Chapter 10
New Perspectives in the Utilization of African Leafy Vegetables

K. A. Taiwo and A. A. Famuwagun

10.1 Introduction

Leafy vegetables may be indigenous or traditional. Indigenous leafy vegetables are native to a particular country, while the traditional vegetables are those that were introduced to a particular country and by the farmer's selection or adoption; they have been naturalized in such a country (Van Rensburg et al. 2007). Leafy vegetables, either indigenous or traditional, are given local names in the regions where such vegetable leaf is being cultivated. With a population of over 200 million in Nigeria, about 40.1% of this populace lives in poverty as of 2020, the high number of people living below the poverty level has caused an increase in malnutrition due to consumption of foods which are deficient in nutrients such as vitamins, minerals and proteins (Udousoro and Ekanem 2013). Studies have therefore indicated that the poor nutrient intake by this large population can be addressed through the consumption of nutrient-dense leafy vegetables. These vegetables are cheap, affordable and ubiquitous and therefore can be easily accessed by the poor communities (Ilelaboye et al. 2013). Leafy vegetables have deposits of numerous useful compounds such as phenolics and phytochemicals that contribute greatly to the antioxidant properties and this provides great effects against various cardiovascular diseases and oxidative damages (Gupta et al. 2005). Access to these leafy vegetables by rural communities would also guarantee good health for the populace. Despite the rich nutrients of these vegetables, the leaves are difficult to preserve due to the high moisture content, ranging between 86% and 92%, and this may

K. A. Taiwo (✉)
Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria
e-mail: ktaiwo@oauife.edu.ng

A. A. Famuwagun
Department of Food Science and Technology, Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria

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account for the high perishability of the leaves (Shukla et al. 2016). Extending the shelf life of the vegetables after harvest and designing efficient machinery for storage may be challenging due to this high moisture content, thus contributing to high post-harvest losses. The utilization of leafy vegetables can be enhanced, especially in the industries by extracting its active ingredients. Leafy vegetables have potentials to be used as ingredients in designing new class of food for enhanced nutrition through extraction of its active ingredients such as protein and polyphenols or using the whole leaf powders and incorporating in convenience food such as bread, confectioneries and fruit juices for the development of new classes of foods. This review therefore discusses the recent efforts aimed at diversifying the utilization of leafy vegetables in an attempt to take this nutrient dense plant resource from the plate to the industries.

10.2 Commonly Consumed Leafy Vegetables in Nigeria

Vast hectares of lands are dedicated to cultivating leafy vegetables (LV_S) in Nigeria, ranging from large farm areas to small portions of land in the backyard garden of individual houses (Gockowski et al. 2003). The three major ethnic groups in Nigeria (Yoruba, Igbo and Hausa) have leafy vegetable that are peculiar to them, such that these LVs are named based on the tribal languages (Table 10.1). Some of the leafy vegetables grown in Nigeria include wild lettuce (*Taraxacum officinale*), African Spinach *(Amaranthus hybridus),* Lagos Spinach (*Celosia argentea*), Water leaf (*Talinum Triangulare),* African eggplant leaf (*Solanum macrocarpon),* Malabar spinach (*Basella alba),* African Basil/scent leaf (*Ocimum gratissimum),* Yoruban bologi (*Crassocephalum rubens),* Fluted pumpkin leaf (*Telfairia occidentalis*)*,* Jute leaves (*Corchorus*)*, Bitter leaf (Vernonia amydgalina), Wild spinach (Gnetum africanum),* Bushbuck (*Gongronema latifolim),* Curry leaf (*Muraya koenigii),* Waterleaf (*Talium triangularea),* Rosewood leaf (*Pterocarpus mildbraedii),* Clove Basil/scent leaf (*Ocimum gratissimum)* and Moringa leaf (*Moringa olefera)*. These leafy vegetables play important roles in alleviating poverty and contribute immensely to the healthy lifestyles of the populace (Abukutsa-Onyango 2003). They are consumed in different forms, either cooked or in the fresh forms, as supporting or main dishes. The taste of some of the vegetables may be bitter, some are aromatic or tasteless (Mensah et al. 2008) and whatever taste it is, consumption of the leafy vegetables is highly valued in the Nigerian culture because of the roles they play in health's promotion and protection of Nigerians.

Table 10.1 Some green leafy vegetables cultivated in Nigeria

	English name	Botanical name	Nigerian name (tribe)
1	Wild lettuce/Dandelion greens	*Taraxacum officinale*	*Efo Yanrin (Yoruba)*
2	African Spinach	*Amaranthus hybridus*	*Efo Tete (Yoruba)*
3	Lagos Spinach	*Celosia argentea*	*Shokoyokoto (Yoruba)*
4	Water leaf	*Talinum Triangulare*	*Gbure (Yoruba)*
5	African eggplant leaf/ garden egg leaf	*Solanum macrocarpon*	*Efo Igbo (Yoruba), akwukwo Anara (Igbo), Ganyen gauta (Hausa)*
6	Malabar spinach	*Basella alba*	*Amunututu (Yoruba)*
7	African Basil/scent leaf	*Ocimum gratissimum*	*Efirin (Yoruba), Nahianwu (Igbo), Daidoya (Hausa*
8	Yoruban bologi	*Crassocephalum rubens*	*Ebolo (Yoruba)*
9	Fluted pumpkin leaf	*Telfairia occidentalis*	*Ugu (Igbo), kebewa (Hausa)*
10	Jute leaves	*Corchorus*	*Ewedu (Yoruba), ayoyo (Hausa), kerenkere (igbo)*
11	*Bitter leaf*	*Vernonia amydgalina*	*Onugbu (igbo), efo ewuro (Yoruba), shakwa shuwaka (Hausa)*
12	*Wild spinach*	*Gnetum africanum*	*Afang leaf (Ibibio), ukazi (Igbo)*
13	Bushbuck	*Gongronema latifolim*	*Utazi (igbo), arokeke (Yoruba),*
14	Curry leaf	*Muraya koenigii*	*Efirin oso (Yoruba), Marugbo sanyan (Yoruba),*
15	Waterleaf	*Talium triangularea*	*Gbure (Yoruba)*
16	Green African spinach	*Amaranthus hybridus*	*Efo tete (Yoruba), tete eleegun (Yoruba), inine (igbo),*
17	Rosewood leaf	*Pterocarpus mildbraedii*	*Oha/ora leaf (Igbo)*
18	Clove Basil/scent leaf	*Ocimum gratissimum*	*Nchuawun (Igbo) Efirin (Yoruba)*
19		*Senecio biafrae*	*Worowo (Yoruba)*
20	Moringa leaf	*Moringa olefera*	Oku-ghara-ite (Igbo) Idagbo monoyé (Yoruba)

