and increase the consumption of leafy vegetables by utilising them for gluten-free vegetable fortified pasta production. We first established that there is a positive consumer acceptance of yellow cassava pasta products and a high consumer preference for amaranth and fluted pumpkin (*Telfairia occidentalis*) leafy vegetables among the consumers (**Chapter 2**). This finding corroborated earlier reports of amaranth being one of the most preferred leafy vegetables in Africa (Maseko *et al*., 2017; van Rensburg *et al*., 2004). Interestingly, consumer preference for fluted pumpkin vegetables was even higher than the well-reported amaranth vegetables (**Chapter 2**).

We then carried out a comprehensive review of African leafy vegetables and identified 146 leafy vegetables being utilised for food in different parts of Africa (Supplementary materials Table S1). We assessed the nutritional profile of the vegetables and found that the consumer choices (amaranth and fluted pumpkin) were also among the most nutritious (Supplementary materials Tables S2-S4). Next, we demonstrated the feasibility of utilising high-quality yellow cassava flour as a gluten-free pasta ingredient, fortified with amaranth leafy vegetables and reported the enhancement of the nutritional composition of the pasta via the incorporation of the vegetables (**Chapter 3**). Furthermore, our use of yellow cassava flour from the newly developed biofortified cassava variety (TMS 05/0793), instead of wheat flour, presented a unique interaction in the food matrix due to the absence of a gluten network. The implications for the structural and techno-functional properties of the developed pasta were discussed extensively in **Chapter 4**. In addition, the approach of fortifying gluten-free yellow cassava pasta with African leafy vegetables enhanced the antioxidant profile of the pasta and as widely reported in the literature, the phenolic compounds present in leafy vegetables played an inhibitory role on starch digestibility (Rocchetti *et al.*, 2018; Giuberti *et al.,* 2020; Kan *et al.*, 2021), thus lowering the glycemic index of the pasta.

This nutritional approach of fortifying yellow cassava with leafy vegetables also presented a sustainable means of combating hidden hunger since the consumption of yellow cassava foods alone is not sufficient to address the challenge of hidden hunger, as yellow cassava is still low in protein, iron and zinc. More importantly, cassava cannot be consumed in its raw form without a form of processing due to the presence of cyanogenic glycosides. It also goes through fast post-harvest physiological deterioration if left unprocessed for more than 72 hours (Ukechukwu-Agua *et al*, 2015). However, several processing techniques result in the degradation of the nutrients. Particularly affected is the beta-carotene retention of yellow cassava products (Taleon *et al*., 2019; Bechoff *et al*., 2017). Thus, we analysed the nutritional value of the functional pasta to ascertain the retention and bioaccessibility of the micronutrients after processing. Our approach in **Chapter 5** was thus to assess the nutritional benefits of the functional pasta product and confirm the effect of fortification on its invitro-glycemic index and bioaccessibility of the micronutrients.

This current chapter evaluates the main findings of the research (Figure 6.1) and addresses some methodological issues, followed by a discussion of implications and recommendations for future research. Specifically, in this thesis, we aimed to:

- establish the overall consumer acceptability and sensorial properties of yellow cassava pasta,
- assess the composition and nutritional values of the functional pasta,
- evaluate the impact of leaf powder addition on the functional and cooking properties of the formulated pasta,
- determine how much of the micronutrients are bioaccessible; and
- analyse the glycemic index of the yellow cassava pasta.

Chapter 2

Consumer attitudes, perceptions, motives for consumption and perceived barriers to cassava pasta and leafy vegetables

- *Health considerations were the drivers of acceptance*
- *Telfairia occidentalis was the most preferred vegetable*
- *Yellow cassava pasta was acceptable to consumers*

Chapter 3

Impact of processing and addition of amaranth leaf powder on the physicochemical, functional, pasting, antioxidant and cooking properties of the white and yellow cassava pasta

- *Leaf powder addition enhanced the dietary fibre and protein contents of pasta*
- *Beta-carotene contents and antioxidant capacities were improved.*
- *Cooking time and gruel solid loss were reduced with the incorporation of leaf powder.*

Chapter 4

Assesment of the techno-functional properties of yellow cassava pasta fortified with fluted pumpkin

- *Fortification enhanced the water solubility, swelling power and oil absorption capacities*
- *Weight gain of pasta increased*
- *Hardness and pasting temperatures & viscosities reduced*

Chapter 5

Leafy vegetable-fortified yellow cassava pasta showed reduced glycemic index and increased zinc bioaccessibility

- *Inclusion of leafy vegetables reduced glycemic index of yellow cassava-vegetable pasta*
- *Zinc bioaccessibility was greatly improved with little effect on iron retention.*
- *Starch digestibility was slower in the fortified pasta*

Fig 6.1 Overview of this thesis and its main findings

6.2 Biofortification and food-to-food fortification: twin-solutions to hidden hunger

In view of the devastating effects of hidden hunger on human health and well-being, several strategies are being implemented worldwide to tackle the problem. Prevention of hidden hunger is combated in several regions of the world, particularly sub-Saharan Africa, through supplementation, fortification, and other food-based approaches including dietary diversification and biofortification (Bailey, 2015).

Supplementation involves the provision of nutrients (vitamins and minerals) in highly concentrated forms (capsules, tablets, syrups or injections) to correct a nutritional deficiency in the short term. Examples are the West African vitamin A supplementation program targeting children of 6-59 months and iron supplementation to pregnant and lactating women (Aguayo & Baker, 2005). Supplementation has the benefit of fast coverage of at-risk populations through the provision of direct, controlled and concentrated doses of the micronutrient to the target groups (Bouis *et al.*, 2011). However, this strategy is extremely difficult to sustain in Africa, particularly among rural dwellers and low-income earners, due to the lack of access to the supplements and lack of compliance (Ba *et al.*, 2019). Several other factors hinder the successful implementation such as the inability to support high coverage over long periods as financial, political, or other health priorities may change rapidly.

Fortification (commercially and at home) refers to the addition of nutrients to food to prevent or correct a demonstrated deficiency in the population. The World Health Organization guidelines on commercial fortification presented three major methods: mass, targeted and market-driven (Allen *et al.*, 2006). Fortification can be implemented by a) restoration of the nutrients lost during food processing to their natural level (e.g. B-vitamins that are lost during milling of cereals), b) increasing the level of a nutrient above that normally found in the food (e.g. iron addition to wheat flour or extra calcium to milk), and c) addition of nutrients that are not normally present in a food item (e.g. vitamin A into sugar and oil, iodine into salt- e.g. the 2002 Nigerian mandatory salt iodisation program). The success of this strategy depends on the usage of an effective food vehicle, such as a staple consumed regularly by the people. It also requires concerted efforts and political will on the part of the government to ensure adequate distribution to the most vulnerable populations with low income and access to commercially fortified food products. Instances of resistance due to mistaken beliefs on the part of the people impede the successful implementation of commercial fortification as well as poor compliance by producers and adulterations.

Home fortification of complementary or semi-solid foods with multiple micronutrient powder (MNPs) is another form of fortification at the community or household level and the point of use (De-Regil *et al.*, 2011). Since 2008, after the Copenhagen Consensus aimed at the delivery of micronutrients to vulnerable populations, home fortification has been implemented in several African countries with varying levels of success (Mugalavai, 2020). The barriers to successful implementation are technical issues such as the prohibitive cost of mixing equipment, inadequate standard of quality control (non-uniformity of mixing), and weak monitoring/distribution systems (Ohanenye *et al.*, 2021).

Thus, another form of fortification, the food-to-food approach, is increasingly utilised in several African countries due to its simplicity and ease of use (Method & Tulchinsky, 2015). Food-to-food fortification emerged as a complementary strategy to the conventional fortification strategy, thus addressing the highlighted shortcomings of commercial fortification (Chadare *et al.*, 2019).

Dietary diversification is a sustainable and long-term approach to alleviating hidden hunger (Gani *et al.*, 2018). Dietary diversification is achieved via numerous food system-based interventions aimed at increasing the supply, distribution and consumption of nutrient-dense foods. Diversifying the diet often entails a change in household food consumption patterns and regular intake of all food groups in sufficient quantity and variety to satisfy the nutritional needs (Nair *et al.*, 2016). It thus requires multi-disciplinary collaboration and adequate consumer knowledge of the nutritional value of foods to be successful (Gibson & Anderson, 2009).

As a result of the challenges encountered in making fortified food accessible and affordable to the low income and at-risk consumers, another nutritional strategy of enhancing the nutritional value of staples by breeding nutrients into the crops, via the sustainable process known as biofortification is being adopted in sub-Saharan Africa. Biofortification is relatively new (initiated in 1990 with the orange sweet potato in Guatemala) but is becoming successful and often regarded as a practical complement to other approaches in addressing hidden hunger (Birol *et al.*, 2014; Saltzman *et al.*, 2016). Biofortification is an effective means of reaching malnourished low-income people in Africa, Asia, and Latin America who have limited access to diverse diets, supplements, and commercially fortified foods. The micronutrients of interest are iron, zinc and vitamin A due to the high prevalence of their deficiencies in the affected areas.

Seven priority biofortified crops, namely orange maize, orange sweet potato, iron pearl millet, zinc rice, zinc wheat, iron cowpea and yellow cassava (Fig 6.2), are now released in the targeted countries. Farmers are cultivating the new varieties, and the consumers are eating the biofortified foods. These crops were specifically targeted based on several criteria such as dietary needs, the consumption by vulnerable populations and the potential to achieve increased levels of micronutrients through conventional breeding (Bouis *et al.*, 2011; De Moura *et al.*, 2015). Ultimately, the success of biofortification depends on the potential retention and absorption of the target micronutrients from the biofortified foods (Bechoff *et al.*, 2017).

Prominent among the biofortified crops of interest in sub-Saharan Africa is the pro-vitamin A carotenoid biofortified yellow-fleshed cassava, simply known as yellow cassava. In our study (**Chapter 3)**, we confirmed the nutritional advantages of yellow cassava over the white-fleshed conventional variety. With higher ash, dietary fibre and beta carotene content, linked to a lower fat content and minimal levels of cyanogenic glycosides, yellow cassava seems, nutritionally, to be a proper panacea to the challenges of hidden hunger. Yet, the retention of the carotenoids is still an issue when processing yellow cassava roots into some of the commonly consumed products, mainly due to the sensitive nature of carotenoids to light, heat and physical handling (Ayetigbo *et al.*, 2018). Furthermore, the adoption of yellow cassava in sub-Saharan Africa has not yet reached the projected levels (Kolapo & Kolapo, 2021) and several countries are yet to release biofortified crops thus there is a limited presence of biofortified foods in most local markets of sub-Saharan Africa (Oteh *et al.*, 2020).

***Fig 6.2** Seven priority crops targeted for biofortification through conventional breeding with the dates of 1st release (adapted from Meena et al., 2018*)

There are several reasons for the lack of widespread adoption of yellow cassava (estimated at 38.72 % by Ayinde & Adewumi, 2016) in sub-Saharan Africa. 1.) Lack of awareness of the nutritional benefit- pro-vitamin A. 2.) Ignorance of the low cyanide content of yellow cassava 3.) Misconceptions about the breeding technique adopted, as it was reported that the people were opposed to transgenic technology (Oparinde *et al*., 2017) 4.) Few studies exist in the public domain about the nutritional value of yellow cassava (Okwuonu *et al.*, 2021).

Furthermore, yellow cassava is plagued by the post-processing degradation of the beta carotene content (Eyinla *et al.,* 2019; Bechoff *et al*., 2018). Despite breeding efforts that have raised the beta-carotene content of yellow cassava to as high as 25 µg/g fresh weight, thereby even exceeding the original breeding target of 15 µg/g, the bioavailability of the provitamin A carotenoid is impeded by carotenoid losses (Taleon *et al*., 2019). Thus, its contribution to the recommended daily intake of vitamin A remains low at less than 50 % (Ceballos *et al*., 2017). In our study**, (Chapters 3 & 4**), we found the total carotenoid content of variety 07/0593 to be below 12 µg/g in the yellow cassava products, while other authors reported even lower values. However, the processing of yellow cassava is imperative as cassava cannot be consumed in its raw form due to the presence of cyanogenic glycosides. Also, post-harvest physiological deterioration of the roots sets in within 3 days after harvest. Yet, different traditional techniques of processing yielded varying effects on the beta carotene contents of the yellow cassava products. For instance, the beta carotene content of biofortified cassava was reduced by ~10 % to 50 % after boiling for ~30 min, and in the range of ~ 40 % to 60 % after frying or roasting (Berni *et al*., 2014; Lawal *et al*., 2015).

Similarly, Bechoff *et al*. (2017) reported that different drying techniques produced varying levels of retention in orange-fleshed sweet potato with boiling/steaming in water having the least degradation effects. Comparable results of carotenoid losses were obtained for other biofortified products such as maize. Ekpa *et al*. (2021), in their study on biofortified maize, reported 20-50 % losses in carotenoid content. This suggests that an increase in the intensity of heat application has a detrimental effect on carotenoid retention in biofortified food products, as processing methods that are harsher on the food matrix (i.e. drying, frying, roasting) result in higher losses of provitamin A carotenoids. Thus, several authors have suggested additional nutritional enhancement of the yellow cassava to ensure it meets the dietary needs of the consumers after processing (Talsma *et al*., 2016, Ayetigbo *et al*., 2018; Eyinla *et al*., 2019).

Processing similarly influences the retention of iron and zinc contents of yellow cassava. Maziya-Dixon *et al*. (2015) evaluated the effect of processing on the retention of iron and zinc in yellow cassava and reported losses due to boiling to be 3.6–20.6 % for iron, and 2.7–21.7 % for zinc. Another study on gari, a popular fermented and roasted cassava product resulted in a modest 22 % retention of iron and a 90 % loss of zinc. It was thus concluded that for the biofortified crops, the fried products present a greater level of degradation, while the boiled products presented better retention. Therefore, additional intervention and processing methods are to be introduced for biofortified staple food products to deliver as high a level of minerals and vitamins to meet the recommended daily requirements (Ayetigbo *et al*., 2018).

Also, the use of agronomic biofortification has been suggested as an additional approach to improve the iron and zinc contents of crops through fertilization of the soil as studies have shown the effectiveness in field studies (Zhang *et al.,* 2010; de Valença *et al.,* 2017). Currently, fertilization programmes in several sub-Saharan African countries utilise NPK fertilizers, but some soils do not respond to NPK due to micronutrient deficiencies of the soils. In such cases, a local adaptation of specific secondary micronutrients to the soil may be more effective (Vanlauwe *et al*., 2015; Voortman & Bindraban, 2015). Sadly, a major hindrance to the implementation of this strategy in sub-Saharan Africa is the lack of knowledge and access to the inputs by the farmers.

Thus, the fortification of biofortified foods with other accessible and highly nutritious foods is a twin strategy aimed at enhancing the nutritional status of the people, particularly the low-income earners. As reported in this thesis, the approach presents a unique and effective solution by combining two well-proven strategies to combat hidden hunger. By fortifying yellow cassava flour with readily accessible leafy vegetables (amaranth and fluted pumpkin), we demonstrated the efficacy of this approach. Fortification and biofortification efforts can effectively be used in improving nutrition in low and middle-income countries.

6.3. Expected contribution to recommended daily intake

One of the goals of food design is to enhance the nutritional benefits of the food to the consumers. In our study (**Chapter 5**), we compared the effect of the inclusion of leaf powders (amaranth and fluted pumpkin) into yellow cassava pasta with the inclusion of broccoli into wheat-based pasta (Silva *et al*., 2013) to achieve a reasonable contribution to the Estimated Average Requirements (EAR) for vitamin A, iron and zinc (Tables 6.2&6.3). In the calculations (Table 6.2), taking account of portion sizes (100 g, 150 g and 200 g for children, adolescents and adults, respectively), we included the percentage retention and bioaccessibility, which differ for each yellow cassava-vegetable pasta product while in (Table 6.3), we used a theoretical estimation based on literature. We also estimated an intake of 100 g of cooked pasta enriched with 10 % vegetable powder, results in the intake of 10 g of dry matter of "pure" vegetable, which corresponds to 100 g of vegetable, considering that water content is approximately 90 %. For the estimation of the vitamin A intake, beta carotene values were converted to retinol activity using the conservative theoretical bioconversion ratio of 12 µg:1 RAE (Institute of Medicine, 2001).

We concluded that the fortified products have a higher contribution to the nutrient intakes for vitamin A, iron and zinc than the unfortified ones (up to 45 % contribution to EAR of vitamin A for children, up to 63 % of the iron EAR for adolescents and as high as 78 % for zinc). Also, the yellow cassava pasta provided up to 119 % EAR of zinc for children and adolescents. This is a marked improvement as the conventional white cassava provided only 5–8 % and 13–14 % EAR for iron and zinc respectively for 1-6-year-old children and non-lactating, non-pregnant West African women (Taylor *et al*., 2016).

