Nutritional quality of wet and dry processed *Moringa oleifera* Lam. Leaves: A Review

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Running title: Wet and dry processed *Moringa oleifera*

Abstract

Moringa oleifera Lam. is a plant species that has found a multitude of applications from health, water clarification and as a food source. In particular, the tree leaves have been consumed in various countries were it is incorporated in the local diet. The advent of food processing technologies have alluded to varies methods to process, preserve and extend the shelf-life of fresh produce. This review focuses on the nutrient quality of *M. oleifera* leaves processed using energy efficient processing technologies such as sun drying, blanching, boiling and fermentation.

Keywords: *Moringa oleifera*, drying, fermentation, blanching

Introduction

Moringa oleifera Lamarck synonym *Moringa pterygosperma* Gaertn. is one of 13 tree species belonging to the Moringaceae family. The species are native to the sub-Himalayan areas of Northern India, Pakistan, Bangladesh and Afghanistan. It is also grown throughout the world in countries like Nigeria, Ghana, China and South Africa [1-3]. *M. oleifera* Lam. is a soft wood tree growing in either dry or moist tropical and subtropical environments of annual rain precipitation between 760 and 2500 mm. Optimum growth is achieved in well-drained sandy or loamy soils of pH range between

5 and 9 [2, 4, 5]. A matured *M. oleifera* Lam. tree is characterised with fast re-growth after pruning, producing approximately 580 t/ha of fresh green shoot biomass annually [6] and reaching an average height of 5 m and a maximum height of 10 m [5]. Among the species in this family, *M. oleifera* Lam. is the mostly cultivated species owing to its fast growth.

M. oleifera Lam. is termed a "miracle tree" due to the versatile use of its leaves, flowers and seeds. The seeds, roots and leaves inherently possess phytochemical, antimicrobial and nutritional compounds, that promote the use of the tree as a medicinal and a food plant [5, 7]. In rural communities the tree is believed to have health benefits and is used in the treatment of ailments such a common cold, inflammation, pain as well as the treatment of diabetes mellitus, hypertension, cholesterol and cancer [8]. *In-vivo* animal studies and *in-vitro* studies have been used to investigate the health benefits of the tree with its potential application as a medicine in treating cancer, diabetes mellitus and hypertension. The trees' leaves and seeds have the highest content of glucosinolate (2; 9). The endogenous plant enzyme, myrosinase catabolizes the gluconosinolates to isothiocyanates, nitriles and thiocarbamates [10] which are reported to lower blood pressure and have muscle relaxant effects [8]. The antioxidant properties of the tree is attributed to the presence of polyphenols such as flavonoids and flavonol glycosides found in the flowers and leaves [10]. Potential antitumor and anticancer activity of the tree is attributed to the o-ethyl-4-(α-(L-rhamnosyloxy) benzyl carbamate, 4-(α-(L-rhamnosyloxy) benzyl isothiocyanate, while niazimicin is reported to be a chemopreventative agent [8, 11, 12].

Moringa oleifera Lam. can be described as a traditional leafy vegetable (TLV) owing to the fact that the tree is not indigenous to Africa compared to African leafy vegetable (ALV) that are native to the continent [13]. The consumption of TLV and/or

ALVs is mainly determined by the poverty status, degree of urbanization and season of the year [14, 15]. In South African rural communities, TLVs or ALVs are consumed as part of the diet in times of short food supply [14], thus can be an important food security crops.

Food cultural practices of a specific region determine the preparation of TLVs or ALVs and the daily intake. *M. oleifera* leaves are consumed as a fresh vegetable or cooked with other food [3, 16]. In Nigeria for instance, the leaves are consumed as a vegetable on their own, used as tea or combined with groundnut cake and spices, mixed with bitter or water leaves, or Egusi (fat and protein rich seeds of cucurbitaceous plants (squash, melon or gourd) [17] or in soup preparation [18]. In Niger, the cooked leaves are flavoured with groundnuts for a midday meal [19]. Whereas in India, the young pods are consumed as a vegetable with rice, whereas mature pods are used in preparation of soups and stews or cooked with pea pulse. The leaves are either used as a condiment, included in salads or cooked with pumpkin or potato. The flower buds, young flowers are mixed in gram flour batter and consumed after deep frying [3].

In developing countries such as those in Sub-Saharan Africa, malnutrition is prominent with the main diet being staples such as cereals, tubers and plantains. These staples are energy dense and lack micronutrients [20]. *M. oleifera* Lam. is reported to possess a multitude of nutrients and the consumption of the edible parts has an influence on the nutritional and health status of the consumer. Dry (natural sun drying, solar drying) and wet (blanching, boiling and steaming) processing technologies and fermentation are most commonly used food processing technologies in households due to their low energy requirements, ease of application and are readily available. It is thus that this review discusses the effect of the above mentioned dry and wet processing technologies on the nutritional quality of *M. oleifera* leaves.

In developing this review manuscript, it became apparent of the lack of reputable literature on *M. oleifera* Lam. A search using ISI Web of Science Core Collection and Scopus revealed 32 review articles, 317 ISI journal research articles and reviews, and 518 research articles and reviews from non-ISI journals over a period of 10 years (2009 to 2019). The lack of peer reviewed literature possibly hinders on strategic research on the topic as there is no reputable literature to refer to or base critical discussion. The credibility of the majority of work in non-ISI papers is questionable and rather challenging to interpret. Furthermore, this exposes the fact that although *M. oleifera* is reported to have a history of domestic use in food [21] and treatment of health ailments [10], the data from such activities including household processing hasn't been captured in a reputable scientific manner or reputable journals. In this review, care has been taken to cite literature found to have undergone peer review and is sourced from ISI indexed journals. In most cases, where there is lack of available literature on *M. oleifera*, discussion are based on other leafy vegetables.

Nutritional value of *Moringa oleifera* Lam. leaves

M. oleifera Lam. is reported to be rich in protein, carbohydrates; and micronutrients such as minerals - calcium, manganese, iron; zinc; and vitamins A, C, E and folic acid, and essential amino acids in comparison to spinach (*Spinacia oleracea*) (Table 1). This is also supported by the review done by Falowo et al. [5], who looked at reported nutritional content of *M. oleifera* leaves. This nutrient rich status of *M. oleifera* is viewed as a good nutrient source to enrich or fortify foods [7]. In food enrichment, micronutrients are added to a food irrespective of whether the nutrients were originally present in the food before processing or not. Whereas, in food fortification an essential micronutrient content is deliberately increased e.g. vitamins and minerals (including trace elements) in a food, so as to improve the nutritional quality of the food supply and provide a public health benefit

Table 1: Nutrient composition of raw *Moringa oleifera* Lam. leaves versus raw *Spinacia oleracea* leaves from USDA [28]

Nutrient	***M. oleifera***	***S. oleracea***
Protein (g/100 g)	9.40	2.86
Fat (g/100 g)	1.40	0.39
Crude fibre (g/100 g)	2.0 (total dietary)	2.2
Ash (g/100 g)	2.26	nt
Carbohydrate (g/100 g)	8.28 (by difference)	3.63
K (mg/100 g)	337	558.0
Ca (mg/100 g)	185	99.0
P (mg/100 g)	112	49.0
Mg (mg/100 g)	42	79.0
Fe (mg/100 g)	4	2.71
Zn (mg/100 g)	0.60	0.53
Mn (mg/100 g)	1.063	nt
Vitamin A	378 µg/100 g (RAE) or 7564 IU/100 g	469 (µg/100 g) or 9377 IU/100 g
Vitamin E (mg/100 g)	nt	2.03 (alpha-tocopherol)
Vitamin C (mg/100 g)	51.7 (total ascorbic acid)	28.1
Vitamin B1 (mg/100 g)	0.257	0.078
Vitamin B2 (mg/100 g)	0.660	0.189
Vitamin B3 (mg/100 g)	2.220	0.724

nt = not tested

Carbohydrate Factor: 3.57, Fat Factor: 8.37, Protein Factor: 2.44 and Nitrogen to Protein Conversion Factor: 6.25 (for the raw *M. oleifera* leaves)

with minimal risk to health e.g. to correct a deficiency disease for a particular nutrient [22].