Sources: Shirley et al. (2019); Borokini et al. (2017); Alexander (2016); Akinwunmi and Omotayo (2016); Otitoju et al. (2014); Adjatin et al. (2013); Davidson and Monulu (2018); and Uraku and Nwankwo (2015)

10.3 Some Nutrients Composition of Leafy Vegetables

10.3.1 Micronutrients in Leafy Vegetables

Human body needs both the macro- and micronutrient to function effectively. Several studies on leafy vegetables have shown they are good repositories of these nutrients (Natesh et al. 2017; Odhav et al. 2007; Shirley et al. 2019). Micronutrients comprise vitamins and minerals, which are normally required in small amounts but vital to the body, while macronutrients include fats, protein and carbohydrates, and are needed in greater amounts than micronutrients to guarantee metabolism, growth and healthy living of the body (Otitoju et al. 2014). There are increasing research evidences of high concentration of vitamins and minerals in various leafy vegetables (LV) cultivated in Nigeria. For instance, LVs such as fluted pumpkin, African eggplant leaf, moringa leaf and bushbuck are widely consumed in different parts of the country where they are found due to extensive and adequate research information on them, whereas consumption of LVs such as Yoruban bolongi, water leaf, Malabar leaf and wild lettuce are not as popular as others in areas where they are found, probably due to inadequate research and knowledge on their nutritive benefits.

Mineral nutrients are important in the body for growth, maintenance of bone health, fluid balance and are useful for several other processes. Food minerals are grouped as macromineral elements (Calcium, Phosphorous, Magnesium, Sodium, Chloride, Potassium and Sulphur) and trace elements (Iron, Manganese, Copper, Zinc, Iodine, Fluoride and Selenium). Many leafy vegetables have been shown to contain substantial amounts of minerals (Arasaretnam et al. 2018; Iheanacho and Udebuani 2009; Otitoju et al. 2014 and Shirley et al. 2019) as shown in Table 10.2. The high amounts of some of these mineral micronutrients suggest that the LVs are a potential source of nutritional intervention in Nigeria for mineral balance. The high amounts of some of the major minerals in the leafy vegetables imply that their consumption would specifically be helpful in the building of strong bone and teeth, assist in the functions of the muscles and contribute to cell membrane structures. The consumption of the leaves would also strengthen the regulation of blood pressure and the control of the reactions of the enzymes, body's fluids balance, help in the proper transmission of nerves and support the body in adequate production of haemoglobin (Nnamani et al. 2009; Adernipekun and Oyetunji 2010).

Vitamins are important for building the body's immune health, blood clotting and energy production, but these are needed in the trace amounts. Vitamins are grouped as water-soluble and fat-soluble vitamins. The water-soluble vitamins such as Vitamin B complex (Vitamins $B_{1, 2, 3, 4, 5, 6, 7, 9}$ and $_{12}$) and vitamin C (ascorbic acid) are soluble in water and not stored in the body, because they are passed out as urine when consumed in excess, as a result, the body needs a lot of them for proper functioning. Fat-soluble vitamins, including vitamins A, D, E and K, are usually soluble in fats for proper absorption and storage in the liver (Ayua and Omware 2013; Arasaretnam et al. 2017). There are substantial evidences that leafy vegetables supply reasonable amounts of the recommended daily required vitamins needed by the body per day (Natesh et al. 2017; Davidson and Monulu 2018; and Otitoju et al. 2014). Consumption of green leafy vegetables are useful for proper vision and health function, helps in immune systems and calcium absorption and bone growth, protect the cells of the body from damage, help in converting nutrient into energy and enhances the creation of neurotransmitter and collagen, the main protein in the skin (Otitoju et al. 2014).

