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Effects of drying on the physicochemical properties and the fatty acids composition of some edible leafy vegetable oils

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Abstract: Green amaranth (Amaranthus hybridus), Scent leaf (Ocimum gratissimum), Basil leaf (Ocimum basilicum), Bitter leaf (Vernonia amygdalina) and Fluted pumpkin leaf (Telferia occidentalis) were subjected to sun-drying and freeze-drying. The Oils extracted from the pulverized samples were divided into two portions, first portion analysed for fatty acids composition while physicochemical properties were determined on the second portion. On the average, freeze-dried leaves' oil contained 76.76% (range 64.99-88.26%) total unsaturated acid which was significantly higher (P<0.05 level) than sun-dried leaves, oil of total unsaturated acid 70.42% (range 58.19-83.57%). Sun-dried leaves' oil contained significantly higher (P<0.05 level) mean free fatty acid (FFA) and mean peroxide value (PV) 2.03% (range 1.3±0.0-2.6±0.1%); 5.13mg/g (range 3.6±0.1-6.8±0.1mg/g) than freeze-dried leaves' oil of mean FFA and mean PV 1.60% (range 1.0±0.0-2.5±0.1%); 4.05mg/g (range 2.8±0.1-5.4±0.2mg/g) while freeze-dried leaves' oil had significantly higher (P<0.05 level) mean iodine value (IV) 122.2mg/g (range 105.0±0.0-139.0±1.0mg/g) than sun-dried leaves' oil of mean IV 113.5mg/g (range 97.0±1.0-131.0±0.0). Of the drying methods, freeze-drying had significantly higher (P<0.05 level) percentage of unsaturation, higher IV, lower FFA and lower PV than sun-drying. Hence, freeze-drying would appear to be more promising.

Keywords: Sun-drying; Freeze-drying.

INTRODUCTION

Veges and fruits offer the most rapid and lowest cost method of providing adequate supplies of vitamins, minerals, calcium and fiber to the people who lives in the tropics[16]. They have a high content of water and abundance of cellulose. The cellulose is in a form which although not digested, serves a useful purpose in the intestine as roughages, thus promoting normal elimination of waste product[16]. Green vegetables have long been recognized[5,23] and more recently[1] as the cheapest and most abundant source of protein due to its ability to synthesize amino acids from unlimited and readily available materials such as sunlight, water, atmospheric nitrogen and carbon IV oxide. Drying has been used traditionally as a method of preserving leafy vegetables in Nigeria and other developing countries. The rationale for drying is to reduce the moisture content to a level, which prolongs shelf life during storage, reduces colonization by microorganisms and as a source of food after rainy season[13]. Fruits and vegetables with high moisture content, provide a favourable condition for the growth of micro-organisms, which lead to their spoilage and wastage[18]. Drying is one of the methods of food preservation adopted in order to decrease losses in quality and quantity, which will otherwise occur[14]. Two processes occur during drying; the addition of heat and removal of moisture from the food[19]. Sun-drying (open air) is the most widely used method of drying agricultural produce in most of the developing countries of the tropical region. It has risk of possible contamination by microorganisms, lack of protection from dust, infestation by insects, rodents and the quality of the products are seriously degraded and sometimes inedible[9]. Freeze drying takes place under vacuum conditions below the "triple point" (6.2 mbar = 4.6 torr). Here, water will only be present in two phases: ice and vapour. By adding energy to the ice, it will sublimate (evaporate) directly into the vapour phase[26] and the afore-mentioned risk is totally avoided. Leafy vegetables are known to be poor sources of fat. Among the proximate compositions, fat content represents the lowest in this category of foods. It is infact unusual to find levels of ether extract exceeding 1.0% in fresh leafy vegetables, although contents of dry sample can range from 1-30% which is comparatively lower than the fat content of vegetable seeds[22]. Extensive work has been done on the proximate composition, mineral content and antinutrient content of sun-dried edible leafy vegetable. Little work has been done on the effects of freeze-drying on the parameters mentioned above. The focus of the present research work was to establish the effects of both sun-drying and freeze-drying on the physicochemical properties (FFA, PV, IV, Saponification value, Specific gravity and Refractive index) and the fatty acids composition.

MATERIALS AND METHODS

Sample collection

Vegetable samples used for the research were purchased at Emure-Ile village market in Owo Local Government Area of Ondo State. The stalks were removed and the leaves were rinsed with distilled water and divided into two portions: the first portion sun-dried and the other freeze-dried in Biochemistry Laboratory, Federal University of Technology, Akure.