Shiriki et al. [23] investigated the preparation of an infant diet using maize, soybean and peanut flour to give a 16 g protein/100 g food. The flours were fortified with 5, 10 and 15% *M. oleifera* Lam. leaf powder. The addition of *M. oleifera* leaf powder improved the protein and ash content of the food preparations (Table 2) when compared to the control and a commercial baby formula. The fortification of maize, millet weaning formulation with 20 and 25% *M. oleifera* Lam. flower resulted in 15.54 and 16.06% protein content, respectively [24] in the complementary food formulations. In the studies by [24] and [21], the formulations surpassed the Reference Nutrient Intake (RNI) (9.1 g/day and 11.0 g/day protein for an infant between 0 to 6 months and 7 to 12 months, respectively[1]). *M. oleifera* leaves contain 9.40 g/100 g of protein (Table 1), which is more than twice the protein content of spinach, thus makes the leaves a suitable food source for protein enrichment.

[1]Based 1.5 g/kg/day for infants

Table 2: Protein and mineral content of dishes fortified with *M. oleifera* Lam. leaves

[1]Food	Protein %	Ash	Cu (mg/100 g)	Fe (mg/100 g)	Mn (mg/100 g)	Zn (mg/100 g)	β-carotene (mg/100 g)	References
[2]Porridge (non-fortified)	nt	nt	0.09 ± 0.11^{a}	0.36 ± 0.49^{d}	0.15 ± 0.17^{b}	0.40 ± 0.46^{f}	0.02 ± 0.00^{a}	[27]
Fortified porridge	nt	nt	0.15 ± 0.13^{c}	2.13 ± 1.64^{g}	0.26 ± 0.08^{d}	0.39 ± 0.21^{f}	1.28 ± 0.04^{c}	[27]
[3]*Waakye* (non-fortified)	nt	nt	0.10 ± 0.03^{ab}	0.96 ± 1.28^{e}	0.10 ± 0.01^{a}	0.56 ± 0.62^{g}	0.08 ± 0.05^{a}	[27]
Fortified *Waakye*	nt	nt	0.14 ± 0.22^{bc}	2.35 ± 3.83^{g}	0.62 ± 0.31^{e}	0.78 ± 0.69^{h}	0.94 ± 0.51^{bc}	[27]
Groundnut soup (non-fortified)	nt	nt	0.10 ± 0.13^{ab}	1.54 ± 0.69^{f}	0.20 ± 0.04^{c}	0.40 ± 0.36^{f}	0.34 ± 0.06^{ab}	[27]
Fortified Groundnut soup	nt	nt	0.10 ± 0.26^{ab}	2.62 ± 2.43^{g}	0.26 ± 0.30^{d}	0.43 ± 0.16^{f}	2.98 ± 0.39^{d}	[27]

nt = not tested

a-h = means with the same superscript in a column are not significantly different ($p<0.05$)

[1] Food - Porridge, *Waakye* and Groundnut soup fortified with 3 g dried *M. oleifera* Lam. leaves per 100 g product

[2] Porridge – composite white maize, groundnut and white cowpea meal

[3] Waakye – boiled red cowpea and rice

Table 2 *cont.*

Food	Protein %	Ash	Cu (mg/100 g)	Fe (mg/100 g)	Mn (mg/100 g)	Zn (mg/100 g)	β-carotene (mg/100 g)	References
M. oleifera Lam. leaves	nt	nt	0.4±0.1	8.6±0.8	2.3±0.4	7.7±0.8	nt	[35]
Maize, soybean, peanut flour (non-fortified)	16.04±0.02	1.40±0.01	nt	nt	nt	nt	nt	[22]
Fortified maize, soybean, peanut flour (5, 10 & 15%)	16.56±0.04, 17.08±0.01 & 17.59±0.01	1.70±0.02, 2.20±0.01 & 2.50±0.01	nt	nt	nt	nt	nt	[22]

nt = not tested

a-h = means with the same superscript in a column are not significantly different ($p<0.05$)

During digestion and absorption, dietary proteins are broken down to amino acids which become building blocks of functional and structural compounds. The role of dietary proteins can be studied through Protein Efficiency Ratio (PER) - relationship between weight gain and the corresponding protein intake, and Net Protein Ratio (NPR) – an indication of the protein quality based on weight changes from the consumption of a test diet. Shiriki et al. [23] studied the PER and NPR of test diet (maize, soybean and peanut at 60:30:10) fortified with *M. oleifera* and a control diet (no fortification). The subjects of the study had PER improve from 1.77 (unfortified) to 1.89 and 1.90, while NPR was from 1.89 (unfortified) to 2.15 and 2.38 for the 5 % and 10% *M. oleifera* fortification, respectively. When compared to a diet of egg:rice, egg:corn, egg:wheat and egg:beans the PER was found to be 2.78, 2.42, 2.23 and 2.16, respectively from a mixture ratio of 30:70 (egg, cereal or vegetable). The digestibility of the egg:rice, egg:corn, egg:wheat and egg:beans mixed diets was 77.8, 78.5, 86.9 and 66.5%, respectively. The PER of an egg only diet was found to be 3.40 with an 88.5% digestibility [25]. Both these protein sources revealed to increase the PER of a food product and improve digestibility. Hassan et al. [26] found that the addition of 0.5% *Moringa oleifera* leaf powder (MOLP) increased the essential amino acids histidine, isoleucine, leucine, lysine, phenylalanine, threonine and valine in yoghurt. Indeed, essential amino acids account for 44% of the total amino acid content in *M. oleifera* leaves [27].

Nutrient bioavailability is defined as the proportion of an ingested trace element in food that is absorbed and utilized for normal metabolic and physiological functions or storage [28]. Table 2 exhibits the fortification of food fed to children between 4 and 12 years with *M. oleifera* leaf powder to improve protein and mineral content. These nutrients are required by the body in the synthesis of tissues and overall well-being of the consumer. The RNI for zinc for children 7 to 9 years is between 3.3 and 11.3 mg/day for

high and low bioavailability (Table 3). Whereas the RNI for iron based on iron bioavailability between 5 to 15% is 18 to 6 mg/day for children between the ages of 7 to 9 years (Table 3). Food products which have high bioavailable nutrients require much less of the nutrient per day. In the case of the Moringa fortified (3 g/100g product) dishes (Table 2), the children (4 to 12 years) consuming the prepared food would not be able to meet the RNI for zinc (as suggested in Table 3) per serving of the food dish even when the dish were to be consumed three times in a day. Whereas the same fortification can meet the iron bioavailability (12 to 15% as suggested in Table 3) when the children are fed the fortified porridge, *Waakye* and groundnut soup three times a day. The feeding of fortified porridge and groundnut soup would provide 213.76 µg RE and 497.66 µg RE of vitamin A, respectively. This provides double the amount of RNI if the food is served three times a day.

Even though *M. oleifera* leaves are in rich protein source, the consumption of the leaves and the subsequent bioavailability of the nutrient(s) are of paramount importance. A low consumption with regards to quantity of a 15% *M. oleifera* fortified formulation resulted in lower PER. The low consumption was attributed to an undesired sensory effect of the formulation at this fortification concentration [23]. However, Arise et al. [24] found that a 20% and 25% *M. oleifera* flower fortification was palatable as did the leaf fortification at 2, 3 or 5g/100 g per food dish e.g. porridge, groundnut soup and *Waakye* [29]. However, caution has to be practised with regards to the concentration to be added. The low or limited bioavailability of the minerals in Table 2, could be attributed to the presence of anti-nutritional factors which bind and make the minerals not bioavailable. The effects of anti-nutritional factors are discussed in later sections.