Table 10.2 Mineral composition (mg/100 g) of some leafy vegetables

	Potassium	Sodium	Phosphorous	Zinc	Calcium	Magnesium	Copper	Iron	Manganese	References
Amaranth viridis	705	4.57	108	2.63	469	175	Trace	19.08	5.95	Shirley et al. (2019)
Amaranthus hybridus	818	Trace	61	0.99	344	166	Trace	8.87	3.66	Shirley et al. (2019)
Talinum triangulare	832	Trace	20	1.23	188	44	0.29	3.72	1.39	Shirley et al. (2019)
Solanum macrocarpon	709	Trace	64	1.01	248	75	0.43	4.39	1.36	Shirley et al. (2019)
Basella alba	7800.00	660.00	20.50	41.00	1050.00	350.00	0.10	1.40	Trace	Borokini et al. (2017)
Ocium gratissimum	2.60	3.10	Trace	2.00	1.40	17.10	8.00	3.10	4.60	Alexander (2016)
Crassocephalum rubens	4469.91	2129.04	1409		3845.88	434.13	2.6	9.6	8.22	Adjatin et al. (2013)
Telfairia occidentalis	6.43	1.53	58.34	1.02	0.96	0.74	0.14	1.77	0.56	Otitoju et al. (2014)
Corchorus olitorius	8.96	13.43	11.54	67.54	28.05	15.23	2.46	27.25	36.55	Akinwunmi and Omotayo (2016)
Vernonia amygdalina	832	Trace	65	1.23	188	44	0.29	3.72	1.39	Shirley et al. (2019)
Gnetum africanum	181.00	17.15	29.47	0.28	34.22	12.23	36.54	1.31	Trace	Davidson and Monulu (2018)
Muraya koenigii	3.13	46.00	Trace	80.67	3.77	Trace	2.40	9.44	3.38	Uraku and Nwankwo (2015)
Amaranthus dubius	52.15	72.50	30.35	95.14	22.30	26.41	3.94	95.4	15.34	Akinwunmi and Omotayo (2016)
Pterocarpus mildbraedii	91.64	202.37	506.87	122.18	128.87	84.00		12.41	2.86	Akinyeye et al. (2010)
Amaranthus cruentus	53.16	79.50	31.36	95.15	22.31	26.42	3.95	97.22	15.35	Akinwunmi and Omotayo (2016)
Senecio biafrae	536.39	14.48	Trace	3.63	242.09	392.27	0.53	4.16	Trace	Ajiboye et al. (2013)
Moringa oleifera	1583.00	36.00	354.00	3.10	2560.00	600.00	1.90	7.30	2.10	Okerulu and Onyema (2015)

10.3.2 Macronutrients in Leafy Vegetables

Macronutrients are required by the body in large amounts. They provide the body with energy, which is measured in calories or joules. The three macronutrients found in foods are protein, fats and carbohydrates. Every part of the human body, such as the brain, require energy to function properly and therefore must be supplied in sufficient quantities. The main source of energy to the body is glucose, which is richly obtained from carbohydrates through various metabolic processes (Natesh et al. 2017). The production of glucose from carbohydrates depends on the type of carbohydrates, either simple or complex. Being a source of glucose, carbohydrates provide four kilocalories of energy per gram (4 kcal/g). Leafy vegetables are rich sources of carbohydrates, ranging between 10% and 71%, making them a good source of energy.

Protein contributes to energy supply of the body in two ways, firstly, as a source of glucose through a pseudo process called gluconeogenesis, a process by which the body obtains its glucose from a non-conventional carbohydrate source. Secondly, protein contributes its energy quota to the body though amino acids (essential or non-essential amino acids). Amino acids, which are the building blocks of protein, are abundant in leafy vegetables. Famuwagun et al. (2020a) reported that the amino acid composition of *Amaranth viridis*, *Telfairia occidentalis* and *Solanum macrocarpon* are well compared with the amino acids in the leguminous seeds. Protein, which performs other functions in the body other than energy provision, such as growth, tissue repairs and muscle mass building, produces equal amounts of calorie as carbohydrate (4 kcal/g of protein food) (Gupta and Prakash 2009).

Fats enhance various functions in the body such as energy storage, organs cushioning, production of certain hormones, absorption of fat-soluble vitamins and maintaining the integrity of the cell membrane (Adernipekun and Oyetunji 2010). Three types of fats have been identified in food, and these include trans-fat, saturated and unsaturated fats. While it is advisable to avoid trans-fat, saturated fats increase the cholesterol level of the body and hence could increase the chance of having heart diseases; unsaturated fat is a good fat and usually obtained from plant sources. Fats supply the highest amount of energy to the body by contributing nine kilocalories of energy per gram of fatty food consumed (9 kcal/g). Due to the provision of the highest quantities of energy to the body, fats have been given a bad reputation. However, selecting a good fat such as unsaturated fats are beneficial to a healthy lifestyle (Otitoju et al. 2014; Ajiboye et al. 2013; Alagbe JO 2013). Fat content of leafy vegetables is usually determined using a Soxhlet extraction apparatus, although it is difficult to estimate using the apparatus, because of the chemical bond that exists between the lipids and chlorophyll in the leaves (Famuwagun et al. 2020a). Determining the fatty acids using gas chromatography may be a better representative of the fats in the leaves. Another type of fat that is found in leafy vegetables is the Omega-3-fatty acids. This fat has been suggested to play important roles in body growth and in the prevention of various cardiovascular diseases (Hamazaki and Okuyama 2001). The presence of this fat (Omega-3-fatty acids) has been reported in some leafy vegetables grown in other parts of the African continent, such as α-linolenic acid with 4 mg/g fresh weight (Simopoulos 2002). The same study also reported the presence of α-linolenic acid in some leafy vegetables such as 1.7 mg/g in spinach, 1.1 mg/g in mustard greens, 0.7 mg/g in red leaf lettuce and 0.6 mg/g in butter crunch lettuce, but omega 3-fatty acids have not been widely reported in most of the leafy vegetables grown in Nigeria.

10.4 Utilization Potentials of Leafy Vegetables in the Development of a New Class of Food

Leafy vegetables have many potentials in the development of a new class of foods, either as functional ingredients, nutraceutical in the fortification and enrichment of other food commodity. Each of these forms of usage comes with its peculiar challenges, prices and advantages. As shown in Fig. 10.1, four different by-products may be obtained from leafy vegetables, which are useful in the enrichment of other food commodities. This section examines the different ways leafy vegetables may be utilized in addition to the conventional uses.