Other authors reported comparable contributions to the recommended daily allowance for vitamin A. Ekesa *et al*. (2012), reported that boiled plantain fortified with fresh beans, amaranth leaves and olive oil would meet 44.3 % and 63.3 % of the vitamin A EAR of a child of 1–5 years and a woman of reproductive age, respectively, while consumption of boiled plantain, with or without olive oil, would meet 28.8–36.7 % and 41.1–52.4 % of the vitamin A EAR, respectively. Our findings on the bioaccessible nutrients and the contribution to the EAR for vitamin A, iron and zinc is key to the further valorisation of yellow cassava. Furthermore, these results can help stimulate further studies as bioaccessibility studies on yellow cassava products are still very few. The supply of sufficient metabolizable micronutrients depends on the amount of food consumed, the quantity of micronutrients retained in the food after ingestion, the proportion of nutrient bioavailable and the proportion effectively converted in an active form (bioefficacy). In the case of yellow cassava-vegetable pasta, these factors were all examined to assess the benefits to the consumers.

As summarised in Table 1.2, a wide range of African leafy vegetables has been utilised as food fortificant to improve the iron, zinc, and vitamin A contents of staple foods. However, other sources of iron and zinc such as the pulp of baobab (*Adansonia digitata*) have also been used extensively (Chadare *et al*., 2008; Adejuyitan *et al*., 2012; Gabaza *et al*., 2019). Within the framework of our study (*in vivo* studies excluded), it can be concluded that yellow cassava pasta is a promising new food to complement other efforts aimed at eradicating the problem of hidden hunger, particularly in sub-Saharan Africa.

Table 6.2 Estimated contribution (%) of cassava-vegetable pasta to the EAR for vitamin A, iron and zinc

Pasta product	**Children (100 g)**	**Adolescents (150 g)**	**Women (200 g)**	**Men (200 g)**
	Vitamin A			
Yellow cassava	27.0	20.6	15.1	0.3
Yellow cassava + amaranth (10 %)	10.3	7.9	5.8	4.6
Yellow cassava + fluted pumpkin (10 %)	44.7	34.1	25.0	20.0
	Iron			
Yellow cassava	17.9	41.3	15.7	20.6
Yellow cassava + amaranth (10 %)	27.4	63.0	23.9	31.5
Yellow cassava + fluted pumpkin (10 %)	19.6	45.2	17.1	22.6
	Zinc			
Yellow cassava	118.7	118.7	40.6	31.6
Yellow cassava + amaranth (10 %)	51.6	51.6	17.7	13.7
Yellow cassava + fluted pumpkin (10 %)	49.1	49.1	16.8	13.0

Table 6.3 Theoretical (%) contribution of cassava-vegetable pasta versus broccoli-wheat pasta to the EAR for vitamin A, iron and zinc

Pasta product	Portion size (g)	Yellow cassava-amaranth fortified (10 %)	Yellow cassava-fluted pumpkin fortified (10 %)	Wheat-broccoli fortified (10 %)
		Vitamin A		
Children	100	10.3	44.7	36.0
Adolescents	150	7.9	34.1	27.0
Women	200	5.8	25.0	45.0
Men	200	5.4	20.0	35.0
		Iron		
Children	100	27.4	19.6	25.0
Adolescents	150	63.0	45.2	28.0
Women	200	23.9	17.1	30.0
Men	200	31.5	22.6	35.0
		Zinc		
Children	100	51.6	49.1	45.0
Adolescents	150	51.6	49.1	55.0
Women	200	17.7	16.8	40.0
Men	200	13.7	13.0	35.0

6.4. The role of leafy vegetable inclusion in the cassava-vegetable pasta matrix

A food-to-food fortification strategy, which involves the incorporation of leafy vegetables into the gluten-free yellow cassava pasta products, presents several interesting scenarios and challenges. Nutritionally, leafy vegetables contain considerable amounts of iron, zinc, and beta carotene but also substantial amounts of inhibitors, including tannins, phenolics, oxalates, and fibre (Khanam *et al.*, 2012). Also, dietary components such as organic acids (e.g., ascorbic and citric acid) and other organic compounds are known to enhance the release of iron and/or zinc from the food matrix through digestion and solubilisation in the gut lumen. Thus, iron and zinc become bioavailable for intestinal absorption and utilisation in the body. Ascorbic acid can also overcome some of the inhibitory effects of phytate and phenolic compounds on non-haem iron bioaccessibility (WHO and FAO, 2006), while citric acid has been shown to enhance zinc absorption (Gibson, 2006). The antagonistic, as well as the synergistic effects of interaction among various phenolic compounds, are thus well reported (Liu *et al.*, 2011; Amoako & Awika, 2016; Obeng *et al.*, 2020).

In our study (**Chapter 5**), the effect of the enhancers of mineral bioavailability is demonstrated with improved iron bioaccessibility (up to 36 %) and an increase in zinc bioaccessibility up to 3-fold when leaf powder was used to fortify yellow cassava pasta. However, beta carotene bioaccessibility was impaired. The type of vegetable fortificant plays a major role in bioaccessibility. Glover-Amengor *et al*. (2017), reported that the inclusion of *Moringa oleifera* in several dishes led to increased levels of protein, iron, zinc and beta carotene in the diets but did not improve iron bioaccessibility. This negative effect was attributed to high calcium content. Thus, the inclusion of leafy vegetables may require the incorporation of enhancers of vitamin A such as ascorbic acid to boost the bioaccessibility of beta carotene in the food matrix. Interestingly, pasta is conventionally (almost always) eaten with some form of sauce or stew as an accompaniment in sub-Saharan Africa. Such a combination as obtained in a meal could contribute to increased micronutrient intake and compensate for the less bioaccessible beta carotene. The food habit is thus expected to potentially bolster the nutritional benefits of the gluten-free cassava pasta to the consumers.

In addition, other rich sources of micronutrients such as yellow and orange vegetables (e.g. tomato, turnip, capsicum, carrots and pumpkins), yellow and orange non-citrus fruits (e.g., mangos, apricots, grapes and papayas), mushroom, seaweed and red palm oil, have been investigated for food-to-food fortification (van der Merwe *et al*., 2019; Adetola *et al*., 2021; Wang *et al*., 2021). Carini *et al*. (2014), added carrot juice to pasta while Gull *et al*. (2015), added carrot pomace powder to a pasta formula. Simonato *et al*. (2019), used 5–10 % olive pomace to fortify pasta while Sobota *et al*. (2020), investigated the use of beet and carrot powder to fortify pasta. Furthermore, red palm oil, which is widely used in sub-Saharan Africa for cooking, could serve as an added source of beta carotene in pasta (500-750 mg/ kg) (Wu *et al*., 2018; Zeba *et al.,* 2006). The fortification of wheat-based cookies (biscuits) with red palm oil was shown to significantly increase plasma retinol and beta carotene concentrations in school-aged children (Ranjan *et al*., 2019). It would thus be interesting to carry out further studies on the use of red palm oil, carrots, pumpkins and other micronutrient-rich fruits and vegetables in the fortification of pasta and ascertain their contribution to vitamin A intake.

6.5 Impact of phenolic compounds of leafy vegetables on starch digestibility of pasta

In recent years, several studies have shown that dietary polyphenols have an inhibitory impact on the digestive enzymes, and therefore retard starch digestion *in-vitro* (Amoako & Awika, 2016; Sun & Miao, 2020; Wang *et al*., 2022). In addition, the inhibitory role of the phenolic compounds in the leafy vegetables on the starch digestibility of the yellow cassava pasta was evidenced in our study. As similarly reported by Kan *et al.* (2020), the presence of phenolics resulted in a reduced glycemic index (up to 20 %) in vegetable-fortified yellow cassava pasta while the unfortified pasta remained high at 71 **(Chapter 5**). Ombra *et al*. (2022), also reported the lowering of the predicted glycemic index of wheat pasta with 3 % onion powder from 72 in the control to 54 in the fortified pasta.

The high dietary fibre content of the leafy vegetables also confers additional health benefits by enhancing the resistant starch content of food. **In Chapter 5**, the incorporation of leafy vegetables contributed to the increase in resistant starch (up to 50 %) in the yellow cassava pasta. Thus, the good glycemic profile of yellow cassava-vegetable pasta makes it an ideal food for diabetics and health-conscious consumers. The different leafy vegetables also exhibited varying impacts on the pasta products, as fluted pumpkin-fortified pasta had a slightly lower glycemic index. The difference in glycemic indices among leafy vegetables implies that the type and variety of leafy vegetable fortificant is an important factor in food-to-food fortification. Loene *et al*. (2018), reported the lowering effect of *Moringa oleifera* leaf powder on postprandial blood glucose response of diabetic subjects. It is thus necessary to evaluate the glycemic indices of several African leafy vegetables for their wider use in healthy food designs. More studies on the beneficial effect of leafy vegetables in Africa will generate more interest in their valorisation and thus enhance consumption.

6.6 Textural and rheological profile of cassava-vegetable pasta

The absence of gluten protein in the cassava pasta food matrix to form a continuous protein network posed a few challenges to producing the textural properties of the cassava-vegetable pasta. The gluten with embedded gelatinized starch in wheat-based pasta products usually provides structure and firmness to wheat pasta, which is absent in the cassava-vegetable matrix. We thus adopted the strategy of pre-gelatinisation of the flour dough at 90-100 °C to create a continuous visco-elastic base (**Chapter 4**). So, when the starch granules were heated, they absorbed water and became swollen, leading to the opening up of the starch granules and the leaching of the amylose. This increased the viscosity of the suspension and caused the formation of a continuous gel phase. In this continuous phase, amylopectin and swollen starch granules got embedded, resulting in a matrix responsible for the pasta structure. Pre-gelatinization provides a robust and rigid network for gluten-free pasta. This approach is also preferred by some commercial gluten-free pasta makers who employ the use of heat-treatment of the gluten-free flour and hot extrusion to obtain the texture of the pasta (Marti *et al*., 2013). Additionally, Waniska *et al*. (1999), reported preheating a mixture of cornflour to successfully extrude pasta. Garcia-Valle *et al*. (2020), reported that pre-gelatinization improved the textural properties of gluten-free pasta of amaranth and mango flour. However, a too high amount of pre-gelatinized starch may also exert high extrusion pressures resulting in high stickiness and gumminess of the pasta. Thus, an optimal level of gelatinization is required for the effective processing of gluten-free pasta. A gelation concentration of about 8 % was reported for wheat flour while for cassava flour it ranged between 12-16 % (**Chapter 4**), which did not deviate much from the 7 – 15 % range recommended for good quality pasta (Silva *et al.*, 2012).

Marti & Pagani (2013), suggested various other techniques, ingredients and non-conventional pasta-making processes to re-organize the macromolecular structure of starch to replicate a texture similar to that found in wheat products. Thus, the use of high protein substitutes for gluten, emulsifiers, hydrocolloids or gums (i.e. arabic gum, xanthan gum, locust bean gum, carboxymethylcellulose) are generally employed in gluten-free pasta formulations. Egg white powder, casein, lupin, and rice proteins have also been explored in gluten-free pasta to reduce cooking loss and to improve the textural properties (Larrosa *et al.*, 2016; Mariotti *et al.*, 2011). However, in our study, we excluded the use of additives in the pasta formulation to avoid alterations of the texture, aroma or flavour of the developed pasta products.

The textural profile of cassava-vegetable pasta was impacted by the incorporation of leafy vegetables as evidenced by the decreased hardness due to the reduced starch content, the decreased retrogradation during gel formation and the emulsifying effect of the protein and fibre of leaf powder. This is one of the distinguishing features of the cassava pasta as the unfortified pasta was slightly harder (**Chapters 3-5**). Achieving the *al dente* (i.e., pasta that is firm to the bite) characteristic of ideal pasta in the gluten-free offerings may be a daunting task unless measures are put in place to improve on the hardness such as introducing high dietary fibre ingredients. Thus, a thorough understanding of the textural properties of the ingredients is important to achieve an ideal gluten-free pasta.

Leaching of solid matter of the pasta during cooking, referred to as cooking loss, is another concern in vegetable-fortified pasta as it causes undesirable textural properties such as stickiness. As a result, most commercially available vegetable–fortified pasta usually contains only 2 – 3 % (w/w) of vegetable powder as a higher fraction of vegetables may affect the texture negatively and consequently make the pasta less attractive to consumers. Texture is one of the key factors determining consumer acceptance of pasta products.

Interestingly, yellow casava pasta is characterised by good cooking qualities as the cooking loss was consistently below 7 %. We attributed the low cooking loss to the formation of resistant starch during the drying and the cooling stage of the pasta, which subsequently inhibited the leaching of solids to the cooking water. Fiorda *et al.* (2013), postulated that a cooking loss of less than 8 % is ideal for good pasta. The spaghetti-type pasta (as developed in this study) with some added functional ingredients generally presents a greater cooking loss than the other pasta shapes. Cooking loss is an important parameter that affects pasta quality and consumer preference.

6.7 Consumer acceptance and perception of yellow cassava pasta

Health considerations are the leading drivers of gluten-free pasta consumption and biofortified food products. This implies that as more consumers in sub-Saharan Africa become better educated about the health implications of their diets, there will likely be a higher demand for healthy foods. Consumer preference and acceptance are crucial factors when introducing functional foods. Thus, an understanding of the attitudes, perceptions, motives for consumption and perceived barriers to consumption of food should precede market entry (Byrne, 2020). Interestingly, various studies conducted on yellow cassava confirmed a preference versus the conventional white variety (Lawal *et al.,* 2020; Oparinde *et al*., 2017; Talsma *et al*.,2017).

In **Chapter 2,** we also gained insights into consumer attitudes and preferences to appropriately recommend yellow cassava food products that will meet consumer needs and expectations through focus group studies, consumer surveys and sensory tests (Fig 6.3). Similarly, we reported *Telfairia occidentalis* (ugwu/ fluted pumpkin) and amaranth to be the most preferred leafy vegetables in our study area in Nigeria. Through consumer studies, we determined that the main driver of yellow cassava and leafy vegetable consumption is the health consideration. The consumers expressed the willingness to try the functional food, as the respondents gave a positive indication that they would welcome the yellow cassava pasta product.

Other authors reported appreciable consumer acceptance of biofortified food crops and that these were sometimes preferred to the conventional ones (Birol *et al*., 2015). In some cases, such as orange sweet potato in Uganda (Chowdhury *et al*., 2011), orange maize in Zambia and yellow cassava in Nigeria (Oparinde *et al*., 2014), consumers liked the sensory attributes of biofortified food products as much as, if not more than, food made with conventional varieties. An exception is the biofortified maize; consumers preferred the conventional white maize and associated the yellow maize with food aid and livestock feed (Meenakshi *et al*., 2012; Banerji *et al*., 2013).

According to Perez-Cueto (2019), the sensory perception of food is dependent on the three determinants of appearance (size, colour and shape), flavour (taste and smell), and texture (touch and sound). We hence examined the organoleptic properties of the developed yellow cassava pasta (**Chapters 2 & 4**) by conducting sensory analyses of the novel food among consumers from sub-Saharan Africa, especially Nigeria. However, more sensory research among local African consumers is required as the preferred sensory parameters may vary among consumers based in different parts of Africa.

The addition of vegetables to pasta however presented some sensorial challenges. In our study, a distinct preference for the unfortified yellow pasta versus the vegetable fortified ones was reported (**Chapter 3**). Jalgaonkar *et al*. (2018), similarly reported a decreased sensory acceptability when 5 %-8 % moringa leaves were added to pasta due to a perceived leafy and bitter taste while 3 % inclusion of the moringa leaf was more acceptable. Other authors reported that a higher (15-30 %) incorporation of vegetables resulted in reduced sensory qualities of pasta (Silva *et al*., 2013), but not necessarily an increase in nutritional value. It is thus imperative to optimise the pasta production process such that the final product retains the sensory and nutritional properties meeting consumers' expectations (Oliviero & Fogliano, 2016).

Another notable finding of this thesis is that the provision of nutrition information improved consumer acceptance of biofortified foods (Fig. 6.4), the provision of nutritional information increased the liking and overall acceptability of cassava pasta. Birol *et al*. (2015), confirmed that once consumers find out about the nutritional benefits of biofortified varieties, their liking for biofortified products increases, while their liking for conventional products decreases. The need for nutritional information to support consumer food choices is generally agreed on (Storcksdieck & Wills, 2012). Effective nutrition education is important, particularly when introducing new healthy foods with unfamiliar tastes since several consumers also choose their food based on hedonic considerations. Fig 6.4 depicted a higher likelihood for consumption of cassava products with information of the nutritional value presented than without it. Therefore, apart from offering a healthy choice, it is important to ensure that a novel food is also the consumer choice. From our study, the acceptance of fortified yellow pasta was lower than the unfortified yellow cassava pasta as the consumers were unfamiliar with the taste and appearance. It is thus imperative that nutrition education among the consumers precedes the introduction of the novel cassava to create awareness and a better understanding of the benefits of the food(**Chapter 3-5**).