Table 3: Recommended Nutrient Intake of minerals adapted from [29]

Age		Calcium (mg/day)	Magnesium (mg/day)	Zinc			Iron			
				High bioavailability (mg/day)	Moderate bioavailability (mg/day)	Low bioavailability (mg/day)	15% Bioavailability (mg/day)	12% Bioavailability (mg/day)	10% Bioavailability (mg/day)	5% Bioavailability (mg/day)
Children	7-9 years	700	100	3.3	5.6	11.2	5.9	7.4	8.9	17.8
Adolescents	Males[4]	1300	230	5.1	8.6	17.1	9.7[6]	12.2[6]	14.6[6]	29.2[6]
							12.5[8]	15.7[8]	18.8[8]	37.6[8]
	Females[5]	1300	220	4.3	7.2	14.4	9.3[5]	11.7[6]	14.0[6]	28.0[6]
							21.8[6]	27.7[7]	32.7[7]	65.4[7]
							20.7[7]	25.8[8]	31.0[8]	62.0[8]

Table 3 cont.

[4] During the growth spurt (10-18 years for zinc)
[5] Pre-menarche, 11 to 14 years (also 11 to 14 years for males)
[6] Menstrual phase, 11 to 14 years
[7] 15 to 17 years

Age		Calcium (mg/day)	Magnesium (mg/day)	Zinc			Iron			
				High bioavailability (mg/day)	Moderate bioavailability (mg/day)	Low bioavailability (mg/day)	15% Bioavailability (mg/day)	12% Bioavailability (mg/day)	10% Bioavailability (mg/day)	5% Bioavailability (mg/day)
Adults	Males 19-65 years	1000	260	4.2	7.0	14.0	9.1	11.4	13.7	27.4
	Females 19-50 years	1000[7]	220[7]	3.0[7]	4.9[7]	9.8[7]	19.6[8]	24.5[7]	29.4[7]	58.8[7]
	Females 51-65 years	1000[9]	220[8]	3.0[8]	4.9[8]	9.8[8]	7.5[8]	9.4[8]	11.3[8]	22.6[8]
Pregnant adults	First trimester	ns	220	3.4	5.5	11.0	[10]	[9]	[9]	[9]
	Second trimester	ns	220	4.2	7.0	14.0	[9]	[9]	[9]	[9]
	Third trimester	1200	220	6.0	10.0	20.0	[9]	[9]	[9]	[9]

ns = not specified

[8] Pre-menopause

[9] Menopausal

[10] It is recommended that iron supplements in tablet form be given to all pregnant women because of the difficulties in correctly evaluating iron status in pregnancy. In the non-anaemic pregnant woman, daily supplements of 100 mg of iron (e.g. as ferrous sulphate) given during the second half of pregnancy are adequate. In anaemic women higher doses are usually required.

Although, *M. oleifera* possess a multitude of nutrients (Table 1), the nutrient content of harvested leaves for consumption is affected by geographical, plant physiology and genetic factors. Ontogenic, innate variability of species, cultivar differences, cultivation soil type, growth environment, leaf maturity, transport and catabolism properties of the individual plant tissues affect the nutrient content of the leaves [2]. The soil type and soil nitrogen content have a direct impact on the plants' protein content. Nitrogen present in the soil is used to synthesize amino acids and proteins, which are stored in the plant parts such as leaves.

Research investigations on the nutrient content of *M. oleifera* Lam. leaves have reported on differing content range [2, 6, 32-35]. Cultivar and variety differences were reported by Nouman et al. [6] and regional differences [35]. Therefore, it can be considered that the nutrient content of *M. oleifera* is best described as a mean range based on available reliable data from analysed leaves. Olson et al. [36] found that uniform growing conditions also yielded nutrient content differences among the 23 *M. oleifera* species tested [36] (Table 5). Furthermore, to fully quantify the nutrient content of *M. oleifera* is challenging due to the different methods used for quantification by the respective researchers, units of measurement, source and time of harvest of the leaves [15].

Table 4: Recommended Nutrient Intake of vitamins adapted from [29]

Age		Water soluble vitamins					Fat soluble vitamins	
		Thiamin (mg/day)	Riboflavin (mg/day)	Niacin[11] (mg NE/day)	Folate[12] (µg DFE/day)	Vitamin C[13] (mg/day)	Vitamin A[14,15] (µg RE/day)	Vitamin E (acceptable intakes)[16] (mg α-TE/day)
Children	7-9 years	0.9	0.9	12	300	35	500	7[17]
Adolescents 10-18 years	Males	1.2	1.3	16	400	40	600	10
	Females	1.1	1.0	16	400	40	600	7.5
Adults	Males (19-65 years)	1.2	1.3	16	400	45	600	10
	Females (19-50 years[18])	1.1	1.1	14	400	45	500	7.5

[11] NE = Niacin equivalents, 60 to 1 conversion factor for tryptophan to niacin

[12] DFE = Dietary folate equivalents; µg of DFE provided = [µg of food folate + (1.7 x µg of synthetic folic acid)].

[13] An RNI of 45 mg was calculated for adult men and women and 55 mg recommended during pregnancy. It is recognised however that larger amounts would promote greater iron absorption if this can be achieved.

[14] Vitamin A values are "recommended safe intakes" instead of RNIs. This level of intake is set to prevent clinical signs of deficiency, allow normal growth, but does not allow for prolonged periods of infections or other stresses.

[15] Recommended safe intakes as µg RE/day; 1 µg retinol=1 µg RE; 1 µg β-carotene=0.167 µg RE; 1 µg other provitamin A carotenoids=0.084 µg RE.

[16] Data were considered insufficient to formulate recommendations for this vitamin so that "acceptable intakes" are listed instead. This represents the best estimate of requirements, based on the currently acceptable intakes that support the known function of this vitamin.

[17] Values based on a proportion of the adult acceptable intakes.

[18] Pre-menopause

Table 4 *conti.*

Age		Water soluble vitamins					Fat soluble vitamins	
		Thiamin (mg/day)	Riboflavin (mg/day)	Niacin[19] (mg NE/day)	Folate[20] (µg DFE/day)	Vitamin C[21] (mg/day)	Vitamin A[22,23] (µg RE/day)	Vitamin E (acceptable intakes)[24] (mg α-TE/day)
Adults	Females (51-65 years[25])	1.1	1.1	14	400	45	500	7.5
Pregnancy		1.4	1.4	18	600	55	800	X[26]

[19] NE = Niacin equivalents, 60 to 1 conversion factor for tryptophan to niacin

[20] DFE = Dietary folate equivalents; µg of DFE provided = [µg of food folate + (1.7 x µg of synthetic folic acid)].

[21] An RNI of 45 mg was calculated for adult men and women and 55 mg recommended during pregnancy. It is recognised however that larger amounts would promote greater iron absorption if this can be achieved.

[22] Vitamin A values are "recommended safe intakes" instead of RNIs. This level of intake is set to prevent clinical signs of deficiency, allow normal growth, but does not allow for prolonged periods of infections or other stresses.

[23] Recommended safe intakes as µg RE/day; 1 µg retinol = 1 µg RE; 1 µg β-carotene = 0.167 µg RE; 1 µg other provitamin A carotenoids = 0.084 µg RE.

[24] Data were considered insufficient to formulate recommendations for this vitamin so that "acceptable intakes" are listed instead. This represents the best estimate of requirements, based on the currently acceptable intakes that support the known function of this vitamin.

[25] Menopausal

[26] For pregnancy and lactation there is no evidence of requirements for vitamin E that are any different from those of older adults. Increased energy intake during pregnancy and lactation is expected to compensate for increased need for infant growth and milk synthesis. Breast milk substitutes should not contain less than 0.3 mg α-tocopherol equivalents (TE)/100 mL of reconstituted product, and not less than 0.4 mg TE/g PUFA. Human breast milk vitamin E is fairly constant at 2.7 mg for 850 ml of milk.

Table 5: Mean protein and mineral content of 23 *M. oleifera* species sourced from America, Africa and Asia grown under uniform conditions adapted from Olson et al. [35].