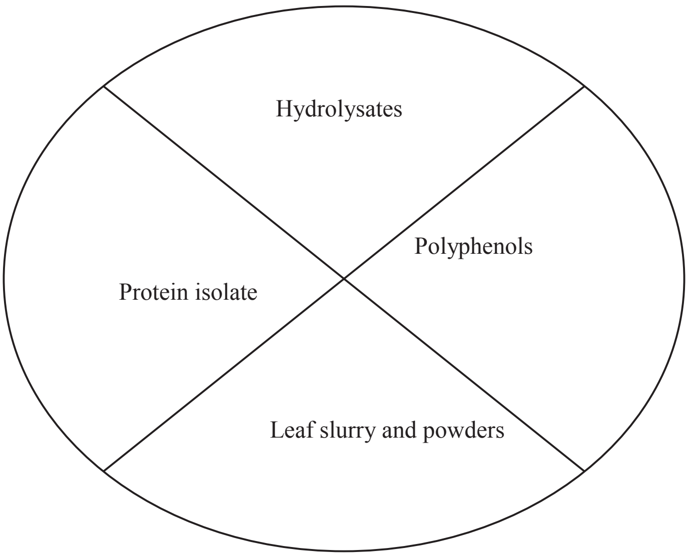

Fig. 10.1 Possible by-products from African leafy vegetable

10.4.1 The Use as Part or Major Soup Ingredients

The vast nutrient in leafy vegetables has increased their utilization potentials in recent times. Leafy vegetables may be consumed fresh in salads, food dressings or as flavouring ingredients (Souzan et al. 2007). In many parts of Africa, LVs are also used as a side dish to accompany a thick starchy gruel that is the primary carbohydrate source. However, the most popular form of consumption is in their use as sources of fibre, minerals and vitamins in various home dishes (Nnamezie et al. 2019). In this case, the leaves are steamed. The leaves, after washing with clean water, are sliced and are immersed in boiled water and steamed for a few minutes before their use in soup preparations. It is important to limit the amount of time the leaves spend in the boiled water, to limit leaching of vital nutrients in the water (Nnamezie et al. 2019). In another technique, the leaves are washed and sliced into pieces; they are then poured in the already prepared sauce to cook them for some minutes. An attempt to widen the utilization potential of these leaves have recently resulted in their use as nutrient-enriching ingredients. This is to ensure that the leafy vegetables can be consumed without necessarily being accompanied by any sauce. The use of fresh and dried leafy vegetables has recently been piloted to enrich the nutrient content of local weaning and other convenient foods (Odunlade et al. 2017; Odunlade et al. 2019).

10.4.2 The Leafy Vegetable Slurry and Powders

Leafy vegetables are recognized as the cheapest and most abundant sources of carotenoids, antioxidant compounds, vitamins, pigments and minerals (Souzan et al. 2007). The technology involved in the preparation of leaf slurry and powder is very simple and can be adaptable. The leaves are washed with clean water and then blended with small amounts of water. Water is needed in the operation to ensure smooth and easy blending of the leaves to produce the slurries as shown in Plate 10.1 (Famuwagun et al. 2019). The dried form of the leaves is produced by drying

Plate 10.1 Slurries produced from leafy vegetables

a b

Dried leaves before milling Powders from leafy vegetables

Plate 10.2 Different forms of leafy vegetables after drying. (**a**) Dried leaves before milling. (**b**) Powders from leafy vegetables

already washed leaves in a hot air oven at a temperature less than 60 °C until no change in the dried leaves, which are later packaged as dried leaves (Plate 10.2a) or fine milled to obtain leafy vegetable powder (Plate 10.2b). The leaf slurry and the powders can be used to enrich many food commodities that would guarantee the supply of nutrients, as though it was fresh leaves that were taken (Odunlade et al. 2019). The leaf slurry can be used to improve the nutrient content of local weaning food, popularly called *ogi*.

Ogi is a popular weaning food in some parts of Nigeria, which is obtained through the fermentation of maize or sorghum. *Ogi* serves as weaning food, convalescent food and a dietary staple for adults (Odunlade et al. 2016). *Ogi* has a characteristically white to creamy colour. The colour of *ogi* depends on the colour of the raw material. *Ogi* is starchy food and therefore does not possess enough nutrient for normal body growth (Abioye and Aka 2015). Attempts have been made by several researchers to improve the nutrient base of *ogi*, one of such is the use of leafy vegetable. Odunlade et al. (2019) successfully supplemented maize *ogi* with fluted pumpkin leaf slurry (Plate 10.3). The picture showed different maize *ogi* with different colours because of addition of different percentages of vegetable leaf slurry compared with the sample without leaf slurry. The study found improvement in the nutritional qualities of the resulting ogi, such as mineral composition and antioxidant properties. The outcome of the research showed that traditional weaning foods, such as the maize/sorghum *ogi*, *are* one of the food vehicles that could be used to deliver the vast nutritional components of leafy vegetables to the target organs in the body. Apart from physically adding the slurry to *ogi*, other strategies that could be used to enrich the weaning food with fresh leafy vegetables include co-fermentation process, in which case the leafy vegetable is fermented together with the grains during the preparation of the enriched *ogi*. Another method is co-milling, where the leaves and the grains are milled together for the preparation of the enriched *ogi*. The leafy vegetable can also be squeezed with water and then sieved. After sieving, the

Plate 10.3 Maize ogi enriched with leafy vegetable slurry

supernatant may then be added to the already prepared *ogi* for the purpose of enrichment. In either of the enrichment processes, the quantity of water in the leaves must always be linked with the amount to be used in the preparation. This is necessary not to overshoot the total amount of water needed for the production of enriched products.