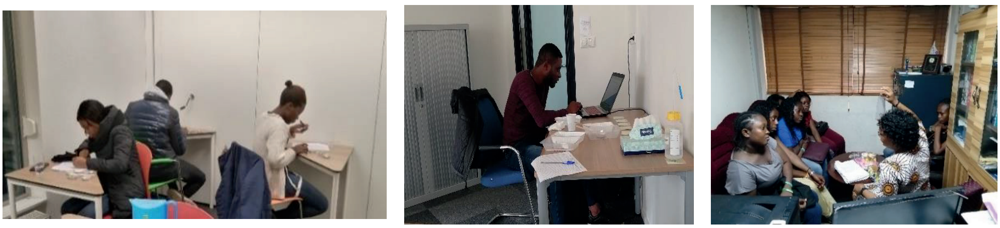

Fig 6.3 Consumer and sensory studies on yellow cassava pasta

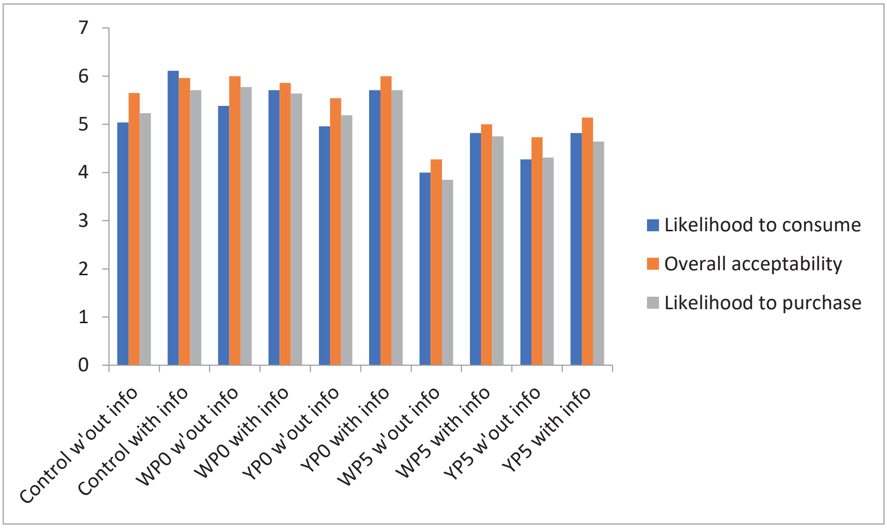

Fig 6.4 Impact of nutritional education on consumer acceptability of cassava pasta

Control: Commercial white cassava pasta, WP0: 100% white cassava pasta, YP0: 100% yellow cassava pasta, WP5: white cassava pasta + 5% ugwu powder inclusion, YP5: yellow cassava pasta + 5% ugwu powder inclusion, W'out info: Samples were evaluated without nutritional information, With info: Samples evaluated with nutritional information

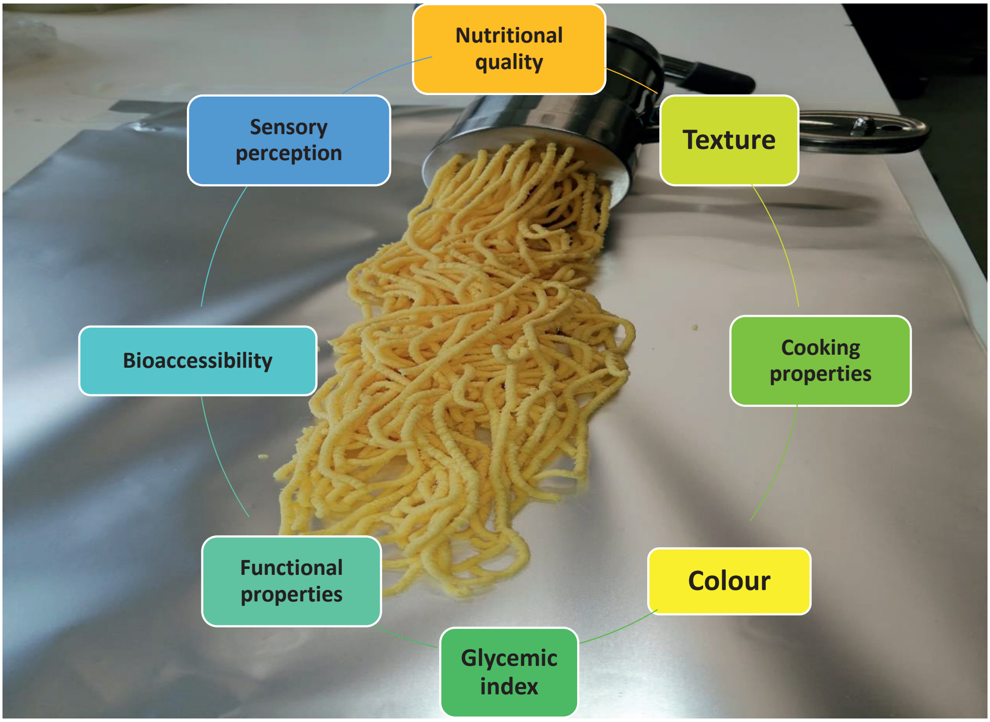

Fig.6.5 Overview of the important elements addressed in the thesis

6.8 Methodological considerations and limitations of the study

We adopted a multidisciplinary approach in this study to gain a better perspective of the novel yellow cassava pasta as a potentially healthy food choice. We started with consumer studies in Nigeria to gain insights into the food choices and habits using structured questionnaires, with both open and closed-ended questions. It would have been interesting to also conduct a consumer study in a canteen setting where the novel and commercial pasta are offered to the consumers to have a deeper understanding of consumer acceptance in a real-life situation. It is also necessary to point out that the studies were mostly conducted in Nigeria and generalisations were made based on the findings. Differences may exist for different regions, which require further studies on the local situations.

The bio-conversion factor used in this study to convert beta carotene values to retinol activity equivalents to estimate the vitamin A intake and contribution to % EAR is 12 μg:1 RAE. This bioconversion ratio follows the Institute of Medicine (IOM) estimates. However, the bioconversion factor varies widely depending on the food matrices. Other authors have suggested other conversion factors such as 21: 1, 24:1, 6:1, 4:1 and 3.2:1

(Howe *et al*., 2009; Muzhingi *et al*., 2011; Van Loo-Bouwman *et al*., 2012 & Palmer *et al*., 2016). Tang (2010), concluded that the vitamin A equivalency of beta carotene could range from 3.6–28:1 in a mixed diet. If we assume that vitamin A containing foods, such as the cassava-vegetable pasta in this study, are eaten with a form of fat and since vitamin A is fat-soluble, the conversion rate could be closer to 6:1. Consequently, a higher contribution than reported in this study seems likely.

As a result of delays in obtaining ethical approval from regulatory bodies in Africa and the current pandemic, it was not possible to use *in vivo* studies. Thus, *in-vitro* models were deployed to mimic the multidimensional physiological and chemical processes of the human body. To compare results obtained in different labs around the world, an *in-vitro* small intestinal digestion method was developed, called the harmonised INFOGEST method (Minekus *et al*., 2014). This method is claimed to reflect the in vivo digestion conditions better than other methods, as it includes all steps starting from the oral cavity to the small intestinal phase. However, the method only determines the release of the component from the matrix in which it is present because of gastrointestinal digestion but does not evaluate the absorption of the released nutrient. The true nutrient bioavailability is beyond the release of nutrients as it has a physiological or metabolic endpoint and is further influenced by host factors such as age, genotype, etc. Thus, when using an *in-vitro* digestion model, inter-individual differences are eliminated. This has the advantage that there is less variability between replicates and differences due to treatments being easier to interpret and normally fewer replicates are needed than in vivo.

The widely adopted *in-vitro* starch digestibility method formulated by Englyst *et al*. (1999), was used in this study to determine the estimated glycemic index of the developed pasta. The *in-vitro* methods provide an optimistic alternative to *in-vivo* methods for rapid screening and cost-effective methods for formulating digestible food ingredients (Germaine *et al*., 2008).

6.9 The gluten-free advantage- commercial cassava pasta catching on

The global pasta market reached a value of US$ 21.8 billion in 2020, mostly led by the big players such as Barilla, Nestlé, De Cecco and Makfa, producing mainly wheat-based dry pasta. However, gluten-free pasta is gradually picking up pace as the quest for healthier food choices increases. Kulshrestha (2021), reviewed about 200 gluten-free pasta products that are currently available in stores worldwide. It is thus projected that by 2025, the gluten-free pasta market would reach $1,289.2 million (Kulshrestha, 2021). The most common commercially-available gluten-free pasta raw materials are rice and corn but research is ongoing for several other plant-based ingredients: legumes, cereals and tubers such as orange-fleshed sweet potato and cassava. As more studies develop on the use of non-wheat, gluten-free materials for pasta, several other pasta products emerge.

Another emerging niche is the vegetable-pasta segment, as the inclusion of vegetables in pasta products is becoming well-accepted, mainly for health purposes (Fig 6.7-6.8). Vegetable pasta is an emerging functional product and its consumption may deliver health benefits by increasing vegetable intake (Wang *et al*., 2021). Initially, the commercial vegetable pasta products were mostly made with durum wheat and tended to contain rather low concentrations of dried vegetables or vegetable pulp (which often contains about 90 % water). However, in recent times, several vegetable pasta products from non-wheat ingredients are commercially available in Europe and the US (Fig 6.9).

In the last decade, several food companies (Nestlé Foods, Barilla, Wholy Greens etc.) have started offering consumers vegetable-pasta with increased protein content, substitutes for gluten, increased indigestible carbohydrates and better nutrient values in response to the consumer quest for more healthy pasta products that incorporate vegetables with gluten-free flour.

Cassava pasta, though not an entirely new food concept, is now receiving more attention as it is gluten-free. It is known as *Mie lethek* (means "ugly" or "dirty" noodles) in Indonesia and has been part of the Javanese, Vietnamese and Chinese diets for several decades (Purwandari *et al*., 2014). However, studies that consider pasta or noodle products made with cassava are less common while none exist for yellow cassava and African leafy vegetables. Borneo and Aguirre (2008), reported leafy vegetable pasta production made with spinach and amaranth and confirmed that incorporation with 35% (w/w) split pea and fava bean powder had a significant effect on these types of matrices, resulting in higher cooking losses and a very firm and rubbery pasta.

We, therefore, project that in the next decade, commercial yellow cassava pasta will be contributing to the selection of healthy food choices available to consumers in sub-Saharan African countries. In the same vein, vegetable-cassava pasta products may not be too far behind.

6.10 Future outlook of yellow cassava gluten-free pasta

Gluten-free products such as yellow cassava pasta are fast gaining acceptance worldwide due to the growing number of sufferers of celiac disease as well as people who want to exclude gluten-based products from their diet for other health reasons. Cassava, which is gluten-free, may attract the interest of food processing companies that seek affordable ingredients to produce gluten-free products. This may initiate public-private investments into diverse new cassava products such as confectioneries, breakfast foods, beverages and drinks besides pasta. As an alternative to wheat flour, yellow cassava can also help to reduce the financial burden of wheat importation in the sub-Saharan region. However, to date, several gluten-free pastas prepared from a single ingredient still have an inferior sensory quality compared to semolina pasta. Efforts to improve gluten-free pasta may require the application of additives, improver materials (e.g., hydrocolloids and emulsifiers), crosslinking enzymes, or material pre-treatment such as starch pre-gelatinisation and fermented flour, which may increase the production costs of gluten-free pasta.

Overall, this study has paved the way for the effective valorisation of yellow cassava flour through new food product development to improve the nutrition security of consumers in sub-Saharan Africa.

Fig. 6.7 Commercially available white cassava pasta products

Fig 6.8 Vegetable noodles launched by Nestlé brand Maggi in October 2015

Fig 6.9 Some commercially available vegetable pastas

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Supplementary Materials

Table S1: Commonly consumed African leafy vegetables

S/N	Scientific Name	English Name	Local Names	Use in food	Part consumed	Origin	Reference
1	*Abelmoschus spp.*	Okra(o), Musk mallow	Ewe ila[Y], Delele-Mandande[SA]	Young leaves eaten in salads, cooked, stir-fried or steamed	Leaves, pods, seeds	*Ethiopia*	*Gemede et al. 2016*
2	*Abrus precatorius*	Rosary pea, coral-bead	*Ido zakari [G], Enfiambo[SA]*	Leaves chewed or boiled with food as a sweetener	Leaves	*Africa*	*Glew et al. 2010*
3	*Acalypha spp.*	Irishpetticoat, Jacobcoat	*Mhacha, Aworoso[Y],Jiwene[H]*	Young shoots eaten as cooked vegetables minus flowers	Leaves, shoots	*Central & East Africa*	*Grubben et al. 2004*
4	*Achyranthes aspera*	Prickly chaff flower	*Aboro[Y]*	Leaves cooked or mixed with other vegetables as sauce/stew	Leaves	*Tropics*	*Nudrat et al 2014*
5	*Acmella oleracea*	Electric Daisy, Ting flower	Jambu, Brede Mafane	Leaves steamed and used in salads, stews & made into tea	Leaves	*Central America*	*Uthpala et al 2020*
6	*Adansonia digitata*	African baobab leaf	Kukah[H],Mwambom[K], Ewe Ose[Y]	Dry leaves &seeds for sauces, condiments, spices, drinks	Leaves, seeds, fruit	*Madagascar*	*De Caluwe 2011,*
7	*Adenia lobata/gummifera*	Adenia, Monkey rope	Donwada[B], Ngole[K],Mulozi[Za]	Tender leaves are finely chopped and cooked as a sauce	Leaves	*Tropics*	*Agoreyo et al. 2012*
8	*Aerva lanata*	Mountain knot grass	Efun ale[Y]	Leaves are cooked as vegetable sauces	Leaves	*Tropics*	*Omoyeni et al. 2009*
9	*Albizia zygia*	West African albizia	Akoro, Pangban[G]Nongo[SA]Siri[I]	Leaves are cooked and added to soups and sauces	Leaves	*Tropical Africa*	*Okunrobo et al. 2017*
10	*Amaranthus caudatus/cruentus*	Amaranth, Green	Tete funtun[Y] ,Fotete[B,G]	Leaves steamed, blanched, cooked	Leaves & stems	*Africa*	*Olumakaye 2011. Ejoh et*
11	*Amaranthus hybridus/viridis*	Local Amaranth	Tete Abalaye[Y]Inine[I]	Cooked, steamed, blanched, made into stew and sauces	Leaves & stems	*Tropical Africa*	*Traore et al 2017*
12	*Ammannia baccifera*	Monarch red stem	Worukoho[B],Ngole	Young leaves cooked or mixed with other vegetables	Tender leaves	*Asia & Africa*	*Achigan-Dako et al 2010*
13	*Aneilema spp.*	Aneilema	Aretekedo[S], Efĩajija[Y]	Leaves are cooked as vegetables	Leaves	*Africa*	*Akpabio & Ikpe,2013*
14	*Annona senegalensis*	Wild custard apple	Bakoko[B], Yogoti, Maroro[Mz]	Fruits eaten raw,leaves and flowers cooked as stew/sauce	Flower, leaf, fruit	*Africa*	*Yisa et al 2011*
15	*Asystasia gangetica*	Tropical primrose	Lobiri[Y],Isihobo[SA]	Leaves, flowers and young shoots - cooked with legumes	Leaves	*China, Kenya, Uganda*	*Essack 2018*
16	*Balanites aegyptiaca*	Desert date leaf	Aduwa[H], Mchunju[K]	Leaves &flowers raw/cooked, fried or made into beverages	Leaf, seed, flower	*East Africa*	*Okia et al 2013*
17	*Basella alba/rubra*	Ceylon/Malabar spinach	Amunututu[Y], Atameme[I]	For a salad, steam, cooked or stir-fried, substituted for tea	Leaves & stem tips	*Tropical Asia*	*Ejoh et al 2019*
18	*Beilschmiedia manni*	Spicy cedar	Atiokwo[I], Tola[SL]	The bark is eaten as an appetizer/leaf pounded in water as a drink	Leaves, bark, flower	*Guinea, DRC, S. Leone*	*Sahore et al 2013*