Nutrient	Nutrient content	Kruskal Wallis statistic (χ[2])	*p*-value
*Total protein (g/100g)	29.1 (24.8-35.3)	78.05	<0.001
*Soluble protein (g/100g)	25.9 (21.3-34.7)	38.32	<0.001
Calcium (µg/g)	16046.7 (8940.0-22284.7)	55.69	<0.001
Iron (µg/g)	97.9 (72.7-124.1)	92.10	<0.001
Magnesium (µg/g)	2833.8 (1738.7-5055.0)	64.65	<0.001
Manganese (µg/g)	59.8 (34.0-97.6)	98.25	<0.001
Sodium (µg/g)	117.4 (56.0-272.7)	123.98	<0.001
Potassium (µg/g)	17450.0 (13544.2-24930.0)	86.45	<0.001
Phosphorus (µg/g)	4827.4 (3665-6829)	85.14	<0.001
Zinc (µg/g)	291 (18.8-58.3)	104.07	<0.001

Parenthesis: range of nutrient content from 23 *M. oleifera* species sampled
All tests were 12 degrees of freedom
*Protein = 11 degrees of freedom

Effect of dry processing methods on the nutrient content of *Moringa oleifera* Lam. leaves

Drying is the removal of unbound and bound water from the surface and interior of a food product till a desired moisture content is reached. Heat and mass transfer occur simultaneously during drying while the product is exposed to the drying temperature conditions and relative humidity. Leaf drying occurs in two stages, firstly is the warm-up preheating period followed by the falling rate drying period. The first stage is characterized by the product absorbing heat and evaporating the bound water as the

unbound water is insignificant in leaves. The second stage of drying - the falling rate drying period, the drying rate decreases as the moisture inside the leaf is slowly transported to the surface by a gradual increasing leaf temperature before it is evaporated from the surface of the leaf. When drying reaches equilibrium moisture content i.e. no increase in exchange of moisture between leaf and the surrounding air, the drying operation is stopped [38].

The above drying mechanism has been observed when bitter leaves (*Vernonia anyadalina*), crain-crain leaves (*Corchorus olitorus*), and fever leaves (*Ocimum viride*) were sun dried [39], and no detailed literature was found for *M. oleifera* leaves. The drying process is determined by the initial quantity of moisture to be removed. Furthermore, the drying time is dependent on particle size, spread thickness of leaves and external factors such as temperature, relative humidity and air velocity [38]. Sobukola et al. [39] observed a slower drying rate and longer drying period for bitter leaves (*V. amyadalina*) compared to the crain-crain leaves (*C. olitorus)* and fever leaves (*O. viride*). The authors found that the broad nature of the *V. amyadalina* leaves allowed for a small surface area which resulted in less water to be removed per time therefore increasing the drying time for these leaves. Increased drying time in addition to the drying conditions (low drying temperature, thickness of spread and small volume of air) influences the nutrient and sensory quality of the product. These factors can lead to quality deterioration of the product [38].

Solar radiation - natural sun and solar drying

Fresh leaves are highly perishable due to a high transpiration and respiration rate under ambient conditions [40]. Natural sun drying is one of the methods used to extend the shelf-life of fresh ALVs were natural solar radiation from the sun is used to evaporate moisture from ALVs by rural communities. Drying methods that utilize solar radiation

are grouped based on the mechanism of drying (Table 6).

Table 6: Solar radiation drying methods or techniques

Solar radiation		**Design and mechanism of drying**	**Reference**
Natural sun drying	Open sun drying	Fresh produce is layered on a flat surface and exposed to solar radiation, wind and other ambient conditions. The fresh produce can either be covered with a cloth or left bare. The moisture is evaporated from the fresh produce as a result of the solar radiation heating the produce and the surrounding air thereby increasing the rate of water evaporation from the surface and interior of the produce.	[40]
Solar drying	Direct solar dryer	A rectangular cabinet fitted with a transparent glass at the top and a black interior surface. The cabinet has holes drilled on the sides to allow ventilation. Solar radiation which heats the fresh produce is transmitted through the glass layer and absorbed by the black surface.	[41]
	Indirect solar dryer	Two stationary systems exists a solar collector and a drying chamber. Solar radiation is collected on a solar radiation collector in which surrounding air is heated then directed to a drying chamber were the heated air dries fresh produce layered on stacked trays.	

Natural sun drying requires low capital, simple to no equipment and low energy input, making it an ideal method for rural communities [39]. The harvested leaves are firstly washed in cold water then placed on drying trays. Often the trays are covered by a mesh cloth to ward off flies and insects. The trays are exposed to sun radiation from morning to the afternoon, equating to about 8 or 9 h of drying time. The evaporation of

interior moisture depends on the particular produce being dried and the air temperature of drying. Solar radiation heat is not controlled, therefore, it is intermittent and varying, thus the leaves may over or under dry [41].

Alakali et al. [44] observed a decrease in ascorbic acid (AA) in *M. oleifera* leaves shade and oven dried at temperatures between 30°C and 70°C. Shade drying resulted in 87.55% reduction of AA content while oven drying at 60°C and 70°C resulted in 88.32% reduction. Carotenoids were less affected by these drying conditions as only 13.09%, 19.73% and 17.7% was lost during shade, oven drying at 60°C and 70°C, respectively. Mosha et al. [45] observed a decrease in AA concentration of pumpkin leaves after open sun drying for 24 h from 49.17±0.62 mg/100g *fwb* to 2.91±0.00 mg/100g *fwb*. This decrease was also observed for the vitamins riboflavin and thiamine, were after drying the content was 10.67±0.16 µg/g *fwb* and 0.033±0.001 µg/g *fwb* from an initial concentration of 17.14±0.11 µg/g *fwb* and 0.093±0.001 µg/g *fwb*, respectively. Heat exposure, light and oxygen are factors that affect nutrient content during drying [46]. Ascorbic acid degradation is affected by moisture and temperature during drying. In the initial drying stages, the effect of moisture content is predominant and the effect of temperature becomes dominant as the drying process proceeds. In the initial stages of drying, the integrity of the cell structure is still intact thereby protecting AA from oxidation reactions as it is imbedded in the cells [47] thus not much degradation is observed. The further decrease in moisture as drying proceeds allows for the heat degradation of AA as a result of high temperature exposure and the degradation of the cellular structure.

Clydesdale et al. [48] noted that dehydration of fruits and vegetables increased the surface area of the produce and led to very poor stability of carotenoids unless the products are protected from air and light. Carotenoids in plants are found in free form or

esterified with fatty acids [49] localized in plastids i.e. in chloroplasts in association with proteins and in chromoplasts were they are deposited in crystalline form or as oily droplets [50]. Due to the hydrophobic nature of carotenoids, water serves as a protective shield to autoxidation of carotenoids by oxygen and light [48]. When water is evaporated off from a product, the carotenoids are then susceptible to degradation. This is attributed to the oxygen present in the cells which catalyses the activity of the peroxidase enzyme which can oxidize the carotenoids. Lavelli et al. [51] found that a_w of 0.31 to 0.54 corresponding to 6 to 11% moisture (wet weight basis) maintained maximum carotenoid stability in blanched and unblanched carrots.

Carotenoids (α- and β-carotene) have a series of conjugated double bonds and exist in the stable all-*trans* configuration with β-carotene dominant than α-carotene. The presence of the double bonds makes carotenoids more susceptible to degradation either through *cis*/*trans* isomerization by light, oxygen, heat, acids or oxidation during processing. The *cis* forms found after degradation of carotenoids are 15-*cis*-β-carotene, 13-*cis*-β-carotene and 9-*cis*-β-carotene. Oxidation of β-carotene on the other hand is either through autoxidation, photo-oxidation or enzymatic oxidation. The degradation mechanism is postulated to be initially through isomerization followed by oxidation [52] (Figure 1). *Cis*-isomers and oxidation products of carotenoids have less vitamin A activity than the all-*trans* form. During the sun drying process, photo-oxidation of both *trans*- and *cis* carotenoids takes place resulting in epoxidation and cleavage to apocarotenals (Figure 1) before fragmentation into a series of low mass compounds thus losing their biological activity [53]. The *cis* form is estimated to have only 38 to 53% of the biological activity of the all-*trans* forms [54]. Specifically, all-*E*-β-carotene, 13-*Z*-β-carotene, all-*E*-α-carotene, 9-*E*-β-carotene, 13-*Z*-α- carotene and 9-cis-α-carotene exhibit provitamin A activity of 100, 53, 53, 38, 16 and 13%, respectively [49].