Bread and some snacks such as *chinchin* and cookies can also be used as a vehicle to deliver the packet of nutrient in the fresh leafy vegetable to the body. Bread is widely consumed in Nigeria and other parts of the world and is a staple food product in many countries, whose method of preparation has been in existence for a long time (Badifu et al. 2005). It is a product made from wheat, which is low in basic nutrient needed to support life. Famuwagun et al. (2019) documented the use of leaf slurry in the enrichment of bread and hence, as a vehicle to transport the nutrient in the vegetable. Care should also be taken while using the slurry in bread due to excess water. The water added during slurring process should be linked to the water needed for baking to avoid excess use of water in the final product. According to the reports, there were losses in the nutrient of the product when compared with the calculated nutrient of the mixed dough which was attributable to high baking temperature, yet, the residual nutrients in the final product were enough to meet the daily requirements and were higher when compared with the baked bread without leafy vegetable. In this case, the bread is being used as vehicle for delivering the nutrients in the leaves to the consumers.

The leaf powders can also be used to enrich bread as shown in Plate 10.4. While using leaf powder in bread, care should be taken because the presence of leaf powder in the bread recipe beyond the optimal level may impede on the rising ability of the dough during the baking processes. Hence, the need to optimize the quantity of

Plate 10.4 Bread enriched with leafy vegetable powder

leaf powder that would not negatively affect the physical and structural characteristics of the dough and the resultant bread. Famuwagun et al. (2016) demonstrated that the inclusion of 3.65% level of leaf powder was optimal when bread was enriched with fluted pumpkin leaf powder. Subsequently, Odunlade et al. (2017) reported greater retention of antioxidant activities and mineral contents of enriched bread when fluted pumpkin, amaranth and eggplant leafy vegetables powders were added to bread recipe by varying the quantities of the leaf powder between 1% and 3%. Plate 10.4 shows different loaves of bread with different colours, resulting from the use of different proportions of leaf powder in the bread recipe.

10.4.3 Phenolic Compounds in Leafy Vegetables

Leafy vegetables are rich sources of phenols, due to the presence of green pigment. Phenolic compounds are identified by the presence of aromatic rings with one or more hydroxyl groups. They are classified into simple phenol and polyphenols, depending on the number of phenol units (Napal et al. 2010; Kennedy and Wightman 2011). The main dietary phenolic compounds in leafy vegetables are flavonoids, phenolic acids, tannins, lignins and hydroxycinnamic acids. Flavonoids are the commonest form of phenolics occurring in the plant kingdom. The flavonoids, together with carotenoids and chlorophyll, determine the colour of plant materials, either blue, purple, yellow, orange or red colours. Flavonoids is a large family of phenols, consisting flavones, flavonols, iso-flavonols, anthocyanins, anthocyanidins, pro-anthocyanidins and catechins. It is a derivative of aromatic compound with three-ringed rings. Each of the family of flavonoids are differentiated by the kind of reactions (hydroxylation, prenylation, alkalinization and glycosylation) they undergo, which cause changes in their basic molecule (Ferreira and Pinho 2013; Rong 2010; Routray and Orsat 2012). The presence of flavonoids has been reported in some leafy vegetables grown in Nigeria, including *Ocimum gratissimum* (0.65%), *Murraya koenigii* (0.60%), *Amaranth hybridus* (0.27%) (Fagbohun et al. 2011; Uraku and Nwankwo 2015; Oulai et al. 2017) and other leafy vegetables. Phenolic acids are another class of phenolic compounds that occur in the form of esters and glycosides. It is a major class of phenolic compound occurring in plant, which is usually in the bound form. Phenolic acids have two main structures; hydroxycinnamic (ferulic, caffeic, *p*-coumaric and sinapic acids) and hydroxybenzoic acid (gallic, vanillic, syringic and protocatechuic acids), which are differentiated by the amount and the position of the hydroxyl groups on the aromatic rings (Pereira et al. 2009). There are phenolics in the cell walls of plants that is made up of lignins and hydroxycinnamic acids (Vanholme et al. 2010; Naczk and Shahidi 2004). They are very important for maintaining proper posture of plant during growth and protect plants against injuries and the actions of ultraviolet lights. Tannins (hydrolysable and condensed tannins) is another group of phenolic compounds that is present in plant kingdom (Soto-Vaca et al. 2012). Though classified as antinutrient in other plant species, its health benefits and the presence of hydroxyl groups in its molecule made to be part of phenolic compound. The hydrolysable form of tannins is easily hydrolysed with acids, alkaline and enzymes and this includes tannic acid (Giada and de Lourdes 2014). Condensed tannin or proanthocyanidins are difficult to hydrolyse with acids, alkaline and enzymes because it is chemically combined with other compounds. Examples include catechins and leucoanthocyanidin. In some cases, hydrolysable tannins share some similarities with condensed tannins in that it is may be esterified with other compounds such as gal oil, resulting in compounds of high molecular weight (Scalbert and Williamson 2000; Sánchez-Moreno et al. 2002; Hollman 2001).