19	*Biden pilosa*	Blackjack	Abere oloko[Y], Boboyo[B]	Leaves shoot dried or cooked fresh for vegetable stews	Leaves & soft shoot	*West & Southern Africa*	*Bartolome 2013*
20	*Blighia unijugata/ sapida*	Triangle top	Mwakamwatu[SA] Akoko-Isin[Y]	Cooked and eaten as a vegetable sauce	Leaves, tree bark	*Nigeria, South Africa*	*Offor et al 2015*
21	*Boerhavia diffusa*	Hogweed	Erimmirii[I], Tataya[B]	Cooked as a pot herb, sauces or added to soups or cereals.	Leaves, seeds	*Tropical Africa*	*Ujowundu et al 2008*
22	*Bombax costatum*	Silk-cotton tree	Bunkungo[S], Kattupa[S],Bumbum[GB]	Leaves & calyxes dried made into sauce, unripe fruit drink.	Leaf, calyxes, fruit	*Africa*	*Catarino et al 2019*
23	*Boscia senegalensis*	Aizen	Aizen[Mt], Hanza[H]	Cooked as a vegetable, used in soups & mixed with pap	Leaf, seed, shoots	*Algeria, Sudan*	*Edwige et al 2014*
24	*Brassica carinata/juncea*	Ethiopian kale	Field pumpkin, Yabesha gomen	Leaves, seeds, fruits & flowers used in potherb, stews.	Fruits, flowers, seed	*Ethiopia*	*Odongo et al 2017*
25	*Brillantaisa nitens/guianensis*	Fiddle leaf	Agbolu-uku[I], Bolobolo[GU]	Leaves cooked as vegetable soups.	Leaves, seed	*Central & West Africa*	*Chinedu et al 2019*
26	*Burkea africana*	Wild seringa	Beeribu[B] ,Mufhulu[SA]	Tender leaves cooked as a sauce.	Tender leaves	*Tropical Africa*	*Mbatchou et al 2011*
27	*Cadaba farinosa*	Herd's boy fruit	Kalkacha[K], Ndeybarga[S]	Leaves & twigs dried & milled for sauce, gruel or as a sweetener	Leaves, twigs	*Tropical Africa*	*Glew et al 2010*
28	*Calotropis procera*	Sodom apple leaf	Bom-bom[H], Bariba,Osha[Su]	Leaves are used in the production of cheese.	Leaves, fruit	*North Africa*	*Sulaibi et al 20202*
29	*Caralluma spp.*	Caralluma	Gubehi[H], Buri nanewi[S]	Leaves are eaten raw, has a slightly acidic flavour	Leaves	*Tropics*	*Ahmad et al 2014*
30	*Cardamine spp.*	Hairy bittercrest	Kisegeju[T],	Leaves are chopped, boiled and eaten as a sauce or mixed with pod	Leaves	*Africa*	*Grubben & Denton,2004*
31	*Cassia tora Linnaecus*	Cassia, Sicke pod	Voamahatsara[MA]	Tender leaves& stems eaten cooked for soups also unripe fruits	Leaves, seed, fruit	*Asia*	*Prabhu and Barrett 2009*
32	*Caylusea abyssinica*	Caylusea	Ukwipa[T], Jerenchi, Mwaka[E]	Leaves are cooked or mixed with other vegetables as sauces	Leaves	*N&E Africa*	*Ruffo et al 2002, Edilu 2015*
33	*Ceiba pentandra*	Kapok	Rimi[H],Mapou[B], Akpu-Ogwu[I]	Leaves & seeds cooked, roasted, pounded made into soups	Leaves, buds & fruit	*Africa & S. America*	*Gandji et al 2019*
34	*Celosia argentea*	Spinach	Sokoyokoto[Y],Avouvo[B] Soman[B]	Leaves blanched, cooked stir-fried into stew and sauces	Leaves, stem	*Tropical Africa*	*Adegbaju et al 2019*
35	*Centella asiatica*	Marsh pennywort	Icudwane[SA] Gotu Kola	Leaves are cooked as a vegetable and dried as tea or juice	Leaves	*Asia*	*James et al 2008 Hashim*
36	*Centrosema plumieri*	Butterfly pea leaf	Akidi[I], Ewa-ahun[Y]	Leaves cooked as sauces or stews.	Leaves	*Tropical America*	*Achigan-Dako et al 2010*
37	*Ceratotheca sesamoides/triloba*	False sesame	Kalkashi[H], Yoroxlan[S], Goubdou	Leaves dried, milled into powder, cooked to makes stew	Leaves, shoots	*Senegal, Zimbabwe*	*Kandonga et al 2019*
38	*Chassalia kolly*	Wild sunflower	Isepe Agbe[Y]	Leaves are cooked as vegetable sauce/stew	Leaves	*Tropics*	*Achidan-Dako, 2009*
39	*Chenopodium album*	Gallant soldier, Fat-hen	Lamb's quarters, Melde	Leaves and young shoots are eaten steamed or cooked	Leaves	*South America, Malawi*	*Sharma et al 2012*
40	*Chromolaena odorata*	Siam weed	Sekou toure[CI], Jabiinde[CAR]	Leaves occasionally used as an aromatic addition to soups	Leaves	*Tropical Africa*	*Nwiloh et al 2009*
41	*Cichorium spinosum*	Spiny Chicory	Lombo[T],Biberetugu[G],Rawaye[Y]	Leaves and shoots cooked as sauce or stews	Leaves & shoots	*Asia & North Africa*	*Petropolous et al 2018*

42	*Citrullus lunatus*	Watermelon leaves	Ewe Egusi[Y], *Makataan*[SA]	Tender leaves cooked as vegetables; fruits eaten raw	Leaves, seed, fruit	*South Africa*	*McGregor, 2012*
43	*Cleome gynandra*	Cat's whiskers	Ekuyako[Y] Tabadamashe[H]Kisii[K]	Made into 'miya' sauce[H]leaves/shoots boiled/ in stews	Leaves, shoots	East Africa	Igor *et al* 2013
44	*Clerodendrum volubile*	White butterfly	Marugbo[Y,] Obenetete	Leaves ground into slurry in addition with herbs and spices	Leaves	*West Africa*	*Ajao et al 2018*
45	*Cnidoscolus aconitifolius*	Tree spinach	Iyana Ipaja[Y], Chaya	Leaves cooked, eaten raw and made into stew and soups	Leaves	*C. America*	*Babalola & Alabi 2015*
46	*Coccinia abyssinica*	Anchote	Ancootee[E], Shushe[E], Ajjo[E]	Young leaves cooked for sauce	Tender leaves	*N & E. Africa*	*Ayalew, 2016*
47	*Cochlospermum tinctorium*	Buttercup tree	Ntiribara, Djandere	Tender leaves cooked as sauces	Tender leaves	*Tropical Africa*	*Achigan-Dako et al 2010*
48	*Colocasia esculenta*	Cocoyam leaves	Ewe koko[Y]	Leaves dried, powdered, kneaded into dough baked, in a stew	Leaves & roots	*Africa*	*Pawar et al 2018*
49	*Combretum micranthum*	Health tree	Kinkeliba[Ma],	Leaves are dried and brewed into a tea	Leaves	*West Africa*	*Welch et al 2018*
50	*Commelina nudiflora*	Creeping Dayflower	Obogu[I], Itopere[Y], Balasa[H],	Leaves are cooked and eaten as vegetable stew	Leaves	*Bangladesh*	*Ujowundu et al 2008*
51	*Corchorus olitorius L/tridens*	Jute mallow/Saluyot	Ewedu[Y]Ademe[T],Atigo[U] Ayoyo[B,G]	Leaves cooked, chopped, made into puree or sauce	Leaves	*Africa*	*Traore et al 2017*
52	*Convolvulus farmosus/arvensis*	Field bindweed	Muduhwi[C],	Leaves are dried to make tea and flavour liquer	Leaves	*Africa, Asia*	*Arora & Mholtra 2012*
53	*Crassocephalum rubens/biafre*	Yoruba Bologi	Gbolo[B],	Semi-succulent mucilaginous leaves used soups & sauces	Leaves	*West and Central Africa*	*Adjatin et al 2013*
54	*Crassocephalum crepidiodes*	Fire weed/thickhead	Ebolo[Y],Okinawa spinach	Leaves are eaten raw or blanched and made into stew, sauces	Leaves & stems	*West and Central Africa*	*Dairo & Adanlawo 2007*
55	*Crateva Adansonii*	Sacred barna	Amakarode[I], Taniya[Y]	Leaves cooked as stew or mixed with cereal	Leaves	*Tropical Africa*	*Agbankpe et al 2015*
56	*Crotalaria burkeana/brevidens*	Slender leaf, Rattle pod	Fore[SA], Mitoo[K],Marejea[E],Alaju[U]	Leaves boiled/fried as stew/soups or mixed with other vegetables	Leaves	*Ethiopia*	*Abukusta et al 2007*
57	*Cucumis metuliferus*	Spiked melon leaves	Gakachika[Z],Uhufafa[SA]	Leaves are cooked as spinach or mixed with maize meal	Leaves & fruits	*Sub-Saharan Africa*	*Aliero& Gumi 2012*
58	*Cucurbita pepo/maxima*	Pumpkin leaves	Elegede[Y] ,Kuwe[SL], Diboke [C]	Fruits, seeds milled & cooked. Leaves &stem dried, cooked	Leaf, seed, flower	*Mexico*	*Borrero et al 2013*
59	*Cyphostemma adenocaule*	Adenocaule	Mwengele [SA],Kabombo[U], Keta[E]	Leaves and fruits are eaten and cooked in stew or tubers	Leaves, fruits	*Tropical Africa*	*Bello et al 2019*
60	*Daniella oliveri*	African balsam	Agba[I],Eepo iya[Y], Zaman[M]	Young leaves are cooked and eaten in times of famine	Leaves, bark	*West and Central Africa*	*Atolani& Olatunji 2016*
61	*Detarium macrocarpum*	Sweet Detar	Igbo[Y], Ofor[I], Dank[S], Abu laila[H]	Cooked as vegetable and condiment in sauces	Seed, Fruit, leaves	*Togo, Senegal, Mali*	*Ene-Obong, 1992*
62	*Dicerocaryum senecioides*	Boot protector/Devilthor	Museto[ZI], Makanagwe[Z]	Leaves cooked as vegetable and potherb	Leaves	*Zimbabwe*	*Steyn et al 2001*
63	*Digera muricata/arvensis*	False amaranth	Kanjero	Leaves and young shoots cooked as a vegetable	Leaves	*Tropical Africa*	*Ghaffar et al 2019*

64	*Diplocyclos palmatus*	Lollypop climber	Native bryony	Leaves, shoots are cooked as a vegetable sauce	Leaves, shoot, fruit	*Tropics*	*Ghane et al, 2017*
65	*Eclipta prostata L*	False daisy	Ahokponu[B],	Leaves and shoots cooked as vegetables	Leaves & shoots	*Asia*	*Gani & Devi 2015*
66	*Ehretia cymosa*	Ehretia	Zomali[B],Labassa[G],Mpelu[T]	Leaves and fruits are eaten and made into sauces	Fruits and leaves	*Tropical Africa*	*Borokini & Omotayo*
67	*Emex australis*	Deil's thorn	Inkunzane[SA]	Leaves are cooked as a vegetable	Leaves	*Africa-Australia*	*Essack et al 2017*
68	*Emilia spp.*	Milne redhead	Odundun odo[Y],	Leaves cooked as sauce or stew	Leaves	*West Africa*	*Nwachukwu et al 2017*
69	*Entada africana*	Entada	Butiare, Samatino[S],Ogurobe[Y]	Tender leaves cooked in sauce and used as a condiment	Tender leaves	*Tropical Africa*	*Yusuf &Abdul 2019*
70	*Ficus elastica/vogaliana*	Fig leaves	Ogbu ike[I], Agbede[B,] Ewe Ipin[Y]	Young leaves are eaten as a vegetable and as a salad	Young leaves	*Asia*	*Umerah &Nnam 2019*
71	*Galinsoga parviflora*	Quickweed,	Kofume[U],Macdonaldi[K]	Young leaves and stem cooked as a vegetable	Young stem, leaves	*C.America*	*Patharaj &Khanan 2017*
72	*Girardinia diversifolia (Link)*	Nilgiri/Himalayan nettle	Ewe ina[Y]	The leaf and inflorescence are eaten as a vegetable	Leaves, seeds	*Kenya, Sudan, Ethiopia*	*Shrestha, 2020*
73	*Gnetum africanum/bucholzianum*	African salad/Gnetum	Ukazi, Afang/EruOkok[C] Fumba	Leaves eaten raw /finely chopped & added to soups /stews	Leaves	*Nigeria, Cameroon, DRC*	*Ndomou, et al., 2014*
74	*Grewia spp*	Raisin bush	Mkole[SA]	Young leaves or the shoots are normally used for soup	Leaves, fruit, shoot	*Africa Anywar et al 2017*	*Gandji et al 2019*
75	*Gssampelus mucanta*	Gssampelus	Obuako-enwe[I],	Leaves chopped and made into stew and sauces	Leaves	*Tropical Africa*	*Umerah &Nnam 2019*
76	*Gongronema latifolium*	Bush buck	Arokoeke[Y]Utazi[I] Gasub[S]	Leaves are eaten as salad and cooked to make sauces &stew	Leaves	*West Africa*	*Aba & Udechuckwu 2018*
77	*Gossypium hirsutum*	Cotton plant	Ewe Owu[Y], Pamba[SA]	Leaves and seeds	Leaves, seeds	*Tropics*	*Arowolo et al 2011*
78	*Guilleminea Kunth*	Mat weed	Guillemina	Leaves are chopped and cooked as a sauce	Leaves	*Tropics*	*Basu et al 2014*
79	*Haematostaphis barteri*	Blood plum	Jinin Kafiri[H]	Leaves are used for seasoning soup	Leaves, seed, pulp	*Tropics*	*Aremu et al 2017*
80	*Heinsia crinita*	Bush apple	Atama[I], Tonoposho, Fumbwa	Leaves cooked as a vegetable; fruits eaten raw	Leaves & fruits	*Tropical Africa*	*Onuminya et al 2018*
81	*Heteromorpha arborescens (S)*	Parsley tree	Wildepitersielie, Umbangandlala[K]	Leaves are eaten as vegetables, roots for children	Leaves, roots	*Tropical Africa*	*Abifarin et al 2021*
82	*Hibiscus sabdariffa*	Roselle/Hibiscus	Isapa[Y], Sure[H],Ngaingai[DRC]	Leaves cooked; calyxes made into beverages	Leaves & calyxes	*West Africa*	*Catarino et al 2019*
83	*Hoslunda opposita* Vahl	Butterberry	Kamyuye[U], Uyaweyawe[K] Oke Ota[I]	Leaves & calyxes with aromatic flavour cooked into a sauce	Leaves, fruit, calyxes	*Tropical Africa*	*Said 2017*
84	*Ipomea batata*	Sweet potato leaves	Ewe odukun[Y]	Leaves and roots cooked into porridges and soups	Leaves and roots	*Tropical America*	*Truong et al 2007*
85	*Jatropha tanjorensis. L*	Catholic Vegetable	Ewe lapalapa, Iyana Ipaja[Y]	Leaves cooked and made into sauces and stews	Leaves	*Sub-tropical America*	*Chigozie et al 2018*
86	*Justicia flava/tenella*	Water willow, Justicea	Geelgarnaalbos, Impela[SA]	Leaves made into soups or stews	Leaves	*Tropical Africa*	*Kitadi et al 2019*
87	*Kigelia africana*	*Sausage tree*	Koya [B],Nufuten[G]	Leaves used in making palm-nut soups	Leaves	*Tropics*	*Glew et al 2010*

88	*Lagenaria siceraria*	*Bottle gourd*	Mbika nkalu[CO]	Young shoots and tender leaves may be eaten as a vegetable	Leaves, young shoots	*Tropics*	*Latham 2014*
89	*Laportea peduncularis*	*River Nettle*	Dzaluma[SA],	Leaves & young shoots cooked and eaten as a vegetable	Leaves	*Tropics*	*Mahlangeni et al 2016*
90	*Lasianthera africana (P.Beav.)*	*Lasianthera*	Editanin[Y],Aluka, Nka nkan[I]	Leaves made into stews and sauces	Leaves	*West Africa*	*Udosen et al 1999*
91	*Launeat araxacifolia*	Dandelion, Wild lettuce	*Yanrin*[Y], Namijin dayii[H], Anoto[G]	Leaves are eaten fresh as salad /cooked in soups and sauces	Leaves	*Tropical Africa*	*Busari et al 2016*
92	*Launaea Cornuta*	Bitter lettuce	*Mutsunga,*	Leaves consumed raw or made into soups	Leaves	*Kenya*	*Orech et al. 2007*
93	*Leptadenia hastata*	Akamongot, Anvara	Yadiya[H], Obi-ogbome[I]	Eaten as cooked vegetables and soups, mixed with legumes	Leaves, shoot, flower	*Mali, Ethiopia, Uganda*	*Muhkta et al 2019*
94	*Lippia javanica*	Lemon bush,	Musukudu[SA], Matswane[SA]	Leaves cooked as a vegetable or dried as herbal tea	Leaves	*South Africa*	*Maroye 2017*
95	*Ludwigia spp.*	Yerba de jicotea	Toloman[B],	Leaves are cooked to make a sauce for maize & porridge	Leaves	*S.America*	*Folorunso & Adelalu 2015*
96	*Luffa cylindrica*	Sponge gourd leaf	Madodoki[K], Kannka-ayaba[Y]	Young fruit eaten raw, leaves cooked into sauce, seed fried	Fruits, leaves, seeds	*West Africa*	*Musibau et al 2013*
97	*Macrosphyra longistyla*	Long-Style Gardenia	Zigidigohoum[B], Wopetele[CI]	Young leaves are cooked and made into sauce	Leaves	*Tropics*	*Elufioye et al 2019*
98	*Maerua angolensis*	Africa bead-bean leaf	Mkuruka[K],	Leaves & fruit used in soups and as a food supplement	Leaves, fruits	*Tropical Africa*	*Cook et al 1998*
99	*Manihot esculenta*	Cassava leaves	Ewe Ege[Y], Hako Mbai[C],Saka saka	Leaves and roots dried, milled, boiled, roasted, stir-fried	Leaves & roots	*South America*	*Koubala et al 2015, Latif &*
100	*Melanthera scandens*	Vine	Ounje-ehoro[Y]	Laves are cooked as soup and potherb	Leaves	*Tropics*	*Omoyeni et al 2012*
101	*Momordica balsamina/foetida*	African cucumber	Nkaka, Nghotoncha[I]	Tender leaves and fruits made into sauces and soups	Leaves, fruits	*Tropical Africa*	*Thakur et al 2009*
102	*Moringa oleifera*	Drumstick	Ukwe-oyibo, Agunmoniye[Y]	Leaves dried, milled made into soups and sauces	Leaves, seeds, stem	*India*	*Razis et al 2014, Nobose*
103	*Murraya koenigii*	Curry leaves	Bizari, mchuzi, mvuje[SA]	Leaves (fresh &dried) is used as soup flavouring	Leaves, stem, roots	*India*	*Jain et al 2017, Njoku 201*
104	*Myrianthus arboreus*	Giant yellow mulberry	Ujuju[I]	Leaves made into soups and fruits eaten raw	Leaves, fruits	*Central Africa*	*Awodi et al 2017*
105	*Napoleona imperialis*	Napoleon's hat	Boribori[I], Mabungi[H],Nkpodu[I] Isi-efe[I]	Leaves are cooked as vegetables and fruits eaten raw	Leaves, fruits	*Tropics*	*Umerah et al 2019*
106	*Nymphaea lotus*	Water lily, Cow cabbage	Iyeye[Y], Bado[H], Ijikara	Eaten raw/cooked, dried & ground into powder as a thickening agent	Leaves	*Tropics*	*Wasagu et al 2015*
107	*Obetia radula/tenax*	Rock tree-nettle	Muvhazwi[SA]	Leaves are cooked and mixed with other vegetables as soup/sauce	Leaves	*Tropical Africa*	*Mokganya &T.2019*
108	*Occimum Gratissimum L.*	Basil african, clove basil	Effirin[Y],Doddoya[H], Aluluisi[I]	Leaves made into soups and sauces	Leaves	*Tropical Africa*	*Idris et al 2011*
109	*Oldenlandia corymbosa*	White diamond flower	Muamba ziwa[T],Nyangulunga[SA]	Leaves are cooked with other vegetables as a softener	Leaves	*Tropics Datta et al 2019*	*Ezeabara et al 2016*