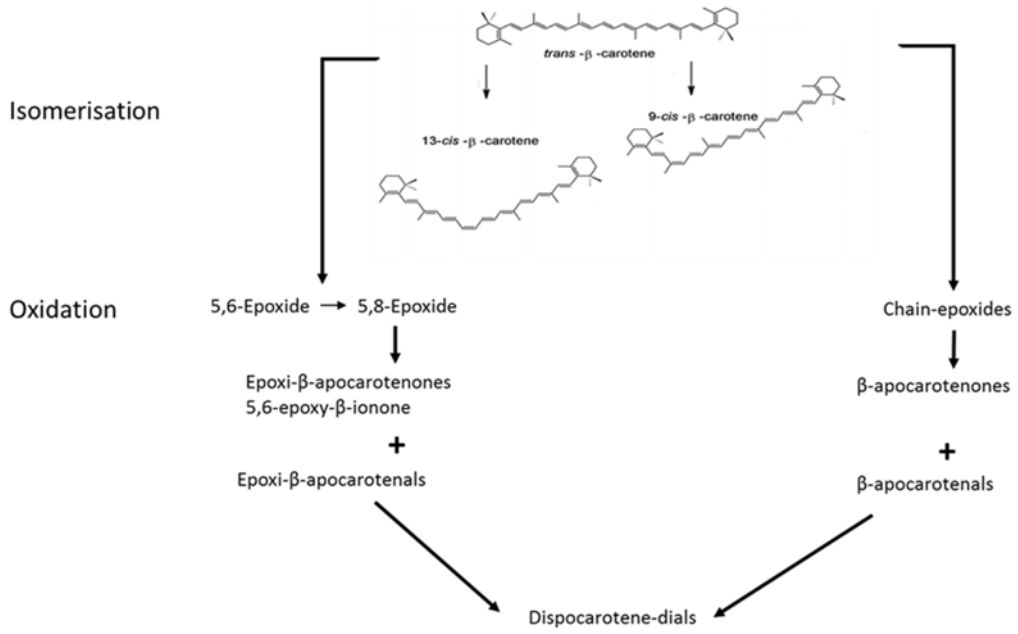

Figure 1. Isomerization and oxidation of all-trans-?-carotene (Adapted from [52].

Sun and solar dried *M. oleifera* leaves (2-3 days) exhibited higher nutrient content than fresh leaves (Table 7) particularly for β-carotene content. Considering the effect of solar drying on nutrient content as discussed above, the difference can be attributed to fact that the leaves were harvested from different regions, and geographical and agronomic differences are reported to influence the nutrient content of fresh produce as seen in Table 7 [2]. It is also notable that open sun and solar drying conditions influence the cell structure differently thus affecting the nutrient content. This is explained by the mechanism of drying, duration of drying and temperature during drying which allowed for the intracellular leaf structure to be exposed thereby releasing and concentrating the micronutrients onto the surface [47, 48]. This then allows for detection and quantification of the micronutrients.

Table 7: Effect of sun drying on the nutrient content of *M. oleifera* Lam. leaves

Plant leaves	**Total carotenoid (mg/100g) *dwb***	**β-carotene (mg/100g) *dwb***	**α-carotene (mg/100g) *dwb***	**9-*cis*-β-carotene (mg/100g) *dwb***	**13-*cis*-β-carotene (mg/100g) *dwb***	**15-cis-β-carotene (mg/100g) dwb**	**Retinol Equivalent**	**Ascorbic acid (mg/100g) *dwb***	**Ash (g/100g) *dwb***	**References**
Fresh	68.81 - 98	18.27	nt	nt	nt	0.69	nt	271.0 - 691	9.5±0.2	[58]
2-3 days Natural sun dried	74.2±4.02 - 183.08	32.33±0.02 - 54.42	0.25±0.00	0.54±0.01	0.36±0.00	2.31	5.57	510.00		[55, 58]
Solar dried (no blanching)	91	21.42±1.67	nt	nt	nt	nt	nt	250	10.7±0.1	[27, 66]
Solar dried (blanched)	132	nt	nt	nt	nt	nt	nt	320	8.5±0.2	[66]

nt = not tested

Table 8: Effect of heating temperature and time on all-*trans*-carotene and *cis*-carotene degradation adapted from [55]

Temperature (°C)	All-*trans*-β-carotene (%)							*Cis*-β-carotene (%)						
	0 h	0.5 h	1 h	2 h	3 h	4 h	D - value (h)	0 h	0.5 h	1 h	2 h	3 h	4 h	D – value (h)
25	100	37.5	29.17	8.33	<4.17	0	2.0517	16.67	39.58	27.08	10.42	10.42	ND	2.7099
35	100	52.94	23.53	14.29	0	ND	1.8016	51.76	52.94	29.41	8.82	0	ND	1.8521
45	100	40	20	3.75	0	ND	1.6489	35	42.5	24	7.5	0	ND	1.4425

When *M. oleifera* leaves were sun dried for 2 days at temperatures between 30 and 40°C for 10 h each day, the leaves retained 67 and 59.6% 15-*cis*-β-carotene and *trans*-β-carotene, respectively [55]. Whereas Djuikwo et al. [53] reported a 47% loss of total carotenoid during sun-drying of *M. oleifera* leaves. Xiao et al. [56] found that an increase in temperature encouraged the isomerization (Figure 1) of all-*trans*-β-carotene to *cis*-β-carotene. This resulted in D-values (time required for 90% degradation of enzyme) between 1.65 h and 2.05 h, and 1.44 h and 2.71 h for β-carotene and *cis*-β-carotene, respectively. The identified *cis*-β-carotene were not detected in the heat-treated samples after four hours for all the temperature treatments (Table 8) [56]. Drying temperature seems to be the key determinant in the amount of β-carotene that can be retained.

Although natural sun drying is easily available, it is disadvantageous as it often leads to discoloring by UV-radiation [57]. The color pigment, chlorophyll *a* and *b*, is found floating in liquid medium within the chloroplast embedded in the thylakoid membrane. During natural sun drying, UV radiation results in the loss of chloroplast integrity because the organized pattern of the grana and stroma thylakoids is lost. This allows for the permeation of intracellular ions out of the thylakoid membrane [58]. Heat generated during drying, further contributes to the disruption of the integrity of the plant cell structures thus exposing chlorophyll to heat, air and intracellular enzymes. Chlorophyllase cleaves the phytol chain of chlorophyll *a* resulting in the formation of chlorophyllide [59]. This cleavage is accompanied by the removal of the Mg^{2+} atom (demetalation) in chlorophyll to form pheophytin. The chlorophyllide is further converted to pheophorbide through the loss of the Mg ion while pheophytin is converted to pheophorbide through the loss of its phytol chain through epimerization [60]. Heat degradation of chlorophyll is reported to follow first-order kinetics [59]. In order to preserve the nutrient content, it is advisable to cover the leaves and dry under mild

conditions to preserve the nutritional and sensory quality of the leaves in addition to preventing contamination by insects and microorganisms [57].

On the other hand, solar drying is an elaboration of natural sun drying were solar radiation is utilized to dry fresh produce (Table 6). In comparison to other energy sources used to dry food products; solar energy is abundant, inexhaustible, non-pollutant, renewable, efficient and cheap [39, 61]. Solar drying can heat large volumes of air to flow over products removing moisture [41] rapidly, uniformly and hygienically [62] and reducing drying time by 50% [57]. Indeed the moisture content of *M. oleifera* leaves that were solar dried was found to be 7.64% after 5 h of drying [29] and 5.5% after 12 h of solar drying at 35°C [63].