10.4.3.1 Preparation of Phenolic Compound from Leafy Vegetables

Pre-preparatory processes of leaves usually precede extracting the active ingredients such as polyphenol from leafy vegetable. This step is very crucial in maintaining the integrity of the active ingredients (Famuwagun et al. 2017). The choice of sample preparation step for phenolic extraction from leafy vegetable usually depends on the type and the sample matrix, the properties of phenols, polarity, concentration and the number of hydroxyl radicals within the molecular structure of the phenolic compounds (Singh et al. 2011). The variations in the chemistry of different types of phenolic compounds in the leaves is a major factor to consider during sample pre-preparation processes. The purity of phenolic compounds to be extracted depends on the level of complexation of phenolics with other nutrients like proteins, carbohydrates and minerals within the sample matrix (Quanhong and Caili 2005). After the receipt of the leafy vegetables, the pre-sample preparation steps follow. The leaves are destalked, sorted and rinsed of dirt with water. The choice to dry or not to dry is the first step in the sample preparation procedures. If the leaves are to be dried, various drying methods, such as oven drying, sun drying, freeze-drying and air-drying may be used to reduce the moisture content of the leaves before extraction. Each of these drying methods has its peculiar effects on the content, yield and the functionality of the phenolic compounds being extracted. To maintain the integrity of the samples vis-à-vis the active ingredient, the use of freeze-drying and air-drying may be preferred (Famuwagun et al. 2017). However, freeze-drying is a capital-intensive process, while air-drying may be time-consuming. The use of a temperature-regulated oven for drying is one of the ways to reduce the time consumed during air-drying process, but the temperature of drying needs to be optimized to prevent disintegration of heat labile phenolic compounds. Usually, a drying temperature of $<60^{\circ}$ °C has been suggested in some studies for drying leafy vegetables. Sun drying process is less expensive and not time-consuming unlike air-drying, but the inability to control the natural drying temperature is a huge challenge for researchers, due to dynamic nature of the weather (Nnamezie et al. 2019). The dried samples are then ground finely with blender or sieved afterwards to obtain homogenous particle size leaf powder for extraction. The milled samples are then packaged in airtight containers before use. For some preparation techniques, the drying step is skipped. After the pre-preparation steps, the leaves are wet milled using laboratory mortar and pestle or grinder (Odunlade et al. 2019). In each of the methods of sample preparation (wet or dry milled), the leaf sample must be mixed with solvent to make a suspension during polyphenol extraction. Organic solvents, such as ethanol, acetone, diethyl ether or a mixture of water with these solvents, have been used (Dai and Mumper 2010). Sample to solvent ratios of 1:5, 1:10, 1:15 or 1:20 may be used at different extraction times, depending on the optimization of extraction variables. After the mixing stage, the suspension is first sieved using muslin cloth and later centrifuged to remove insoluble materials. The supernatant is then evaporated to dryness using a rotary evaporator or can be freeze-dried with a freeze dryer in case of aqueous extraction (Famuwagun et al. 2017). The dried material is referred to as crude polyphenol extract (CPE). The CPE may be further purified to different phenolic fractions, using sophisticated equipment such as high-pressure liquid chromatography (HPLC) in order to purify and separate the crude polyphenols into individual phenolic compounds. Studies have reported the bioactivities of polyphenol extracts from leafy vegetables and confirmed their potentials as functional ingredients.

The use of crude and purified phenols in the development of a new class of food may be done in two ways; first, as nutraceuticals, in which case the functional ingredient may be palleted or tableted in to shapes to tackle any perceived illness. The second way is the use in the development of functional foods. The phenols can be added to food commodity suitable to transport the ingredient unhindered to the target organ in the body. However, works still need to be done to ensure the vehicle does not interfere with the perceived bioactive properties of the phenols.

10.4.4 Protein and Peptides in Leafy Vegetables

Leafy vegetables are consumed majorly for their high concentration of phenolic acids, vitamins, especially ascorbic acid (Famuwagun et al. 2017). There is a dearth of information on the protein content vis-à-vis the peptides in the leafy vegetables, as only few publications are available in the research space. However, evidence from recent publications on leafy vegetables have shown that food proteins function as nutrients and also have physiological roles in the body (Famuwagun et al. 2020b). Peptides are small fragments of proteins, which can only be released by enzyme actions (Aluko 2015). The physiological roles of protein in the body are performed by the peptides, which are usually present within the primary protein structure. Due to the biological functions of peptides, they are called bioactive peptides. The food-derived peptides are obtained from food materials through a series of value addition processes of the crude proteins. The leafy vegetables grown in Nigeria have reasonable amounts of proteins, which can enhance their chance for peptide extraction (Famuwagun et al. 2020a). The protein is first isolated from the leafy vegetable powders or slurry in order to increase the protein content. The protein isolate is subjected to enzymatic actions to hydrolyse the protein by breaking the peptide bonds to produce hydrolysates (Girgih et al. 2011). This is called crude protein hydrolysates. The crude protein hydrolysates are then fractionated or purified into peptides of small molecular weights. However, the higher the crude proteins in the food materials, the higher the amounts of protein hydrolysates that could be obtained, though this sometimes depends on the solubility of the proteins to the actions of the enzymes (Udenigwe and Aluko 2011). The quantity of crude proteins in the fresh leafy vegetables is usually low (<5.0%), due to high moisture content, hence the need to reduce the water content of the leaves though various modes of drying techniques, in order to obtain high protein yield. Hydrolysates and resulting fractions can be used as functional ingredients in the development of functional foods or as nutraceuticals (Aluko 2007), which in turn will expand the fortunes of the leafy vegetables in the effort to take it to the industries.

10.5 Isolation of Proteins and Preparation of Hydrolysates from Leafy Vegetables

The sample preparation steps leading to the production of peptides from leafy vegetables is similar to that of polyphenols in terms of destalking, washing and drying or macerating. After this stage, the leaf powder or slurry is subjected to protein isolation process using the combined processes of solubilization, precipitation and neutralization (Ajibola et al. 2011). Prior to these steps, the dried and milled leaf powder is subjected to acetone extraction to reduce load of compounds (polyphenols) that may interfere with the smooth extraction of proteins. Since the protein and polyphenol in the leaf are chemically bonded together (Famuwagun et al. 2020a), care must be taken during the partial removal of chlorophyll from the leaf to avoid loss of proteins. In this case, a 5% suspension of leaf powder in acetone is stirred continuously for 1 h and the acetone layer is filtered using muslin cloth. For the very green leafy vegetables, the process of chlorophyll removal may be repeated for two or three times using fresh absolute acetone. The residual acetone from the depolyphenolic material is removed by spreading under a fume hood for 48 h. For some processes, the leaves are macerated without going through the drying process. In such a method, removal of polyphenol using acetone is not necessary. The leaf slurry is subjected to protein solubilization without regard for polyphenol interference in the process.