110	*Opilia amantacea*	Opilia	Mkandekande[B],Gbanro[B]	Fruits eaten & the leaves cooked or mixed with other vegetables	Fruits, leaves	*Tropical Africa*	*Magid et al 2017*
111	*Oxygonum sinuatum*	Stars Talk	Untabane, Kindri[T]	Leaves are cooked as vegetable sauces	Leaves	*Africa*	*Essack et al 2017*
112	*Passiflora foetida*	Red passionflower	Gbatotwe	Leaves used as an ingredient in soups	Leaves	*Tropics*	*Melho et al 2018*
113	*Pennisetum purpureum*	Elephant grass	Tige de Sissongo[C],	Leaves are made into soups and tea	Leaves	*Africa*	*Ukpabi et al 2015*
114	*Pergularia daemia*	Trellis-vine	Mufungi[Z], Eriko[N],Leshwe[SA]	Leaves cooked as a vegetable	Leaves	*Tropics*	*Van Wyk 2011,*
115	*Physalis viscosa*	Sticky gooseberry	Uqadolo	Leaves are cooked into sauces	Leaves, fruits	*S. America*	*Essack et al 2017, Odhav*
116	*Piper guineense*	Black pepper leaves	Uziza[I], Ewe Iyere[Y],Cubeb	Leaves made into soups & the seed used as spice/condiments	Leaves & seeds	*West Africa*	*Chiwendu et al 2016*
117	*Pistia stratiotes*	Water lettuce	Nyamayingiya[T]	Leaves are parboiled and added to other vegetables	Leaves, seeds	*Tropics*	*Ruffo et al 2002*
118	*Portulaca oleracea*	Water leaf purslane	Ntioke[I],	Leaves used in salads, stir-fried or cooked as soups	Stem, leaf & flower	*Western Asia*	*Uddin et al 2013*
119	*Pouzolzia mixta*	Soap nettle/bush	Muthanzwa	Leaves are chopped&cooked as vegetables or mixed with coconut	Leaves	*Africa*	*Mokganya &T.2019*
120	*Psychotria spp.*	Psychotria	Aya-azu[I],aka nta [I] Katamutu [G]	Leaves cooked as vegetables	Leaves	*Tropical Africa*	*Otitoju et al 2014*
121	*Pterocarpus mildbraedii* Hamus	Black Oha, Ora	Oha/Ora, Mkula[SA] Urube	Tender leaves used as a cooked vegetable	Leaves & stems	*West Africa*	*Akpayung et al 1995*
122	*Pterocarpus santalinoides* DC	Red sandalwood	Nturukpa, Gbengbe[Y]	Leaves and stems consumed as cooked vegetables	Leaves	*Tropics Nwokorie et al*	*Ogbonna et al 2018*
123	*Senna alata/occidentalis/tora*	Senna, Coffeeweed	Upulutu[I],Tasba[C]	Leaves and seeds roasted, milled or cooked for soups	Leaves, seeds	*Tropical Africa*	*Oladeji et al 2020*
124	*Sesamum angustifolium/indicum*	Sesame leaf	Samsam[H]Wild simsim	Leaves chopped and cooked with other vegetables	Leaves, seeds	*Kenya, Uganda*	*Mbaebie et al 2010*
125	*Sida acuta*	Broom/grass, fireweed	Jakoto, Sowa[G]	Leaves made into tea substitute and cooked as a vegetable	Leaves	*Tropics*	*Raimi et al 2014*
126	*Solanecio biafrae*	Sierra Leone Bologi	Worowo[TY]Ota eke, Lambe[SL]	Leaves made into sauces and stews	Leaves	*Sierra Leone*	*Ajala 2009,*
127	*Solanum aethiopicum/macrocarpon*	African eggplant leaf	Gbagba,Nyanya, Igba[Y]Anyara	Steamed/ fried with other ingredients as a sauce.Bitter taste	Fruits & leaves	*Tropical Africa*	*Ojo 2015, Akubgwo2008*
128	*Solanum nigrum/scabrum*	African Nightshade	Odu[Y],Gautan Kaji[H] Jamajama	Leaves steamed or cooked in soups and sauces	Leaves	*Australia*	*Traore et al 2017*
129	*Sonchus asper/oleraceus*	Prickly Sow Thistle	Seralha branca	Young leaves& stem tops eaten raw in a salad or cooked as stew	Leaves, stems	*North Africa*	*Grubben, 2004*
130	*Stachytarpheta jamaicensis*	Blue porter weed	Kandikandilaan	Leaves are cooked alone /mixed with other vegetables.	Leaves	*Tropical America*	*Liew&Yong 2016*
131	*Struchium sparganophora(Linn.)*	Water bitter leaf	Ewuro odo[Y] Wata-bitas[SL]	Leaves added to soup or consumed as a vegetable	Leaves	*Tropical America*	*Oloyede et al 2014*
132	*Strychnos innocua*	Kaffir Orange leaf	Mgulungungulu[T],Kokiya[H]	Fruit eaten raw/cooked, leaves cooked& mixed with cereal	Fruit, leaves	*Tropical Africa*	*Isa et al 2014*
133	*Talinum triangulare* Wild	Water leaf	Shee[G],Gbure[Y],Gurai[H], Ngbolodi[I]	Mixed with other vegetables to make stews and sauces	Leaves, stem	*All over Africa*	*Okpalanma & Ojimeluk*

134	*Tamarindus indica L*	Tamarind leaf	Djabbé[C],EnkogeOmuhuwu[U] Some[M]	Young leaves eaten raw, added to a salad or cooked	Leaves, pod, flower	*West Africa*	*Van der Stege et al 2011*
135	*Taraxacum officinale*	Wild lettuce	Yanrin[Y]	Leaves, stems, flowers, and roots are cooked& eaten	Leaves	*Tropics*	*Dias et al 2014, Martinez*
136	*Telfairia occidentalis*	Fluted pumpkin	Ugu[I], Nyae[C], Agboroko[Y]	Leaves and stem, chopped and cooked into stew/soups	Leaves	*Southern Nigeria*	*Okonwu et al 2018*
137	*Uraria picta*	Prishnaparni, Dabra	Alupayida[Y],	Leaves are cooked as sauce or potherb	Leaves	*Tropics*	*Oyesiku et al 2013*
138	*Urtica massaica*	Stinging nettle	Mpupu[U], Rise[K]	Leaves cooked as vegetable esp. during a famine	Leaves	*North Africa*	*Mahlangeni et al 2016*
139	*Vaccinium parvifolium*	Red huckleberry	Ewa[I]	Leaves and fruits used to prepare soups and sauces	Leaves, berries	*North America*	*Umerah&Nnam 2019*
140	*Vernonia amygdalina*	Bitter leaf	Ewuro[Y], Onugbo[I], Shuwaka[H],	Washed to reduce bitterness, cooked to make stew &soup	Leaves	*Tropical Africa*	*Oboh&Masodje 2009*
141	*Vigna Unguiculata L. Walp.*	Cowpea leaf	Ewe Ewa[Y], Niebe[C]	Leaves and seeds cooked into sauce, stews /soups	Leaves & seeds	*West & Central Africa*	*Enyiukwu et al 2018*
142	*Vitex doniana*	Black plum leaf	Dinya[Y] Mfudu [SA], Ucha koro[I]	Leaves, twigs &fruit consumed as vegetable & tea substitute	Leaves, shoots, fruits	*Tropical Africa*	*Osum et al. 2013*
143	*Wahlenbergia undulata*	African blue bell	Ushwaga [SA]	Leaves are chopped and made into stew or sauces	Leaves	*South Africa*	*Odhav et al. 2007*
144	*Waltheria indica*	Sleepy morning	Meidebossie [SA], Delelemukula [Bo]	Leaves are made into sauces and potherbs or dried as tea	Leaves	*Africa*	*Zongo et al 2013, Afisu*
145	*Warburgia spp*	Wood pepper, Fever tree	Muwiya[U], Mkaa[K],Chibaha[Mz]	Fresh/ dried leaves are used in dishes to add aroma & peppery taste	Leaves, bark	*S. Africa*	*Maroyi 2014*
146	*Xanthosoma sagittifolia*	Cocoyam leaf	Kontomire[G], Eke koko[Y]	Leaves are consumed as vegetables	Leaf, fruit	*Africa*	*Kwenin et al 2011*

I(Igbo- S.E Nigeria), H (Hausa- Northern Nigeria), Y (Yoruba-S.W Nigeria), G(Ghana), S (Senegal), T(Tanzania), K (Kenya), SL (Sierra Leone), SA (South Africa), GU (Guinea), C(Cameroon), U(Uganda), B(Benin), CI (Cote D'Ivoire), Mz(Mozambique), Za(Zambia)CAR (Central African Republic), M(Mali), MA(Madagascar), SU(Sudan), CO(DRC), Mt (Mauritania)

Table S2: Nutrient composition of green leafy vegetables

S/N	Scientific Name	M.C (%)	Protein	Fat (%)	Ash (%)	Crude fibre	CHO (%)	Beta	Vit C (mg)	Ref.
1	*Abelmoschus esculentus*	81.5	2.8-8.6	0.19-0.6	0.86-1.5	3.2-17.6	41.3	38.5	0.27-9.5	[5], [96]
2	*Abrus precatorius*	10.5-11.0	5.8-8.0	4.4-6.5	7.0-9.0	2.0-6.0	65.5		0.6	[85]
3	*Achyranthes aspera*	53.3	26.0	0.9	15.7	14.34	46.3		1.5	[41]
4	*Acmella oleracea*	89.9	24.1	1.5	10.9	62.6	63.4			[14]
5	*Adansonia digitata*	76.7	3.5-13.1	0.5-5.0	2.8-11.6	7.2-15.0	9.2-65.0	5.0-18.0	47.0	[2] [11] [36]
6	*Adenia lobata/gummifera*	14.7	24.7	24.6	14.5	13.4	21.8			[69]
7	*Aerva lanata*	6.4	19.5-22.6	2.5-6.4	31.2	6.8	39.5			[24],[86]
8	*Albizia zygia/glaberrima*	75.9	25.3-43.9	2.3-4.5	7.6-13.5	1.4-21.5	15.3-24.6			[11] [12]
9	*Amaranthus*	85.0	9.4	0.4	3.1	2.1	6.0		46-106.8	[16] [35]
10	*Amaranthus hybridus/viridis*	84.3-84.5	17.9-25.2	4.7-7.6	13.8-16.3	8.6-16.4	25.7-52.2	24.3-38.6	52.2	[15] [35]
11	*Ammannia baccifera*	39.4	5.0	17.6	10.5	26.4	1.3			[17]
12	*Aneilema spp.*	84.9	17.1	29.7	5.9	1.3	46.0		0.7	[92]
13	*Annona senegalensis*	12.2	8.8	2.4	12.1	17.6	25.3			[18]
14	*Asystasia gangetica*	70.2-82.6	3.7-7.8	1.2-2.0	12.7	8.2-25.6	10.6			[25] [41]
15	*Balanites aegyptiaca*	16.3	9.6	0.4	9.1	3.8		6.9	6.9	[26], [36]
16	*Basella alba/rubra*	86.4	50.0	6.4	4.7	1.7	16.7	3.9	2.8	[12]
17	*Beilschmiedia manni*	16.0	5.9-7.0	0.5-2.0	3.9-5.7		75.8-92.1			[88]
18	*Biden pilosa*	86.5-88.0		0.6	1.8-2.8	2.9-3.9	3.7-8.4	18.0		[87]
19	*Blighia unijugata/ sapida*	60.45	24.1	3.8	6.0	12.6	41.3			[11] [39]
20	*Boerhavia diffusa*	78.9	2.3	1.6	1.0	2.4	10.6		4.4	[27] [28]
21	*Boscia senegalensis*	92.5	24.2	1.1	1.2		45.3			[93]
22	*B. costatum/buonopozense*	89.4	14.2	2.7	7.7	15.6	40.0			[11]
23	Brassica carinata/juncea	84.0	28.4	0.93		3.6	8.75		1.2-3.0	[29], [115]
24	*Brillantaisa nitens/guianensis*	88.4	3.5	0.01	2.4	5.2	0.5		1.9	[30]
25	*Burkea africana*	7.5	66.2	3.0	4.3	11.3	7.77			[31]
26	*Calotropis procera*	61.0	16.2	4.5	20.0	18.0	88.0			[33] [34]
27	*Caralluma spp.*	58.3-72.3	5.3	1.4-8.1	14.1	6.2-19.4			1.4	[89],[90]
28	*Cardamine spp*	92.9	4.0	0.3	1.8	1.7				[94]
29	*Cassia tora Linnaecus*	12.8	12.5-27.3	2.0-6.0	7.5	33.7	36.6		19.6-84.2	[97],
30	*Ceiba pentandra*	94.7	18.8	9.4	10.4	21.7	34.4			[11]
31	*Celosia argentea*	87.6	3.3-32.0	0.2-1.1	2.7-32.4	3.5-11.7	32.8	6.5	1.8-26.0	[1],[11], [99]
32	*Centella asiatica*	85.3	1.8-8.4	0.5	1.4	3.5-17.0	43.8	5.98	15.0	[13],[119]
33	*Centrosema plumieri*		23.2		9.1	8.8				[104]
34	*Ceratotheca*	85.0	2.0	2.1	2.3	2.1	8.3			[47]
35	*Chenopodium album*	92.6	5.0-26.4	0.8	2.9	1.9-16.7	8.3	22.4	3.1-10.0	[13], [47], [61]
36	*Chromolaena odorata*	59.5	16.2	0.3		26.6				[113]
37	*Citrullus lunatus*	81.3	3.5	0.4	1.7	3.8	13.1			[133]
38	*Cleome gynandra*	85.0	4.8	0.8	3.0	4.3		26.0	13.0-50.0	[115]
39	*Clerodendrum volubile*	86.0	14.0	1.8	9.6	13.5	52.6			[58]
40	*Cnidoscolus aconitifolius*	84.0	4.8	1.6	1.6	1.0				[20]