Ascorbic acid and carotenoid content of *M. oleifera* leaves was reduced when dried using solar drying (Table 7). The same study revealed that solar drying coupled with blanching retained 46.30% AA in comparison to only solar drying. This was not observed for minerals measured as ash content as coupling of blanching and solar drying reduced the ash content of the leaves. The reduced ash content is attributed to mineral leaching or diffusion during blanching as reported by Xiao et al. [74]. The leaves were blanched at 95°C for 10 min to inactivate enzymes that would otherwise contribute to the degradation of the nutrients. It is safe to say that this was achieved based on the retained AA and total carotenoids in comparison to only when the leaves were solar dried. The overall effect of solar drying and blanching increased the carotenoid content to 132 mg/100 g *dwb* of the treated leaves in comparison to the fresh leaves (68.81 to 98 mg/100 g *dwb*) (Table 7). Glover-Amengor et al. [29] used solar dried *M. oleifera* leaves to fortify porridge, rice, soup and bean amongst other meals. The dried leaves exhibited β-carotene, zinc and iron content of 21.42 mg/100 g *dwb*, 6.79 mg/100 g *dwb* and 20.96 mg/100 g *dwb*, respectively. The cooked food exhibited β-carotene content that could meet the RNI.

Effect of wet processing methods on the nutrient content of *M. oleifera* Lam. leaves

Blanching and boiling

Blanching is a wet thermal processing method carried out for 1 to 10 min between temperatures of 75°C and 100°C using either hot water or steam [46, 64, 65]. During hot water blanching, the product is held in hot water for a specified time then passed to a dewatering cooling section. Whereas, with steam blanching; the product is passed through an atmosphere of saturated steam followed by cooling using either cold air or cold-water sprays [66]. Boiling is another wet thermal processing method often used in the preparation of ALVs [14, 17, 67], where the leaves are immersed in boiling water for 5 min [68]. Most often, the boiling water is discarded in order to remove the bitter anti-nutritional factors (phytates, tannins and trypsin inhibitor) present in the ALVs [69, 70].

The main purpose of blanching is to reduce the activity of enzymes such as peroxidase and lipoxygenase prior to further processing of fresh produce to prevent undesirable changes in odour, colour, flavour, texture and nutritive value. The inactivation of these enzymes depends on the heating method, temperature, concentration of the enzyme, size, shape and conductivity of the product [71]. Blanching also removes intercellular gases in the fresh produce thereby reducing potential oxidative changes and further reduces microbial load on the surface of fresh produce [56]. Furthermore, during hot water blanching and cold-water cooling (in steam blanching), heat sensitive and water-soluble nutrients, respectively may be leached to the effluent potentially reducing the nutrient content of the food. Blanched fresh produce is subjected to thermal stress, thus it is mandatory to cool fresh produce immediately after blanching to minimize damage to the fresh produce cells [46].

Thermal processing causes the initial loss of cell firmness (softening) due to disruption of the cell wall and plasmalemma of the leaves. Cell-wall components such as pectic polysaccharides, hemicellulose and cellulose serve as structural components and protect the plant cell; providing shape, cell adhesion and transportation of cellular fluids. The pectic polysaccharides are concentrated in the outer region of the cell wall and in the middle lamella of the cell wall where they control cell wall porosity. In aqueous environments, such as those found during blanching and boiling, the cell wall structure collapses as exhibited by wilting and/or softening of the leaves. The collapse of the plant cell wall is attributed to the β-eliminative degradation of pectic polysaccharides. The pectic polysaccharide glycosidic linkages are broken through catalytic reactions of the hydroxyl-ions and these polysaccharides are said to be depolymerized at this stage which is influenced by pH, presence of ions and organic acids [72]. The turgid tissue structure contracts in addition to the cell wall becoming porous [56, 64]. This results in the free diffusion of water, leaching of water-soluble pectin and nutrients by mass diffusional loss during high temperature and long blanching times [64, 72-74].

Studies by Babalola et al. [68] and Patel et al. [71] observed a loss in AA content when increasing the blanching temperature and time in green leafy vegetables including *M. oleifera* leaves. Ascorbic acid is a vitamin that is heat labile, water-soluble, sensitive to light, oxygen and oxidizing agents [64]. Extended exposure to an aqueous environment for extended periods of time can accelerate the leaching of the vitamin. Increasing the blanching time by a minute resulted in AA loss between 1.86 and 12.41%, whereas a 10°C temperature change resulted in a loss between 5.08 and 8.45% in ALVs for water blanching, which was also observed during steam blanching (Table 9).

Table 9: Retention of ascorbic acid (vitamin C) content (%) as affected by blanching temperature and time

Treatment	Temperature and time	*Spinacia oleracea* leaves	*Trigonella* leaves	*M. oleifera* leaves	*Amaranthus gangeticus Linn.* leaves	References
Water blanching	80°C, 1min	83.20	71.92	78.54	73.46	[74]
	80°C, 2min	70.79	61.58	76.68	66.18	
	80°C, 4min	55.94	52.54	57.23	57.47	
	90°C, 4min	50.12	47.46	51.53	49.02	
Steam blanching	95°C, 1min	nt	nt	82.7	nt	[92]
	95°C, 2min	nt	nt	72.3	nt	
	95°C, 3min	nt	nt	61.3	nt	
	95°C, 4min	nt	nt	53.1	nt	
	95°C, 5min	nt	nt	40.1	nt	
	95°C, 6min	nt	nt	35.8	nt	
Water blanching with added chemicals	80°C, 1min	84.89	72.42	79.27	80.89	[74]
	80°C, 2min	86.56	72.73	81.27	82.79	

nt = not tested

Ascorbic acid is used as an indicator nutrient to evaluate the effect of food processing. Cooking losses of vitamins depend on the degree of heating, leaching into the cooking medium, surface area exposed to water and oxygen, and pH [64]. Factors such as water to leaf ratio, cooking time; light, heat sensitivity, water solubility, mass transfer, oxygen and enzymatic oxidation play a more significant role in mineral and vitamin losses during processing [46, 48, 64]. The water to leaf ratio during cooking is of paramount importance as excessive water content exposes the water-soluble vitamins to leaching. In a study by Sreeramulu et al. [75], the authors found that excess boiling water had a remarkable impact on AA loss. Ascorbic acid loss for *M. oleifera* was 98.5% (3.0±0.10 mg/100 g material) and 85.4% (29.7±0.51 mg/100 g material) for excess boiling water and minimal boiling water, respectively from a fresh content of 204.0±1.18 mg/100 g material [75]. High AA loss was also observed in boiled Soko (*Celosia argentea*), Green/Tete (*Amaranthus hybridus*) and Bitter leaves. Recorded losses of 74.50%, 91.50% and 94.90% from an initial AA content of 15.66±3.29, 24.00±7.32 and 42.40±4.95 mg/100g fresh weight in the raw Soko, Green/Tete and Bitter leaves, respectively were observed by [68].

Apart from leaching, AA can also be degraded through oxidation were temperature, light, oxygen, metal ions, pH, water activity and enzymes e.g. AA oxidase and ascorbic peroxidase influence the rate of degradation [76]. In plant tissues, AA is reversibly oxidized to dehydroascorbic acid (DHAA) under aerobic conditions by the enzyme AA oxidase [77] and is mainly associated with the cell wall [78]. Ascorbic acid and DHAA possess antiscorbutic activity when taken orally as well as antioxidant activity. In water, DHAA is found as a hydrated hemiacetal which is unstable [79] and is hydrolysed to 2,3-diketogulonic acid during thermal processing which has no antiscorbutic activity [6].