In the protein isolation process, the protein solubilization involves adjusting the pH of the suspension of the leaf powder or slurries to that point where the protein is most soluble using either 1 M NaOH or 2 M NaOH (Adebowale et al. 2009). The point at which protein is most soluble must have been pre-determined using Lowry methods of protein determination. For most leaf proteins, the point of highest protein solubility is usually between pH 9 and pH 10. These pH values are maintained for 1 h with continuous stirring. The solution is centrifuged and the residue is discarded. This process removes the insoluble carbohydrate and fibre from the leaf materials. To the supernatant, the pH is adjusted to the point at which the protein is least soluble, also called isoelectric point, whereby the positive and negative charges of the solution is zero. The isoelectric pH value of most leaf proteins is between 3.5 and 4.5. Acidic solutions, such as 1 N HCl or 2 N HCl is usually used to adjust the pH of the solution and this is called precipitation process. The pH value is maintained for another 20 min to allow the solubilized proteins to precipitate, after which the mixture is centrifuged. The supernatant is discarded while the slurry is the precipitated proteins. The significance of this stage is the removal of soluble carbohydrates and minerals. The slurry is then washed with deionized water and pH of the slurry is neutralized prior to freeze-drying to obtain the protein isolate.

Usually, the protein isolate has about >70% protein content. Hydrolysates are mixtures of oligopeptides or polypeptides that are produced using hydrolysis. Hydrolysis is the process of breaking the peptide bonds in the intact proteins either with enzymes or acids. Enzymatic hydrolysis is one of the safest methods to produce crude hydrolysates because it protects the integrity of the peptides (Aluko 2015). In this case, proteases of different grades and specificities (Pepsin, Pancreatin, Alcalase, Chymotrypsin, Trypsin, etc.) are used to break the peptide bonds in the proteins to yield hydrolysates (Udenigwe and Aluko 2011). This is because the peptides are usually present within the structure of the primary proteins. Smaller fractions of the peptides may be produced by subjecting the hydrolysate solutions to ultrafiltration membrane process of different molecular weights, such as <1 kDa, 1–3 kDa, 3–5 kDa or 5–10 kDa. These small fractions of peptides can be further purified using high pressure liquid chromatography (HPLC).

10.5.1 Application of Peptides as Functional Ingredient in Fruit Juice

Hydrolysates and the resulting fractions of some leafy vegetables, such as the fluted pumpkin, amaranth and eggplant leaves have been evaluated to exhibit various bioactivities such as antioxidant, antidiabetic and antihypertensive properties (Famuwagun et al. 2020a, b). The studies, which further produced small molecular weight peptides of different sizes (1 kDa, 1–3 kDa, 3–5 kDa, 5–10 kDa) with greater potentials to function as substances that can be used to tackle various degenerative diseases, resulting from potential effects of free radicals. The study by Graves et al. (2016) showed that active ingredients of this nature can be used as nutritional interventions for various illnesses ravaging the world either through their use as nutraceuticals, in which case they are tableted or as functional ingredients that require the use of food vehicles to transport the peptides to the target organs in the body. Another work by Famuwagun et al. (2019) evaluated the stability of <1 kDa peptide size obtained from three different leafy vegetables in orange juice. The strategies for its use involved the addition of the freeze-dried peptides (after evaluating them for bioactivities) to the fruit juice using the calculated effective or inhibitory concentrations that scavenged/chelated 50% of the radicals (EC50) or inhibited 50% of the Alpha amylase and glucosidase enzymes (IC50). The added peptides were mixed thoroughly with the juice and evaluated for various bioactive properties. The results from the study showed that leaf peptides were stable for about 10 weeks, suggesting that the peptides are good candidates of functional ingredients and fruit juice could be used as a veritable vehicle to deliver bioactive properties of the ingredients to the body.

10.6 Challenges in the Use of Leafy Vegetables or Their By-Products

The interest of the final consumer (s) regarding any new class of food is of paramount importance when such food is being developed. Consumers may shy away from buying any new class of food if such food does not in any way resemble any conventional brand, irrespective of the nutritional constituents. This aspect of marketing poses a great challenge to the manufacturers in a bid to develop a new class of food containing any functional ingredient.

Colour is one of the most important sensory characteristics that influences the decision of a consumer to purchase food commodity. The consumer expects that the colour of a new class of food should not be totally different from the existing ones, to create rooms for comparison. Irrespective of the nutritional constituent of new a class of food, the colour of such food commodity need to be attractive. Leafy vegetables are green in colour, and this is a great challenge for its use for nutrient enrichment. Apart from the form of use of the leaves or its by-products in the preparation of dishes or as part of ingredients in soup preparations, every other product where leafy vegetable is used for enrichment has colour challenge. The use of leaf powder in enriching bread and cookies appeared to be green unless little quantity is used. The study by Odunlade et al. (2017) explained that despite the high nutrient content of bread fortified with 3% vegetable leaf powder, some consumers scored the product very low during the sensory evaluation. Although, the use of lower percentage, such as 1% level of inclusion improved the nutritional status of the enriched bread, this was significantly lower ($p < 0.05$) when compared with that of 3%.