41	*Coccinia abyssinica*	74.9	3.3	0.2	2.2	2.6	16.9			[60]
42	*Cochlospermum tinctorium*	94.1	2.8	4.7	5.1					[132]
43	*Colocasia esculenta*	83.0	2.9	0.6	9.0	2.2	2.3	18.0		[62]
44	*Commelina nudiflora*	88.6	1.7	1.4	1.1	1.5	5.7			[28]
45	*Corchorus olitorius L/tridens*	80.2	7.3	0.2	2.4	3.4	6.5	27.0	153.6	[1][2] [16]
46	*Crassocephalum*	86.7	21.3-27.1	2.8-3.5	18.7-19.7	8.8	42.2			[134]
47	*Crassocephalum crepidiodes*	85.0	3.5		1.96	0.12	9.43	5.8-9.0		[2]
48	*Crotalaria burkeana/brevides*	78.2	1.8		0.3					[63]
49	*Cucumis metuliferus*	87.0	4	0.7	2.73	2.42	5.6			[47]
51	*Cucurbita pepo/maxima*	85.6	2.9	0.2	1.51				198	[21] [35]
52	*Daniella oliveri*	95.5	16.9	0.8	9.6	32.0	18.0			[39],[64]
53	*Detarium microcarpum*	89.4	26.5	15.2	3.5	11.1				[65]
54	*Digera muricata/arvensis*	13.9	8.8	5.0	18.1	41.0	13.3			[66]
56	*Ehretia cymosa*	88.7	16.5	0.2	12.6	5.2				[67]
57	*Emex australis*	89.0	5.0	0.6	2.6	1.6	2.7			[47]
58	*Emilia spp.*	83.2	3.1	0.9	2.8	2.8	7.0			[68]
59	*Ficus spp.*	68.2	18.7-51.7	5.2	6.2-9.4	1.8-28.9	14.4-30.9			[11][12]
60	*Galinsoga parviflora*	89.0	4	0.5	1.74	1.24	5.29			[47]
61	*Gnetum africanum/bucholzia*	83.8	3.2	1.9	0.4	4.0	6.7			[135]
62	*Grewia spp*	88.0	18.7	5.2	9.9	16.1	50.0			[11][12]
63	*Gssampelus mucanta*	80.2	0.5	0.6	1.1	1.2	16.4			[39]
64	*Gongronema latifolium*	9.5	26.0	4.6	8.5	12.4	39.0			[70]
65	*Gossypium hirsutum*	9.9	27.3	27.8	4.6	22.9	30.5			[71]
66	*Heinsia crinita*	10.0	26.4	7.2	5.3	6.8	43.2			[72]
67	*Heteromorpha arborescens*									[129]
68	*Hibiscus sabdariffa*	86.7	2.8	0.2	1.2	5.0	4.1			[2]
69	*Ipomea batata*	68.1	2.1	0.4	0.04	2.1	27.2		11.0-70.0	[39], [115]
70	*Jatropha tanjorensis. L*	80.3	24.6	4.52	8.1	9.7	58.8		502.2	[73]
71	*Justicia flava/tenella*	84.0	3	0.4	3.3	1.4	8.8			[47]
72	*Kigelia africana*	9.70	16.3	9.2	7.6	21.1	36.1			[74]
73	*Laportea peduncularis*		2.8 (0.3)	8.2 (0.7)	44.4 (0.6)	0.23 (0.1)	44.4			[75]
74	*Launeat araxacifolia*	90.6	4.3	2.2	1.3	1.3	2.8	5.4		[10], [58]
75	*Launaea Cornuta*		3.9	0.4		1.4	45.0		15.9	[128]
76	*Leptadenia hastata*	78.9	4.7	1.1	8.7	0.2	6.5			
77	*Luffa cylindrica*	5.8	35.8	33.9	6.1	4.6	13.7			[76]
78	*Maerua angolensis*	7.9	33.2		12.9	15.0	28.4			[42]
79	*Manihot esculenta*	9.9	7.0	1.4	2.9	2.0-4.0			311.0	[77], [115]
80	*Melanthera scandens*	7.4	25.1	9.4	14.2	6.6	37.4			[78]
81	*Momordica*	71.0	11.3	2.7	18.0	29.0	39.1		4.0	[37],[115]
82	*Moringa oleifera*	71.7	20.5	2.6	5.1	19.3	43.8	6.8		[8],[53]
83	*Myrianthus arboreus*	83.9	18.7	11.6	16.4					[38]
84	*Napoleona imperialis*	68.2	5.3	0.7	1.3	1.01	23.5	34.7		[39]
85	*Nymphaea lotus*	9.7	21.7	5.1	8.3	13.3		5.1		[22]
86	*Occimum Gratissimum L.*	81.4	3.3	8.5	13.7	9.5	65.0			[40]

87	*Oldenlandia corymbosa*	60.3	10.5	2.2	8.3	7.3	9.1			[41]
89	*Opilia amantacea*	9.2	14.8		21.1	16.1	34.9			[42]
90	*Oxygonum sinuatum*	89.3	3.0	1.4	11.9	28.6	2.9	0.2		[43], [47]
91	*Passiflora foetida*	1.9	25.9	2.9	28.7	9.6	40.6		0.03	[44]
92	*Pennisetum purpureum*	89.0	27.0	14.8	18.2	9.1	30.9			[45]
93	*Pergularia daemia*	17.9	9.7		13.8	36.1				[46]
94	*Physalis viscosa*	81.0	6.0	0.8	2.3	2.0	9.81			[47]
95	*Piper guineense*	6.1	15.2	1.9	12.0	21.0	43.9			[48]
96	*Pistia stratiotes*	4.5	7.0	2.2	35.2	17.5	38.2			[49]
97	*Portulaca oleracea*	93.0	3.0	0.3	1.9	1.21	2.7			[47]
98	*Psychotria spp.*		11.8	3.7	3.1	1.5		5.3		[20]
99	*Pterocarpus mildbraedii* H.	85.1	25.8	5.2	6.4	7.6	44.7			[50], [51]
100	*Pterocarpus santalinoides*	60.8	1.1	1.0	2.7	0.9	30.6	36.0		[39], [39]
101	*Senna alata/occidentalis/tora*	77.0	7.0	2.2	4.2	2.6	9.4			[47]
102	*Sida acuta*	81.2	18.0		9.7	6.24		0.8	30.2	[52]
103	*Senecio biafrae*	6.4	15.3	5.1	8.7	17.8	46.9	4.0		[53]
104	*Solanum macrocarp.*	89.5	2.2	0.5	0.9	3.0	4.1	27.0	240.0	[2] [16][35]
105	*Solanum nigrum/scabrum*	84.7	24.9	4.6	10.2	6.8	53.5		2.0	[54],[115]
106	*Sonchus asper/oleraceus*	89.9	13.3	7.8	18.8	18.3	42.0		25.0	[61],[115]
107	*Strychnos innocua WW*	60.2	4.0	20.0	0.5	Trace	75.5		18.0	[55]
108	*Talinum triangulare* Wild	91.2	1.9	0.1	3.0	0.9	2.9	2.3-5.0	260.0	[2], [35]
109	*Tamarindus indica L*	11.2	9.15	6.2	6.3	7.2	60.0			[56]
110	*Taraxacum officinale*	9.1	6.89	9.6	21.3	34.0	19.1			[57]
111	*Telfairia occidentalis*	80.8	6.1	0.6	1.7	1.7	10.5	1.2	358.0	[2], [20], [35]
112	*Uraria picta*	8.7	17.8	1.5	9.9	16.3	45.8			[58]
113	*Urtica massaica*	90.0	33.8	3.6	16.2	9.1	37.4			[59]
114	*Vaccinium parvifolium*	77.0	1.0	2.2	2.8	0.1	16.9			[39]
115	*Vernonia amygdalina*	82.8	4.4	0.6	1.6	5.1	5.5	2.8-14.1	13.4-198.0	[2],[115]
116	*Vigna Unguiculata L. Walp.*	82.4	4.7	0.6	1.8	5.8	10.5	9.9	50.0	[115]
117	*Vitex doniana*	77.0	8.7	2.9	1.7	2.8	7.6			[23] [59]
118	*Wahlenbergia undulata*	80.0	5.0	0.3	2.1	1.3	12.8			[47]
119	*Xanthosoma sagittifolia*		29.4	10.6	8.2	35.2				[121], [123]

Proximate compositions based on % dry weight basis

Table S3: Mineral composition of green leafy vegetables

S/N	Leafy Vegetable *(mg/100g DW)*	Fe	Zn	Ca	Mg	K	P	Cu	Mn	Na	References
1	*Abelmoschus esculentus*	12.0		55.0-142.0	232.5	167.5					[95]
2	*Abrus precatorius*	24.1-74.5	6.1	6820µg/g	21.1-25.7	150-246.9	775	0.1-0.6	28.8	94.1-107.0	[81]
3	*Adansonia digitata*	27.0-38.4		35.3-410.0	16.7-35.3	30.2	7.6-35.3	nd			[83],[115]
4	*Aerva lanata*	11.0	4.5-5.3	18.4-51.7	29.0-41.5	17.5-47.9	18.7-19.8	nd	1.0	12.7	[82], [86],
5	*Albizia zygia*			48.8	124.2	18.5	28.3			11.2	[107]
6	*Amaranthus caudatus/cruentus*	40.0-97.0	4.4-8.4	52.0-73.0	118.0-120.0	86.0-128.0	31.0-32.0		5.5-6.9	65.0-76.0	[109]
7	*Amaranthus hybridus/viridis*	13.6	3.8	44.2	231.2	54.2	34.9			7.4	
8	*Ammannia baccifera*	25.0	2.0	21.7	38.5	94.6	0.14	0.12	0.5	15.9	[17]
9	*Aneilema spp*	15.5	3.3	23.3		14.8	5.1	0.07	4.2	6.2	[92]
10	*Annona senegalensis*	18.0	18.0	13.5	2.4	4.7		0.3	0.1		[18]
11	*Asystasia gangetica*	0.6-3.7	0.1-1.1								[115]
12	*Basella alba/rubra*	3.7-6.3	2.4-6.9	41.4-58.0	25.1-70.0	28.9-146.0	24.0-36.8		11.8	11.2-52.0	[106],[107],[109]
13	*Beilschmiedia manni*	29.0		10.4	7.1	87.2				6.1	[88]
14	*Biden pilosa*	6.0-23.0	0.9-2.6	34.0-162.0	79-135.0		39.0				[87]
15	*Blighia unijugata/ sapida*	3.5	2.1	96.2	4.4	23.2	126.2	2.3	0.7	0.7	[39]
16	*Boerhavia diffusa*	6.1	5.2	219.6	28.2	61.9	685.6	1.0	0.1	1.4	[39]
17	*Boscia senegalensis*	8.3	2.6	0.04	0.01	0.4	0.1	0.4	0.9		[93]
18	*Brassica carinata/juncea*	0.5-3.5	0.9-1.3	27.0-31.0	13.0						[115]
19	*Brillantaisa nitens/guianensis*	1.4	1.4	40.0	41.0	16.2	216.0	0.2	2.6	0.3	[39]
20	*Cadaba farinosa*	33.5		122.0	1410	13200	1730	14.6	129.0	223.0	[81]
21	*Caralluma spp.*	22.3	3.9	37.0	6.0	13.0	1040	0.06	12.1		[89], [90]
22	*Cardamine spp.*	6.1	0.3	6.2	5.6	104.6		2.6	0.6	100.6	[94]
23	*Cassia tora Linnaecus*	4.5-16.5	1.9-5.7	1.9-3.8	108.0-151.0	0.9-1.2	0.4-0.8	0.3-2.5	1.9-3.1	0.07	[98}
24	*Ceiba pentandra*	3.6-8.0	5.3	261.5	15.2	46.2	154.1	0.1	0.6	2.1	[39]
25	*Celosia argentea*	15.3-56.0	2.0-8.0	27.8-178.1	39.6	3.9-69.0	28.0-38.0	3.8	1.7-3.6	35.3	[99], [100],[109]
26	*Centella asiatica*	18.5-74.3	11.3-19.4	11.5	240.7	3.7-5.8	30.7	2.6-6.4		1.1-2.5	
27	*Ceratotheca sesamoides/triloba*	2.9-14.6	0.5	0.3	5.5	0.3	0.1	ND	12.0	0.1	[115]
28	*Cnidoscolus aconitifolius*	8.6	1.8	17.6	12.0	85.1	80.6	0.1	0.8	4.0	
29	*Coccinia abyssinica*	5.5	2.2	119.5	79.7		34.6				[60]
30	*Colocasia esculenta*	7.0	nd	160.0	28.7	33.8				33.8	[106]
31	*Corchorus olitorius L/tridens*	16.0-53.0	3.7-6.9	116.0-238.0	17.5-57.2	36.0-167.0	28.0-128.2	0.7	2.3-8.2	2.3-21.0	[39],[106],[109]
32	*Daniella oliveri*	2.0	1.3	281.9	16.2	56.1	187.9	0.9	4.0	0.9	[39]
33	*Ehretia cymosa*	18.0	11.0	1300 mg	420 mg				2.3 mg		[67]
34	*Ficus spp.*	10.6	0.4	18.2-49.7	42.3-89.2	30.9-54.0	27.2-44.4	0.4	1.3	2.1-16.4	[39],[107]
35	*Gnetum africanum/bucholzia*	2.7		128.0							
36	*Gssampelus mucanta*	2.8	3.2	374.0	16.1	57.2	122.2	1.0	0.9	2.2	[39]
37	*Gongronema latifolium*	13.3	4.7	304.0	16.8-54.8	244.8-659.0	119.0	1.91	2.5	44.7	[70],[117]
38	*Hibiscus sabdariffa*	41.0-54.0	5.4	17.9-59.0	53.7-110.0	43.0-107.0	21.5-65.0		11.4	6.0-25.0	[2], [83], [107], [109]

39	*Ipomea batata*	*1.9*	*4.0*	*140.0*	*20.1*	*49.6*	*257.2*	*3.1*	*1.7*	*2.0*	*[39]*
40	*Manihot esculenta*			*22.2*	*87.9*	*13.5*	*3.7*			*60.0*	
41	*Piper guineense*	*9.6*	*5.9*	*29.7*	*513.0*	*582.0*	*132.0*	*1.7*	*1.98*	*35.9*	*[70]*
42	*Pterocarpus santalinoides* DC	*3.1*	*1.8*	*34.4-52.7*	*17.7-52.6*	*28.8-80.1*	*26.4-85.0*	*0.4*	*0.3*	*1.0-9.2*	*[39],[107]*
43	*Senecio biafrae*		*3.6*	*24.2*	*392.3*						
44	*Solanum aethiopicum/macrocarp*	*7.9-52.0*	*4.4*	*37.8-48.1*	*98.0-178.0*	*40.1-59.0*	*23.0-38.0*		*12.0*	*21.0-23.0*	*[2], [106], [109]*
45	*Solanum nigrum/scabrum*			*49.5*	*91.4*	*21.6*					*{116}*
46	*Struchium sparganophora(Linn.*	*18.8*	*6.0*	*227.8*	*26.7*	*25.9*				*17.9*	*{116}*
47	*Strychnos innocua*			*166.1*	*30.6*	*93.5*				*nd*	*[106]*
48	*Talinum triangulare* Wild	*32.0-39.0*	*8.1*	*48.0-193.0*	*100.0*	*143.0-155.0*	*22.0-26.0*			*45.0-89.0*	*[2],[106],[109]*
49	*Tamarindus indica L*			*21.6*	*10.5*						*[56]*
50	*Taraxacum officinale*			*2.0*	*0.6*						*[57]*
51	*Telfairia occidentalis*	*9.6-41.1*	*4.8-11.0*	*75.0*	*14.3*	*154.0*	*20.0*			*68.0*	*[2]*
52	*Uraria picta*	*2.7*	*0.02*	*60.0*	*3.5*		*75.0*		*0.1*		*[58]*
53	*Vaccinium parvifolium*	*5.3*	*0.8*	*43.0*	*38.4*	*71.2*	*339.1*	*1.6*	*2.3*	*1.1*	*[39]*
54	*Vernonia amygdalina*	*2.8*		*162.0*		*437.0*	*67.0*			*6.0*	*[2]*
55	*Vigna Unguiculata L. Walp.*	*45.8*		*335.7*	*615.8*	*2245.9*	*682.8*				*[83]*
56	*Waltheria indica*	*19.7*	*4.6*	*0.3*	*0.3*	*0.8*	*0.3*	*0.5*	*2.5*	*0.2*	*[118]*