Gil et al. [77] found that AA and DHAA were lost when fresh spinach was exposed to air for 3 and 7 days. Cooking the spinach for 10 min in boiling water at 90°C, resulted in 60% and 40% recovery of AA in cooking water and cooked tissue, respectively. Storage of the cooked tissue in air and in modified atmosphere packaging encouraged further degradation of AA to DHAA at 10°C [77]. These experiments clearly indicated the oxygen and heat sensitivity of AA. Furthermore, photo-oxidation of AA was reported by Faboya et al. [80] in leafy vegetables. The author reported on 66 and 59% loss of AA in *Talinum triangulate* leaves, and 66 and 62% in *Celosia argentea* leaves kept for 8 h in direct sunlight and uncovered in a laboratory (exposed to fluorescence light), respectively. Santos and Silva [47] reported that light can be a source of energy, thus promoting degradation.

Other nutrients are also affected by high temperatures during blanching and boiling (Table 9 and 10). Gidamis et al. [70] found that cooking in boiling water reduced protein and fibre content in young *M. oleifera* leaves. Structural proteins are denatured by high temperature such as those encountered during cooking, which leads to nutrient losses as the proteins dissociate into the cooking water. On the other hand, minerals and vitamins are low molecular weight compounds dissolved in cellular juices of the plant. Minerals and vitamins are reported to leach out or physically separated from the plant cells, rather than being degraded by processing conditions [48]. Mziray et al. [69] reported on mineral content reduction after boiling amaranth leaves for 15 min. Heat treatment encourages the leaching of micronutrients due to the compromised state of the cell wall structure of the leaves which can no longer contain the cellular juices. Therefore, due to the collapse of the cell wall, cellular juices leak from the cell into the cooking medium in which the minerals leach out into.

Table 10: Effect of boiling for 15 minutes on nutritional content of *M. oleifera* Lam. leaves [69]

Macronutrient (mg/100g) *dwb*	**Before boiling**	**After boiling**
Crude protein	33.82[a]	31. 99[b]
Carbohydrates	0.02886[c]	34.92[d]
Micronutrient – vitamins (g/100g) *dwb*		
β-carotene	4404.75±44.80[a]	4145.33±31.30[b]
Ascorbic acid (g/100g) *dwb*	47.63±0.23[c]	46.31±0.30[d]
Micronutrient – minerals (mg/100g) *dwb*		
Calcium	630.71[a]	582.82[b]
Iron	11.85[c]	12.40[d]
Zinc	3.24[e]	2.93[f]

Carotenoids (α- and β-carotene) found in fresh produce are vitamin A precursors that have a series of conjugated double bonds and existing in the stable all-*trans* form. During wet and dry heat processing, the all-*trans* configuration is isomerized to the *cis*/*trans* configuration. *Cis*-isomers of carotenoids and oxidation products have less vitamin A activity than the all-*trans* form. In the study by Mulokozi and Svanberg [81], African leafy vegetables blanched (2 to 3 min in boiling water without food additives) yielded between 526 and 917 µg/g *dwb* of all-trans-β-carotene, and between 12 and 39 µg/g *dwb* of all-trans-α-carotene. The 9-*cis*-β-carotene and 13-*cis*-β-carotene constituted 15% and 5%, respectively of the all-*trans*-β-carotene content in the blanched ALVs [85]. This would have serious nutritional consequences as the consumption of the less active form of the carotenoid can possibly result in inadequate intake, leading to less available Vitamin A. According to the Institute of Medicine [82] between 500 to 800 µg RE/day of vitamin A is recommended for children, adolescents, adults and pregnant women (Table 4).

However, nutrient loss or leaching can be prevented through the use of additives and/or shorter blanching periods. During blanching, the simultaneous addition of chemical additives protects the plant cells from enzymatic activity. Baloch et al. [83] found that sulphiting protected the stability of β-carotene in carrots thereby prolonging the storage life and optimizing the blanching of the carrots. Blanching *Spinacia oleracea*, *Trigonella*, *M. oleifera*, *Amaranthus gangeticus* Linn. leaves at 80°C for 1 and 2 min in a chemical solution of 0.5% potassium metabisulphite±0.1%, magnesium oxide±0.1% and sodium bicarbonate retained AA than blanching with only water (Table 9). The food additives potassium metabisulphite, magnesium oxide and sodium bicarbonate inactivate oxidases that are responsible for AA loss and improves colour of fresh produce by prevention of oxidation reactions [84, 85].

Increasing the temperature of blanching water and reducing the blanching time resulted in the retention of nutrients as seen with the β-carotene and AA content of savoy beet (*Beta vulgaris var bengalensis* cv.), amaranth (*Amaranthus tricolor* cv.) and fenugreek (*Trigonella foenum graecum* cv.) leaves after blanching at 95°C for 30 to 180s [86]. The retained nutrient content is attributed to the reduced activity of the peroxidase enzyme in the leaves to negligible amounts at this high temperature [86]. In the presence of oxygen and endogenous hydrogen peroxide [87], peroxidase oxidises β-carotene to apocarotenals, thereby reducing its content and biological activity [52]. These are in agreement with the findings by Akpapunam [88] who found that blanching at 98°C for 3 min retained the total carotenoid and AA content in vegetables. Whereas blanching *M. oleifera* leaves at 80, 90 and 95°C resulted in a decrease in AA content with increasing time (Table 9). Boiling the leaves for 15 min yielded further reductions in the nutrient content of the leaves (Table 10). Steam blanching at 95°C for 1 min proved to be optimum condition to blanch *M. oleifera* leaves as the leaves retained 82.7% of AA (89). The

temperature is sufficiently high enough to destroy enzymes within the exposure time without destroying AA. Furthermore, steam blanching is a gentler method with no extensive water use or effluent.

The degradation of nutrients in fresh produce is affected by pre-harvest and postharvest factors such as variation of species and cultivars, climatic conditions, maturity at harvest, temperature, humidity, storage and processing factors and conditions. In the case of leafy vegetables, the leaves are exposed to oxygen post-harvest and even during storage prior to processing. It is at this conditions that care has to be taken to minimize nutrient degradation as the fresh produce is still metabolically active [90]. Enzymatic activity of the primary enzymes (ascorbic acid oxidase, peroxidase, lipoxygenase and catalyse) found in fresh produce is dependent on the concentration, presence of catalysts and the cultivar of fresh produce. Thus any blanching or boiling temperature should be sufficient enough to inactivate the enzyme(s) in the shortest possible time to minimize leaching as a result of long exposure in hydrolytic conditions and thermal degradation of nutrients.

Fermentation of *Moringa oleifera* Lam. leaves

Fermentation in food processing has been used since ancient times as a method to extend the shelf-life of food products. In most developing countries, fermentation is the primary food processing method followed by cooking using heat. Different types of fermentation are often employed based on the product. These include alcoholic fermentation; and non-alcoholic fermentations such as lactic acid fermentation, acetic acid fermentation and alkaline fermentation [91, 92]. The main advantages of fermentation in food processing are flavour enhancement, improving nutritional quality of food, preservation of food, detoxification of the food products and provide antimicrobial properties to the food [93].

The indigenous microbiota present on the food product are the main drivers of fermentation in spontaneous fermentations. Microorganisms are able to degrade plant polysaccharides and polyphenols through enzymatic reactions and in a symbiotic interaction [94, 95]. Furthermore, microorganisms secrete enzymes such proteases, amylases and lipases that hydrolyse food complexes into products with desirable texture, aroma and taste [93]. Plant associated micro-environments contain a diverse population of beneficial and pathogenic microorganisms. The lactic acid bacteria (LAB) population is found at a low population of 2-4 log cfu/g in plants. This population is comprised of both hetero-fermentative and homo-fermentative species belonging to genera *Lactobacillus, Pediococcus*, *Leuconostoc*, *Lactococcus* and *Weissella* [96]. The species *Lactobacillus plantarum* is frequently isolated than other LAB species. Lactic acid bacteria have developed adaptation traits in order to proliferate in plant environments. The traits include but are not limited to the down regulation of central metabolism genes, the induction of alternative substrates transport and metabolism, the stimulation of specific responses functionally related to the inherent features of plant materials and the expression of general stress response genes [97]. It is this inherent metabolic traits that render LAB as compatible microorganisms to carry out fermentation of plant based food products.