Leafy vegetable has high moisture content of about 80–94%, hence the leaves cannot be preserved for a long time in its fresh form (Hossain et al. 2017). Drying of the leaves is one of the most effective ways of preservation, though in its dry form. Drying leads to loss of nutrients because most of the active ingredients in the leafy vegetables are heat labile (Nnamezie et al. 2019). For instance, there is considerable amount of loss in the vitamin C and mineral contents of fluted pumpkin leaves when dried at 60 °C (Nnamezie et al. 2019). However, drying of some leafy vegetables, such as fluted pumpkin, amaranth and eggplant leaves increased the protein content of the leaves from <5.0% to about 20–35% (Famuwagun 2019). Therefore, the fear of nutrient loss during drying is one of the challenges faced in a bid to use leaf vegetables/their by-products in the development of new class of food.

10.7 Recommendation on the Use of Leafy Vegetables and Their By-Products

The use of leafy vegetables in the enrichment process will, in no small measure, increase utilization potentials of the leaves, which will further enhance productivity. Not only will it improve the utilization but also reduce the colossal post-harvest losses that are usually being experienced during production glut. Although the use of leafy vegetables in the development of new class of food comes with many challenges, especially with the colour and loss of nutrients during drying, the immense benefits should be considered while making the decisions. The consumers of the new class of food should be aware that, most of the time, development of nutritious or medicinal food commodity may affect one or more of the sensory attributes. For instance, in the production of enriched bread and cookies, product that contained higher level of vegetable leaf inclusion resulted in darker or greener appearance, though most nutritious. It is therefore important for consumers to make a choice between healthy living and sensory pleasures. The manufacturers or the producers of the new class of food also need to consider the fact that it is only what the consumer sees good in their sight that can go through the mouth, regardless of how nutritious such food commodity is. Hence, the need to maintain a balance between what the manufacturer wants and the choice of the consumers.

The manufacturers of the new class of food containing leafy vegetable may also need to optimize the level of inclusion of the vegetable leaf by-products in the enriched food commodity, using the sensory characteristics as responses. By doing this, the manufacturer of the new class of food may be able to clear the doubts of the consumers and produce that class of food that will satisfy the sensory and the nutrition needs of the consumers.

Producing a purer form of the active ingredients in the leafy vegetable may also be a way out to satisfy the need of the consumers. For instance, the vegetable leaf powder is usually green in colour. When the protein is isolated, especially the fluted pumpkin, amaranth and eggplant leafy vegetables, with about 95–98% protein content (Famuwagun et al. 2020a), the colours of the proteins are dark (Plate 10.5), which may not still be attractive to some consumers. When the proteins are hydrolysed using proteolytic enzymes, the colour of the hydrolysates are either chocolate

Plate 10.5 Protein isolates from leafy vegetables

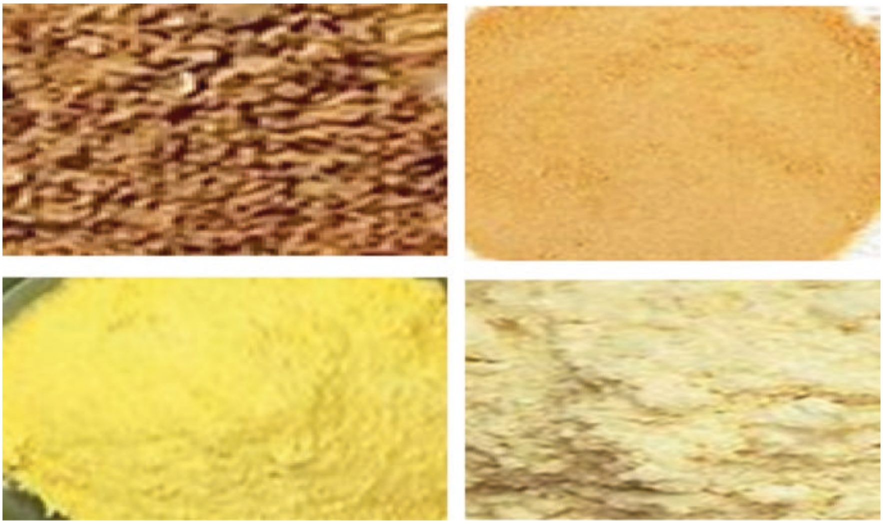

Plate 10.6 Protein hydrolysates obtained by enzymatic hydrolysis by different proteases

in colour or creamy, depending on the enzyme used for the hydrolysis (Plate 10.6). The hydrolysates when used in the enrichment process will result in a new class of food with acceptable colour and this will satisfy both the sensory and the nutrition needs of the consumers. Apart from proteins, polyphenol is another active ingredient that can be purified in leafy vegetables. When the crude polyphenol is obtained from the leaf powder, the colour is usually either green or dark, depending on the type of vegetable. Purifying the crude polyphenol into different phenolic fractions removes the dark or green colour to produce brown colour, which can be used in enrichment process to produce an acceptable functional food.

10.8 Conclusion

Leafy vegetables are a green area of research that has not been well tapped by both the researchers and the manufacturers. Despite the numerous leafy vegetables on the African continent, it is still surprising that the crops have not moved beyond the *pots*. This chapter has discussed the various by-products from the leafy vegetables that can be used to produce a new class of food as well as possible food commodity that can serve as vehicles to transport the nutrients in these by-products to the target organs in the body. However, either most of the leaves are seasonal crops, which are mostly available during the rainy season, transforming the leafy vegetables into another form, through drying or production of functional ingredients, will still afford the consumers to enjoy the benefits of the leaves when not in season. It is therefore important for researchers and manufacturers to work together and make leafy vegetable the economic crop that it truly is.

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