References: Odukoya *et al* 2007[1], Aworh 2015[2]; Olajire & Azeez, 2011[3;] Oboh *et al*., 2005[4] ,USDA[5 ,] Sakpere *et al* 2015[6], Ikewuchi *et al* 2010[7] Nisha *et al* 2012[8];Gunathilake & Ranaweera 2016[9],Oloyede *et al* 2011[10],*Lykke & Padonou[11],Agiang et al 2016[12],Biodiversity for Nutrition (BFN) repository[13], Neves et al 2019[14],Traore et al 2017[15],Nyadanu & Lowor,2014[16], Poornima & Jeyam 2016[17], Yisa et al 2010[18],Orlando et al 2019[19], Otitoju, et al 2014[20], P van Jaarsveld, M Faber & I van Heerden et al 2013[21], Stephen, Adebisi, Chinedu & Samuel et al 2017[22], Nnamani, Oselebe & Agbatutu et al 2009[23], Goyal et al 2011 [24], Khalil et al 2016[25], Favier et al 1993[26], G R Juna Beegum, S Suhara Beevy, V.S. Sugunan et al 2014[27], Ujowundu et al 2008[28], Sanlier et al 2018[29], Chinedu,et al, 2019[30], Mbatchou et al 2011[31], Askira et al., 2016[32], Silva et al 2001[33], Abubakar et al. 2013[34], Olaiya & Adebisi et al. 2009[35], Wakawa et al. 2018[36], Hamad & Usman et al. 2014[37], Amata et al. 2010[38], Umerah et al 2019[39], Idris et al. 2011[40], Sudeshna et al. 2019[41], Pak et al. 2011[42], Anywar et al. 2017[43], Odewo et al. 2014[44], Okaraonye & Ikewuchi et al 2009[45], Chizzotti et al., 2005[46], Odhav et al 2007[47], Chinedu et al 2018[48], Wasagu et al 2013[49], Akpanyung et al 1995[50], Arthur et al 2020[51], Alagbe et al. 2020[52], Ajiboye et al. 2013[53], Akubugwo et al. 2007[54], Hassan et al. 2014[55], Ishaku, et al. 2016[56], Sa'id et al. 2019[57], Gbadamosi et al 2014[58], Adhikari et al 2015[59], Gemede et al 2015[60], Afolayan et al 2009[61], Azubuike et al., 2018[62], Sahou et al. 2014[63], Adamu et al 2019[64], Igbadul et al 2012[65], Khan et al. 2013[66], Pío-León et al. 2012[67], Morshed et al 2021[68], Obi-Abang et al. 2019[69], Adeyeye,& Olaleye et al. 2020[70], Okonkwo et al. 2016[71], Okezie et al. 2017[72], Bella et al. 2008[73], Oseni et al. 2018[74], Mahlangeni et al 2015[75], Ogunyemi et al 2020[76], Eleazu et al 2012[77], Omoyeni et al. 2011[78], Ademoyegun et al 2013[79], Gul et al 2013[80],Glew et al. 2009[81], Fagbohun et al 2010[82], Patricia et al 2014[83], Glew et al 2010[84],Paul et al 2013[85], Omoyeni & Adeyeye 2009[86],Bartolome et al, 2013[87],Sahore et al 2013[88], Ahmad et al 2014[89], Padwal et al 2016[90], Shrestha, 2020[91],Akpabio &Ikpe,2013[92], Edwige et al 2014[93], Basumatari & Narzary, 2017[94],Caluete et al 2015[95], Kamble and Dhage,2019[96],Muhammad et al 2018[97],Rathore &Kumar 2019[98],Onwordi & Wusu 2009[99],Ayodele & Olajide, 2011[100],Ejoh et al 2019[101], Adeyeye &Omolayo[102], Makkar & Becker 1996[103], Nworgu & Egbunike 2013[104],Kubmarawa et al 2009[105], Acho et al 2014[106], Agiang et al 2017[107],Bamidele et al 2017[108],Faboya ,1983[109],Mepba 2007[110], Mbaebie et al 2010[111],Chinyere & Obasi, 2011[112],Igboh et al 2009[113], Omokhua et al 2016[114], Uusiku et al. 2010[115], Ejoh et al 2007[116], Dike et al 2010[117],Basiru et al 2016[118], Afolayan & Jimoh ,2009[119], Kubmarawa et al.2008[120],* Ayalew *et al* 2017[121], Lumu& Katongole 2011[122], Athanase *et al* 2018[123], Adesina & Adeyeye 2013[124], Omoyeni *et al* 2015[125],Suarez *et al* 2020[126], Bamali *et al* 2014[127], Lyimo *et al* 2003[128], Abifarin *et al* 2021[129], Sami *et al* 2013[130], Moloto *et al* 2020[131], Affonfere *et al* 2021[132], McGregor 2012[133], Adjatin *et al* 2013[134], Ndomou *et al* 2014[135], Mnzava &Olsson 1990[136], Poonia& Upadhayay 2015[137], Ugadu *et al* 2019[138], Nagy *et al* 1978[139], Inyang, 2016 [140]

Table S4: Amino acid profile of selected African leafy vegetables

	Vegetables g/100g	Alanine	Arginine	Aspartic acid	Cystine	Glutamic acid	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine	References
1	*A. esculentus*	6.0	6.7	2.9	1.7	1.7	3.8	2.4	2.2-3.1	5.6	5.0	0.7	3.6-5.2	14.0	4.6	4.4-5.3	3.0	3.4	[123][130]
2	*A. precatorius*	4.3	4.1	10.2	2.2	12.4	3.0	2.2	3.0	5.7	3.9	0.6	3.7	5.3	5.8	4.7	2.2	4.3	[84]
3	*A. digitata*	14.7	14.8	24.9	nd	24.9	11.7	5.8	8.9	14.7	11.6	4.5	11.6	31.1	10.3	10.3	8.9	11.6	[100]
4	*Amaranthus*	3.4	3.9	5.4	0.5	3.7	3.6	2.2	3.4-4.7	6.7	3.0	nd	3.8-4.0	2.8	3.0	2.6	2.3	3.5	[70][102]
5	*B. aegyptiaca*	1.8	4.2	7.9	0.8	10.8	9.7	2.8	3.5	6.2	4.5	0.7	4.8	1.9	2.0	2.9	3.2	4.1	[120]
6	*Basella alba*	5.6	5.0	9.6	1.0	13.4	4.6	3.1	5.5	7.3	5.0	1.3	4.7	4.7	3.4	5.1	4.5	5.8	[124]
7	*B. carinata*	2.5	6.0	11.1	1.3	11.5	5.3	2.4	5.0	9.5	6.0	1.4	2.5	4.8	4.7	5.0	0.8	6.3	[136]
8	*C. farinosa*	8.2	9.3	25.2	2.3	20.5	5.5	3.7	5.3	10.8	7.8	1.2	6.5	8.1	6.6	4.7	3.2	7.2	[84]
9	*C. Argentea*	2.4	4.9	8.0	1.1	10.7	4.0	2.1	3	6.5	5.1	1.1	4.3	2.6	2.9	3.3	3.3	4.3	[93]
10	*Cent. asiatica*	4.7	3.2	9.4	1.4	13.4	3.8	11.6	9.6	6.5	9.7	1.5	8.5	3.1	5.9	3.7	7.8	4.5	[119]
11	*C. album*	6.3	6.6	9.7	0.9	11.0	5.6	2.8	5.2-5.4	9.2	7.2	1.8-2.0	5.8	5.3	4.9	5.2-8.2	4.5	6.4	[137],
12	*C. odorata*	4.5	5.6	8.5	1.3	12.0	4.4	2.5	4.4	8.1	4.4	1.4	4.4	4.0	4.2	3.5	3.2	4.5	[113]
13	*C. aconitifolius*	4.0	3.7	8.5	0.6	10.0	3.0	2.2	3.4	7.8	4.6	0.8	3.8	3.0	1.9	3.3	2.5	3.7	[125]
14	*C. abyssinica*	5.0	3.8	7.5	2.9	7.9	4.7	1.4	3.2-5.1	5.2	4.0	0.8-1.0	2.9-3.4	2.1	4.3	3.2-3.8	1.7	4.0	[122]
15	*C. olitorius*	3.9	4.0	9.1	0.5	10.1	3.6	2.0	3.0	7.4	4.2	0.8	3.7	2.7	2.0	3.0	3.2	3.3	[123,125]
16	*C. maxima*	3.6	9.7	11.9	2.1	12.7	5.0	3.7	4.7	5.4	4.0	0.6	5.3	5.3	4.7	3.5	4.1	5.7	[124]
17	*Ficus sur*	4.0	4.3	8.3	0.9	8.8	3.2	2.0	4.0	7.6	3.6	0.9	3.6	2.2	1.9	3.9	3.3	3.7	[138]
18	*G. africana*	4.8	5.2	8.8	0.8	13.3	4.9	2.7	3.0	8.6	4.0	1.1	4.1	3.7	5.0	3.4	2.9	4.5	[112]
19	*G. latifolium*	7.6	7.7	13.8	0.4	11.9	10.2	1.5	4.7	9.2	5.8	0.8	6.4	1.2	6.2	3.8	3.4	7.7	[112]
20	*H. barteri*	2.3	5.1	6.7	0.8	9.5	1.0	2.0	3.0	5.7	3.0	0.9	3.9	2.1	1.7	2.3	2.9	3.3	[105]
21	*H. crinata*	3.6	4.4	5.2	1.4	10.5	4.1	1.7	6.2	5.3	3.8	2.1	5.4	3.1	3.2	4.2	3.6	4.1	[140]
22	*I. batatas*	9.3	9.2	27.8	0.4	21.1	8.3	4.4	6.7-8.8	4.2	2.6	0.6-1.4	3.5-11.1	6.1	7.3	3.2-8.9	2.3	4.1	[123]
23	*L. africana*	4.1	5.7	4.9	1.6	10.6	3.9	1.6	6.3	4.6	3.6	3.0	4.1	3.2	3.1	4.4	3.6	3.9	[140]
24	*M. esculenta*	7.5	6.7	10.3	1.2	13.0	5.8	2.4	5.2	9.9	6.7	1.6	5.9	5.3	4.2	4.8	4.0	6.6	[139]
25	*M. oleifera*	7.3	3.8	8.8	1.4	10.2	5.5	2.4	4.0-4.5	7.7	5.6	1.7	6.2	5.4	4.1	4.7	3.9	5.7	[103]
26	*Ocim.gratissi*	5.9	6.3	11.0	0.5	12.2	7.4	2.0	3.9	8.9	4.9	0.9	5.2	2.2	5.1	3.5	3.5	5.9	[112]
27	*P. santalinoide*	3.9	5.2	9.1	1.1	10.6	3.5	2.4	3.9	8.3	4.3	1.2	4.3	3.0	2.3	2.9	2.9	4.6	[138]
28	*S. indicum*	2.1	4.7	8.1	0.1	10.0	0.9	2.6	3.7	8.7	4.9	1.1	5.0	3.0	2.0	3.6	3.3	4.9	[120]
29	*S. biafrae*	4.3	4.5	8.6	0.5	12.6	3.5	2.1	3.0	7.8	3.6	0.9	3.9	2.9	3.0	3.4	3.1	4.1	[125]
30	*S.macrocarpon*	3.8	3.9	8.7	0.7	10.7	3.0	2.1	3.0	8.1	4.4	0.9	4.0	3.2	3.1	2.9	2.8	3.8	[125]
31	*T. triangulare*	3.9	4.4	8.0	0.5	11.3	3.3	2.2	3.0	7.1	4.0	0.8	4.1	3.0	2.9	3.3	2.6	3.7	[125]
32	*T. occidentalis*	3.3	5.8	0.4	0.6	9.1	2.0	1.1	2.2	5.4	4.6	nd	3.2	0.9	2.3	1.7	1.3	2.5	[102]
33	*V.amygdalina*	5.0	5.9	9.1	1.4	14.3	5.6	3.3	4.0-4.5	7.0	5.2	1.6-2.0	5.0-5.9	4.2	4.9	4.0-4.6	2.1	5.2	[116]
34	*V. uguiculata*	1.4	1.8	3.5	11.3	3.1	1.3	0.5	1.2-1.7	2.1	1.4	1.4	1.4-2.5	1.1	1.3	4.4	1.2	4.1	[126],[13
35	*Vitex doniana*	7.7	1.7	6.3	12.3	15.3	4.8	6.5	6.6	5.9	5.4	5.4	18.0	20.6	5.5	5.6	3.5	5.9	[138]

36	*X. sagittifolia*	1.5	1.5	1.9	0.1	2.4	1.2	0.4	1.0	2.0	1.6	0.2	1.3	1.1	1.0	nd	1.0	11.3	[121,122]

References: Odukoya et al. 2007[1], Aworh 2015[2]; Olajire & Azeez, 2011[3;] Oboh et al. 2005[4] ,USDA[5], Sakpere et al. 2015[6], Ikewuchi et al. 2010[7] Nisha et al 2012[8];Gunathilake & Ranaweera 2016[9],Oloyede et al. 2011[10],Lykke & Padonou[11],Agiang et al. 2016[12],Biodiversity for Nutrition (BFN) repository[13], Neves et al. 2019[14],Traore et al. 2017[15],Nyadanu & Lowor,2014[16], Poornima & Jeyam 2016[17], Yisa et al 2010[18],Orlando et al. 2019[19], Otitoju, et al 2014[20], van Jaarsveld et al. 2013[21], Stephen, Adebisi, Chinedu & Samuel et al. 2017[22], Nnamani, Oselebe & Agbatutu et al. 2009[23], Goyal et al. 2011 [24], Khalil et al. 2016[25], Favier et al. 1993[26], Juna et al. 2014[27], Ujowundu et al 2008[28], Sanlier et al. 2018[29], Chinedu,et al, 2019[30], Mbatchou et al. 2011[31], Askira et al. 2016[32], Silva et al. 2001[33], Abubakar et al. 2013[34], Olaiya & Adebisi et al. 2009[35], Wakawa et al. 2018[36], Hamad & Usman et al. 2014[37], Amata et al. 2010[38], Umerah et al. 2019[39], Idris et al. 2011[40], Sudeshna et al. 2019[41], Pak et al. 2011[42], Anywar et al. 2017[43], Odewo et al. 2014[44], Okaraonye & Ikewuchi et al. 2009[45], Chizzotti et al. 2005[46], Odhav et al. 2007[47], Chinedu et al. 2018[48], Wasagu et al. 2013[49], Akpanyung et al 1995[50], Arthur et al. 2020[51], Alagbe et al. 2020[52], Ajiboye et al. 2013[53], Akubugwo et al. 2007[54], Hassan et al. 2014[55], Ishaku, et al. 2016[56], Sa'id et al. 2019[57], Gbadamosi et al. 2014[58], Adhikari et al. 2015[59], Gemede et al. 2015[60], Afolayan et al. 2009[61], Azubuike et al. 2018[62], Sahou et al. 2014[63], Adamu et al 2019[64], Igbadul et al. 2012[65], Khan et al. 2013[66], Pío-León et al. 2012[67], Morshed et al. 2021[68], Obi-Abang et al. 2019[69], Adeyeye,& Olaleye et al. 2020[70], Okonkwo et al. 2016[71], Okezie et al. 2017[72], Bella et al. 2008[73], Oseni et al. 2018[74], Mahlangeni et al. 2015[75], Ogunyemi et al. 2020[76], Eleazu et al 2012[77], Omoyeni et al. 2011[78], Ademoyegun et al. 2013[79], Gul et al. 2013[80],Glew et al. 2009[81], Fagbohun et al. 2010[82], Patricia et al. 2014[83], Glew et al 2010[84],Paul et al. 2013[85], Omoyeni & Adeyeye 2009[86],Bartolome et al. 2013[87],Sahore et al 2013[88], Ahmad et al. 2014[89], Padwal et al. 2016[90], Shrestha, 2020[91], Akpabio & Ikpe,2013[92,] Edwige et al. 2014[93], Basumatari & Narzary, 2017[94],Caluete et al 2015[95], Kamble & Dhage ,2019[96],Muhammad et al 2018[97],Rathore &Kumar 2019[98],Onwordi & Wusu 2009[99],Ayodele & Olajide, 2011[100],Ejoh et al 2019[101], Adeyeye &Omolayo[102], Makkar & Becker 1996[103], Nworgu & Egbunike 2013[104],Kubmarawa et al. 2009[105], Acho et al. 2014[106], Agiang et al. 2017[107],Bamidele et al. 2017[108],Faboya ,1983[109],Mepba 2007[110], Mbaebie et al. 2010[111],Chinyere & Obasi, 2011[112],Igboh et al 2009[113], Omokhua et al. 2016[114], Uusiku et al. 2010[115], Ejoh et al. 2007[116], Dike et al. 2010[117],Basiru et al. 2016[118] Afolayan & Jimoh ,2009[119], Kubmarawa et al. 2008[120], Ayalew et al. 2017[121], Lumu& Katongole 2011[122], Athanase et al. 2018[123], Adesina & Adeyeye 2013[124], Omoyeni et al. 2015[125],Suarez et al. 2020[126], Bamali et al. 2014[127], Lyimo et al. 2003[128], Abifarin et al. 2021[129], Sami et al. 2013[130], Moloto et al. 2020[131], Affonfere et al. 2021[132], McGregor 2012[133], Adjatin et al. 2013[134], Ndomou et al. 2014[135], Mnzava & Olsson 1990[136], Poonia & Upadhayay, 2015[137], Ugadu et al. 2019[138], Nagy et al. 1978[139], Inyang, 2016 [140]

Summary

Hidden hunger, due to regular consumption of a diet deficient in micronutrients, may persist unabated, especially in low-and-middle-income countries of the world, unless more attention is focused on developing healthy and nutritious foods to alleviate the burden. Several nutritional interventions have been implemented for decades but one of the most sustainable food-based solutions proffered in recent times is the twin-strategy of biofortification and food-to-food fortification. In response to the challenge of combating hidden hunger, the yellow cassava biofortified with pro-vitamin A carotenoids was introduced to sub-Saharan Africa. However, yellow cassava is still low in minerals such as iron and zinc, hence the need to incorporate micronutrient-rich and affordable food ingredients such as the African leafy vegetables. To ensure an adequate intake of the much-needed micronutrients, we developed pasta, a widely consumed, healthy and affordable food product with the yellow cassava with two leafy vegetables, amaranth and fluted pumpkin incorporated. The increasing demand by consumers for gluten-free pasta as a functional food also focused attention on cassava as a gluten-free ingredient. This thesis, therefore aimed at evaluating the nutritional, sensorial, technological and functional aspects of the novel vegetable fortified yellow cassava pasta to gain more insights into its contribution to a healthier living for the consumers.

In **Chapter 2**, using consumer survey focus group discussions and sensory studies, we evaluated the acceptance of yellow cassava pasta and African leafy vegetables. We found the main driver of intended consumption among the consumers in Nigeria to be related to health concerns. Also, yellow cassava pasta was acceptable to the consumers while fluted pumpkin (*Telfairia occidentalis*) was the most preferred leafy vegetable followed by amaranth. The low awareness of yellow cassava among the consumers was also highlighted with solutions proffered to boost awareness of the nutritional benefits of the novel food product. The findings of this study highlighted a need to conduct experimental studies on the nutritional composition of the novel pasta products to ascertain the health benefit to consumers.

The main focus of **Chapter 3** was to ascertain the nutritional value of the novel cassava pasta product. We conducted experiments to gain insights into the nutritional and functional properties of the cassava- vegetable pasta before and after being processed. We found that the incorporation of amaranth leaf powder boosted the antioxidant capacities of pasta products while the cooking time and gruel solid loss were also reduced upon the addition of the leaf powder. Furthermore, the protein, fibre, iron and zinc contents of the formulated pasta were enhanced with the addition of amaranth leafy vegetables.