Thierry et al. [98] fermented *M. oleifera* leaf powder with *L. plantarum* at 30 and 37°C over a period of 120 h. This resulted in improved protein content and protein digestibility (Table 11). An increase in protein digestibility contributes to the amount of protein that would be bioaccessible and bioavailable. *M. oleifera* leaf powder is often added to finished food products or as part of a meal to enhance the nutrient content of those foods. When *M. oleifera* leaf powder was supplemented (between 5 to 15%) in fermented sorghum flour, the nutrient content of the flour increased between 1.59 and

10.7% for the micronutrients calcium, potassium and iron [99]. Considering that sorghum is one of the staple cereals in most African countries, the supplementation of *M. oleifera* leaf powder would thus be beneficial in meeting the RNI of consumers. The positive contribution of fermentation on amino acid composition was observed by Osman et al. [100]. The authors found that fermentation of Sicklepod (*Cassia obtusifolia*) leaves resulted in an increase in alanine, valine, cysteine, isoleucine, leucine and methionine.

Table 11: Effect of fermentation on the nutrient content of fermented *M. oleifera* leaf powder [96]

Fermentation period	Protein content g/100 g *dwb*	Protein digestibility (%)	Iron (%)
0h at 30°C	38	39.97	31.86
0h at 37°C	38	39.97	31.86
120h at 30°C	42	55.57	47.94
120h at 37°C	44	63.97	52.14

Apart from supplementation, *M. oleifera* leaf powder has been used to fortify fermented products such as yoghurt and cheese. In both these products, the addition of *M. oleifera* improved the nutrient content of the food as well as antioxidant and antimicrobial properties as reviewed by Oyeyinka and Oyeyinka [7]. The production of Labneh cheese with 3% dried *M. oleifera* leaves resulted in an increase in the minerals Ca, Fe and Zn; and vitamins A, B1, B2 and E. The same treatment resulted in a net protein utilization of 75 and 69.40 for the Labneh+Moringa diet and the Labneh only diet, respectively [101]. Vanajakshi et al. [102] produced a fermented beetroot (*Beta vulgaris* L.) beverage with a probiotic *L. plantarum* species over a period of 48 h. The fermented juice was supplemented with different ratios of *M. oleifera*. The fermented juice was found to have antimicrobial activity against the foodborne pathogens *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* as well as 20.79% antioxidant activity. This was attributed to the presence of the *M. oleifera* and the role of

fermentation in releasing the active compounds in *M. oleifera*.

M. oleifera Lam. possess anti-nutritional factors (ANFs) such as phytate, oxalate and trypsin inhibitors [70]. Anti-nutritional factors are naturally occurring secondary metabolites that protect the plant against biological stress such as pests [103]. Trypsin inhibitor (TI) inhibits the activity of pancreatic enzymes trypsin and chymotrypsin thereby reducing digestion and absorption of dietary proteins by the formation of complexes that are indigestible even in the presence of high amounts of digestive enzymes. Trypsin inhibitors are thermosensitive, being totally inactivated at temperatures around 108°C for 15 to 30 min [103]. Phytates are responsible for the formation of insoluble complexes with proteins and minerals. Phytic acid chelates mineral cations and proteins, forming insoluble complexes, which leads to reduced bioavailability of trace minerals and reduced digestibility of proteins.

Clydesdale et al. [48] defined various mineral interactions that might affect bioavailability of micronutrients. The interactions include the displacement of a mineral from a complex with another mineral to form a soluble (available) or insoluble (unavailable) complex, the addition of a second or third mineral to a soluble mineral-ligand complex forming a poly mineral-ligand complex and causing precipitation, the addition of a mineral to a mineral-ligand complex, which then binds another substrate (ligand) and forms a poly mineral poly-ligand complex and the formation of a poly mineral-ligand complex. However, during fermentation *Lactobacillus plantarum* produces the phytase enzyme which reduces phytate. Fermenting *M. oleifera* leaves at 30 and 37°C reduced phytate from 1182 mg/100g *dwb* to 542 mg/100g *dwb* and 3.91 mg/100g *dwb*, respectively to release ions thus allowing an increase of mineral availability [98]. Osman et al. [100] observed a 37 to 56% reduction in phytate content in Sicklepod leaves after fermentation. This reduction was accompanied by *in-vitro* protein

digestibility of 34 to 56%.

Concluding remarks

M. oleifera leaves are rich nutrients and are used in food preparation in many communities as a readily available vegetable. The ease of access in rural communities renders the leaves as a common ingredient in the making of food products such as vegetable soup. The nutrient content of *M. oleifera* leaves compares well with that of spinach, and could be an alternative substitute in meal preparations. Based on the relatively high protein content of *M. oleifera*, the leaf powder is often incorporated in nutritional interventions either as an enrichment or fortificant. This incorporation offers value addition to the plant as well as the food products it is incorporated into. Sub-Saharan diets are often composed of porridge type meals prepared from cereals or grains with the occasional addition of a protein source from meat. Evaluating the protein content of *M. oleifera* and the malnutrition and protein deficiency burden that is rampant in sub-Saharan countries, *M. oleifera* seems a potential solution to correct such nutrition deficiencies through targeted interventions. One key nutrition intervention is increasing the RNI of macro and micronutrients in the diet of consumers. This can be met through the incorporation of *M. oleifera* in the diets of consumers in a systemic and scientifically sound manner. Convenience products such as snack bars, noodles, instant soups or porridge seem to the possible solutions to target nutrition deficiency while at the same time providing consumers with foods that require less preparation.

Food processing technologies such as solar drying and fermentation are energy efficient and most suited for conditions found in rural communities. In particular, fermentation affords the benefits of enhanced nutrient content, reduction of anti-nutritional factors, inhibition of spoilage microorganisms and consequently extended

shelf-life. This presents a winning solution for rural communities. In this case no additional processing aids or equipment is need and thus a nutrient rich and safe food product can be produced economically. Prepared *M. oleifera* leaves are of often perceived as bitter as a result of the anti-nutritional factors present in *M. oleifera* leaves. This compromises the sensory quality of food and such any products that can be prepared from the leaves. This would prove quite a challenge in introducing fermented *M. oleifera* foods to children. However, through the fermentation the anti-nutritional factors can be reduced. Furthermore, in preparing convenience foods targeted at children, food grade flavourings can be added to mask the bitter taste. This is quite popular in extruded products such as chips. Fermentation also has the added advantage of releasing micro nutrients which are bound anti-nutritional factors making them not bioavailable. Bioavailability and bioaccessibility are essential in nutrition interventions as these determine the success on such an intervention.

Solar drying on the other hand, also energy efficient and accessible, has limited use based on stability of the nutrients and repeatability of the experiment as it is affected by prevailing weather conditions. This technology does need improving such as temperature control and possible designs of drying fresh produce in inclement weather, using stored energy. This highlights the need for more research on solar drying conditions that would cater for fresh produce drying under all weather conditions. These could also be assessed whether a combination of processing technologies can be used in a single unit such that nutrients are retained to the maximum possible content.

Overall, the drying of leafy vegetables has been researched using solar drying and microwave. Indeed leafy vegetables are sensitive plant structures, and thus any processing technology should cater to maintain the structure and nutrient composition. Depending on the dosages applied when using microwave energy, the morphology of the plant tissue

is left intact or with minimal morphological changes. With solar drying on the other hand, there are observed changes that can alter the further use or application of the product. The discussion above highlighted on the use of blanching methods to retain the nutrients. This pre-treatment is successful in retaining nutrients. Taking on the developing food products with increased protein content, research should be directed at processing plants such as *M. oleifera* investigating on the influence of such processing technology on structural morphology, nutrient content, nutrient bioaccessibility, nutrient bioavailability and possible hybrid technologies employing more than one energy source. These would help in developing products specific for particular consumers such as nutrient deficient consumers, immune compromised consumers and/or athletes using novel technology.

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