As a follow up to the study in Chapter 3, another popular leafy vegetable, fluted pumpkin was used in food-to-food fortification of yellow cassava pasta in **Chapter 4.** We conducted experiments on both the white and yellow cassava flours and pasta to evaluate the functional, textural, pasting, thermal, cooking and sensory properties of the novel pasta with the inclusion of fluted pumpkin leaf powder. We found that the inclusion of the leaf powder reduced the cooking time and hardness of cassava pasta products. Also, the pasting temperatures were lowered with leaf powder incorporation. Interestingly, we observed that the provision of nutritional information enhanced the likeness of the leaf powder-fortified cassava pasta products.

In **Chapter 5**, we focused on the nutritional value of both Amaranth-fortified and fluted-pumpkin-fortified yellow cassava pasta highlighting the added health benefits of the gluten-free cassava pasta. We conducted *in-vitro* studies to evaluate the impact of leaf powder addition (amaranth and fluted pumpkin leaves) on the retention and bioaccessibility of beta carotene, iron and zinc in the pasta and the impact on the starch digestibility and *in-vitro* glycemic index of the functional pasta. We found that the estimated glycemic index of yellow cassava pasta was reduced with the inclusion of the leaf powder while the zinc was found most bioaccessible. Also, the functional pasta was estimated to contribute a substantial percentage to estimated average requirements for vitamin A, iron and zinc.

Chapter 6 provided the rundown of all the results presented in the previous chapters and put them into perspective with similar works from literature while enumerating prospects of the novel gluten-free cassava pasta. We demonstrated the potential of the functional food to alleviate micronutrient deficiencies.

Ní àkótán

Ebi tíó farasin ni o le maa wáyé nígbà dégbà látàrí jíjẹ ouńjẹ tí kò dára tó, tí ó sì ṣe ṣe kí ọwọ́ má ka, pàápàá l'áwọn orílẹ̀ẹ̀dè tí ọwọ́ wọn kò ṣe bẹ́ẹ̀ t'ẹ́nu ní àárín àwọn ojúgbà wọn l'ágbáyé àyàfi tí wọ́n bá gbájúmọ ọ̀nà àti mú ìdàgbàsókè débá ouńjẹ tó l'óókun dáradára láti rọ̀ wọ́n lẹ́rù. Ọ̀pọ̀lọ́pọ̀ ìgbésè ni wọ́n ti gbé l'átẹ̀yín wá fún ọ̀pọ̀lọ́pọ̀ ọdún ṣùgbọ́n ọ̀nà àbáyọ èyí tó fẹsẹ̀ múlẹ̀ jùlọ nípa ti ọ̀rọ̀ oúnjẹ ni ọ̀nà àbáyọ oníbejì èyí tí olóyìnbó pè ní *biofortification* àti ríró ouńjẹ lalágbára pelu ounje miran. Fún kíkojú ìṣòro ebi tíó farasin, ẹ̀gé pupa èyí tí ó ní aṣara lóóre Fítámìn A wúlò lọ́pọ̀lopọ̀.Ohun tí a mọ̀ sí , ẹ̀gé pupa èyí tí ó ní *carotenoids* ni wọ́n fi ṣ'ọwọ́ sí ilẹ̀ adúláwọ̀. Sùgbọ́n ẹ̀gẹ́ pupa kòní agbára tó bí ti iron àti zinc ìdí rẹ̀ nìyí tí wọ́n fi gbọ́dọ̀ fun ní àwọn èròjà àti ohun-èlò bíi ewébẹ̀ ilẹ̀ adúláwọ̀. Lójúnà àti jẹ ohun tí yíò ṣe ara lóore, ìdí nìyì tí wọ́n fi ṣàgbékalè ohun tí wọ́n ń pè ní pasta èyí tó ń ṣe ara lóore pẹ̀lúu ẹ̀gẹ́ pupa pẹ̀lú ẹ̀wébẹ̀ oríṣi méjì, tí a mọ̀ sí tẹ̀tẹ̀ àbáláyé àti úgú. Bíi àwọn oníbárà tó ń wá *pasta* tí kò-ní-*gluten* gẹ́gẹ́ bi ohun tó dàra fún àgọ́ ara ṣe ń pọ̀si lójojúmọ́ bẹ̀ẹ̀ni àkíyèsí ṣe ń pọ̀si lórí ẹ̀gẹ́ tí kò ní èròja gluten. Látàrí èyí, ìwé àpinlẹ̀kọ yí gbájúmọ́ isẹ́ àti ohun aṣara lóore tí ó wà nínú ewébẹ̀ àti pasta tí wọ́n fi ẹ̀gẹ́ pupa se àti ipa tí ó kó lórí ìgbé-ayé alálaafíà f'àwọn oníbáárà.

Ní orí kéji ìwé yí, nípa ṣíṣàmúlò àgbéyẹ̀wò ìjíròrò àwọn oníbárà, a wo bí àwọn ènìyàn ṣe tẹ́wọ́gba pasta tí wọ̀n fi ẹ̀gẹ́ pupa ṣe àti ewébẹ̀ ilẹ̀ adúláwọ̀. A ri wípẹ̀ ìdí pàtàkì tí àwọn ènìyàn fi ń jẹ ouńjẹ yí lórílẹ̀ẹ̀dè Nàíjíríà ni nítorí ìlera ara wọn. Bákan náà àwọn ènìyàn tẹ́wọ́gba pasta tí wọ̀n fi ẹ̀gẹ́ pupa se ti *úgú* sì jẹ́ ewébẹ̀ tí wọ́n ní ìfẹ́ jùlọ ti tẹ̀tẹ̀ àbáláyé sì tẹ̀le. A tún ṣe àgbéyẹ̀wò aìsí ìtanijí nípa ìwúlò ẹ̀gẹ́ pupa àti ọ̀nà ààtọ̀ láti ṣe ìgbéláárugẹ ìtanijí lórí àwọn èròjà aṣara lóore tí ó wà nínú oúnjẹ náà, ìwáàdí inú iṣẹ́ yí ṣàfihàn pàtàki ṣíṣe àyẹ̀wò l'ọ́nà ìgbàlódé láti mọ àwọn èròjà aṣara lóore tí o sọdun sinu *pasta* naa, láti le è mọ pàtàkì rẹ̀ fún ìlera ara àwọn to n rà á.

Àfojúsún orí kẹ́ta ìwé yi ni láti wo èròjà tó wà nínú *pasta* tí wọ́n f'ẹ̀gẹ́ se. A ṣe àyèwò láti ní òye lẹ́kúnrẹ́rẹ́ lórí wíwo èròjà tó wà nínú *pasta* tí wọ́n fi ẹ̀gẹ́ àti ẹ̀fọ̀ ṣe kí wọ́n tó ṣètò rẹ̀ àti lẹ́yìn tì wọ́n ṣetò rẹ̀ tàn. A ri pé ṣíṣàmúlò ìyẹ̀fun ewé tẹ̀tẹ̀ àbáláyé ṣèrànwọ̀ fún ìrólàgbára *pasta* yi. Àsìkò sísè rẹ́ ni àdíkún yíò débà ní kété tí wọ́n bá ti fi ìyẹ̀fun ewé náà si. Bákan náà ohun aṣara lóore, *protein*,*fibre*,*iron* ati *sinki* ni ìdàgbàsóké débá pẹ̀lú àfikún ewé ẹ̀fọ́ tẹ̀tẹ̀ àbáláyé . Ní àfikún sí orí kẹ́ta, ẹ̀fọ́ *úgú* ni wọ́n ń lò fún ìrólágbára *pasta* tí wọ́n fi ẹ̀gẹ́ pupa se.

Ní orí kẹ́rin, a ṣe àyèwo lórí ẹ̀gẹ́ pupa àti ẹ̀gẹ́ funfun láti mọ àwọn ìyàtọ̀ tó wà ní àárín wọn fún sísè pasta àti tí wọ́n bá fi ìyẹ̀fun ewé úgú si. A ri pé ṣíṣàfikún ìyẹ̀fun ewé úgú ma ń mú àdínkù bá iye ìséjù tí wọn yio fi sè é àti rírọ̀ *pasta* tí wọ́n fi ẹ̀gẹ́ se. Gbígbóná *pasta* yí yio lọ lẹ pẹ́lú àfikún ìyẹ̀fun naa. Ohun tíó dún mọ́ni nínú jùlọ ni wípé ìròyìn nípa àwọn èròjà asara l'óore ohun lómú kí àwọn ènìyàn fẹ́ràn pasta tí wọ́n f'ẹ̀gẹ́ se pẹ̀lú ìrólágbára ìyẹ̀fun ewé naa.

Ní orí karún a gbájúmọ́ èròjà aṣara lóore tí ó wà nínú pasta tí a f'ẹ̀gẹ́ pupa se èyí tóní tẹ̀tẹ̀ àbáláyé àti úgú gẹ́gẹ́ bí ohun tí wọ́n fi ro lágbára,tí ó sí ń ṣàlàye lẹ́kùnrẹ́rẹ́ bí *pasta* tí wọ́n f'ẹ̀gẹ́ ṣe ṣe ń sàǹfàní fún àgọ́ ara. A ṣe àyẹ̀wò ipa tí ìyẹ̀fun ewé méèjì yi tẹ̀tẹ̀ àbáláyé àti úgú (*amaranth* ati *fluted pumpkin*) kó, lórí sísàmúdúró àwọn èròjà bíi *beta carotene, iron* àti *sinki* nínu *pasta* àti pàtàkì *starch* nínu bí *pasta* ṣe n si'sẹ́. A ri pé iye *carbohydrate* tíó wà nínu ẹ̀gẹ́ pupa ni àdínkù débá bí wọ́n ṣe lo ìyẹ̀fun tíó sì kópa nínú ìdá vitamin A, iron àti sinki ní àgọ́ ara.

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-Hellen Keller

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Curriculum Vitae

Oluranti Mopelola Lawal was born in Ibadan, Nigeria on 16th of October 1973. She obtained her Bachelor's degree at the University of Lagos where she was awarded the University of Lagos Scholar Awards for the best student in the Faculty of Environmental Sciences for 4 consecutive sessions (1990-1995), She later got a Master's degree in Food Science and Technology in 2016 from the Federal University of Technology, Akure in Nigeria and was the best graduating post-graduate student in the department. Upon completing her Master's program, she was employed as a lecturer at the Department of Food Science and Technology of the Federal University of Technology, Akure Nigeria. Oluranti is passionate about developing healthy and nutritious plant-based foods to fight hidden hunger in low-income countries using common staples such as cassava, maize and leafy vegetables. She founded the Ondo State Sunshine Women in Agriculture (OSSWA) in 2009 and the Ondo State Women Food Processing Industry (OWFPI) in 2010, both women cooperative organisations established to boost agribusiness and reduce post-harvest losses incurred by smallholder women farmers in Ondo State, Nigeria. She is also involved in several collaborative research studies such as the 'Veg-on-Wheels' nutritional intervention project aimed at increasing consumption of leafy vegetables in Nigeria which has been very successful and has attracted commendations and international recognition (https://issuu.com/wageningenur/docs/ww2019_01_eng/52). She has also produced, in conjunction with other researchers in the field of nutrition, a Massive Open Online Courses (MOOC) video on Food Environments for Healthy Sustainable Diets https://www.wur.nl/en/Education-Programmes/Wageningen-Academy/What-we-offer-you/Courses/Food/show/Online-Course-Food-Environments-for-Healthy-Sustainable-Diets.htm In 2019, Oluranti was awarded the Tertiary Education Funds (TETFund) scholarship to complete her PhD in Food Quality and Design at the Wageningen University and Research, the Netherlands. She was a member of the Advanced Studies in Food Technology, Agrobiotechnology and Health Sciences (VLAG) PhD Council and the President of the Nigerians in Wageningen (NIW).

Oluranti's hobbies are reading, writing, travelling, cooking and dancing. She can be contacted by email: ranti2lawal@gmail.com.

List of publications

Publications in this thesis

Lawal, O. M., Talsma, E. F., Bakker, E., Fogliano, V., & Linnemann, A. R. (2021). Novel application of biofortified crops: Consumer acceptance of pasta from yellow cassava and leafy vegetables. *Journal of the Science of Food and Agriculture*, jsfa.11259. https://doi.org/10.1002/jsfa.11259

Lawal, O. M., Stuijvenberg, L., Boon, N., Awolu, O., Fogliano, V., & Linnemann, A. R. (2021). Technological and nutritional properties of Amaranth-fortified yellow cassava pasta. *Journal of Food Science*, 1750-3841.15975. https://doi.org/10.1111/1750-3841.15975

Lawal, O. M., Sanni, O., Oluwamukomi, M., Fogliano, V., & Linnemann, A. R. (2021). The addition of fluted pumpkin (Telfairia occidentalis) leaf powder improves the techno-functional properties of cassava pasta. *Food Structure*, *30*, 100241. https://doi.org/10.1016/j.foostr.2021.100241

Lawal, O.M., Fogliano, V., Rotte, I., Fagbemi, T.N., Dekker, M. & Linnemann, A.R. (2021). Leafy vegetable-fortified yellow cassava pasta had a decreased glycemic index and increased zinc bioaccessibility *Food & Function*

Other scientific peer-reviewed publications

Lawal, O. M., Wakel, F., & Dekker, M. (2021). Consumption of fresh Centella asiatica improves short term alertness and contentedness in healthy females. *Journal of Functional Foods*, *77*, 104337. https://doi.org/10.1016/j.jff.2020.104337

Lawal, O.M., Badejo, A.A. & Fagbemi, T.N. (2020). Processing and Storage Impact on Carotenoid Contents and Functional Properties of Yellow Cassava Traditional Food Products. *Journal of Food Technology Research*, *7*(2), 154–162. https://doi.org/10.18488/journal.58.2020.72.154.162

Lawal, O.M, Idowu-Adebayo, F, Enujiugha, V.N. (2018). Nutritional Assessment of Nigerian Ethnic Vegetable Soups (Marugbo, Tete and Ila). (2018). *Journal of Nutrition, Food and Lipid Science*, *1*(1), 32–39. https://doi.org/10.33513/NFLS/1801-05

Lawal, O. M., & Enujiugha, V.N. (2016). Chemical composition, functional and sensory properties of maize-based snack (Elekute) enriched with African oil bean seed (Pentaclethra macrophylla benth). *African Journal of Food Science*, *10*(12), 379–384. https://doi.org/10.5897/AJFS2016.1461

Awolu, O. O., Odoro, J. W., Adeloye, J. B., & **Lawal, O. M**. (2020). Physicochemical evaluation and Fourier transform infrared spectroscopy characterization of quality protein maize starch subjected to different modifications. *Journal of Food Science*, *85*(10), 3052–3060. https://doi.org/10.1111/1750-3841.15391

Overview of completed training activities

Category A: Discipline-specific activities

Name of the course	**Organizing institutes**
Horticulture sector development for emerging markets	WUR (*The Netherlands*), 2019
Healthy and sustainable diets: Synergies and trade-offs	VLAG (*The Netherlands*), 2019
Stable Isotope methods in Nutrition Research	VLAG (*The Netherlands*), 2019
Sustainable Food Systems-Performing by Connecting	EFFoST (*The Netherlands*), 2019
Food Composition Database in Food Nutrition	VLAG (*The Netherlands*), 2019
Bioethics and Research	Ministry of Health (*Nigeria*), 2020
4th International Conference on Global Food Security	Elsevier (*The Netherlands*), 2020
Plant-based food protein	European Food Conference (*The Netherlands*),2021

Category B: General courses

Name of the course	**Organizing institute**
VLAG PhD week	VLAG (*The Netherlands*), 2019
Scientific Artwork – Vector graphics and images	WGS (*The Netherlands*), 2019
Scientific Publishing	WGS (*The Netherlands*), 2019
PhD Workshop carousel	WGS (*The Netherlands*), 2019
Introduction to R for Statistical Analysis	VLAG (*The Netherlands*), 2019
Searching and Organising Literature for PhD candidates	WGS (*The Netherlands*), 2019
Competence assessments	WGS (*The Netherlands*), 2019
Adobe In Design	WGS (*The Netherlands*), 2019
The Essentials of Scientific Writing and Presenting	WGS (*The Netherlands*), 2019
Research Data Management	WGS (*The Netherlands*), 2019
Philosophy and Ethics of Food Science and Technology	WGS (*The Netherlands*), 2020
Working on your PhD research in times of crisis	WGS (*The Netherlands*), 2020
Multivariate Statistics	WMIMEK PER&C (*The Netherlands*),2020
Writing grant proposal	WGS (*The Netherlands*), 2021

Category C: Optional

VLAG Proposal	VLAG (*The Netherlands)*, 2019
PhD council (lunch meeting committee)	VLAG *(The Netherlands)*, 2019-2021
Weekly group meetings	FQD *(The Netherlands),* 2019-2022
PhD study tour	FQD *(Spain)*, 2022

VLAG: Graduate School for Nutrition, Food Technology, Agrobiotechnology and Health Sciences
WGS: Wageningen Graduate School
WMIMEK PER&C: Wageningen Institute for Environment and Climate Research
FQD: Food Quality and Design

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