Sample preparation

The preparation of the samples for analysis generally involves reduction in amount and simultaneous reduction in particle sizes and thorough mixing of the product so that the portion used represents the average composition of the entire mixture. The sun-dried and the freeze-dried vegetable samples were blended separately using an electronic kenwood blending machine. The samples were ground to small uniform particles of a 40mm mesh sized sieve to aid analysis and stored by transferring the granulated samples to an air tight and moisture proof containers.

Mass extraction was carried out to obtain enough oil for the physicochemical test. This involved the addition of hexane (2.5 litres) to each of the pulverized samples described above for about 1 week. The hexane turned yellowish due to the dissolution of the vegetable oil. Then the solvent consisting the oil was decanted and filtered to remove all the sediments. The oil was separated from the solvent (hexane) using simple distillation apparatus.

ORIGINAL ARTICLE

Analysis of the seed oils

The physico-chemical properties of the crude oil extracted viz: the acid value, saponification value, peroxide value, iodine value and free fatty acid (as oleic acid) were determined[2].

Analysis of free fatty acid

5g of the homogenized oil was weighed into a 500ml flask and 80ml of 2M KOH in water-ethanol. (1:1) was added and refluxed for 45mins. Excess thanol was boiled off and then cooled by adding 10ml of water, The hydrolysate was then acidified with 10M H_2SO_4 and the released fatty acids were extracted with petroleum spirit (3×30ml). TLC on asilica gel and solvent mixture of diethylether- petroleum spirit-acetic acid (50:50:1) were used and spotted along maker acids. UV visible absorbances on Unicam-Uv-visible spectrometry-vision soft ware V3-32 were measured in ethanol. The ethanol was measured on nujol. Determinations were done in triplicate and Analysis of Variance (ANOVA) was carried out using SPSS (version 15). The means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

TABLES 1 & 3 clearly showed that the acid value (AV) and the free fatty acid (FFA) for the sun-dried samples were significantly higher (at $P<0.05$level) than that of the freeze-dried samples. The sun-dried leaves had mean acid value 4.06mg/g (range 2.6-5.3±0.1mg/g) which is significantly higher than the freeze-dried leaves of mean acid value 3.19mg/g (range 2.1±0.0-5.0±0.1mg/g). The FFA was highest in sun-dried *T. occidentalis* (2.6±0.1%) and lowest in freeze-dried *O. gratissimum* (1.0±0.0%). All the vegetable oils had high saponification values. The saponification value which measures the amount of alkali that is required to combine with fatty acids and the hydrolyzed esters using a solution of potassium hydroxide in alcohol was found to have no significant difference when sun-dried and when freeze-dried leaves were analyzed. Therefore, both freeze-dried and sun-dried oil samples exhibited high saponification values ranged from 170.2±1.7mg/g in freeze-dried *O. gratissimum* to 200.0±0.8mg/g in freeze-dried *O. basilicum*.

The iodine value which is the measure of the degree of unsaturation was significantly higher in freeze-dried samples of range 104.5-138.8mg/g and mean value 122.2 mg/g than in sun-dried samples of range 96.5mg/g–130.5mg/g and mean value 113.5mg/g. Peroxide value is an indication of deterioration. TABLE 3 showed that both groups of oils had low mean peroxide values 5.13mg/g for sun-dried leaves which was significantly higher than 4.05mg/g for freeze-dried leaves. The peroxide value was highest in sun-dried *T. occidentalis* (6.8±0.1mg/g) and lowest in freeze-dried *A. hybridus* (2.8±0.1mg/g). Drying methods had no significant effect on the refractive index of the vegetable samples' oils. The refractive index of freeze-dried samples' oils ranged from 1.430 to 1.450 which was the range of the refractive index of sun-dried samples' oils.

TABLE 1 : Physicochemical properties of Selected vegetable types as affected by drying method.

Name of Vegetable	Drying Method	AV (mg/g)	FFA (%)	SV (mg/g)	IV (mg/g) wij	PV (mg/g)	SG @20^{0}C	RI @25^{0}C
Amaranthus hybridus	Sun-drying	$4.1±0.0^c$	$2.0±0.0^{ab}$	$183.7±2.7^b$	$114.0±0.0^g$	$3.6±0.1^g$	$0.928±0.003^d$	$1.440±0.010^{ab}$
	Freeze-drying	$3.3±0.0^d$	$1.7±0.0^{bc}$	$182.8±1.9^b$	$118.0±1.0^e$	$2.8±0.1^i$	$0.928±0.001^d$	$1.440±0.000^{ab}$
Ocimum gratissimum	Sun-drying	$2.6±0.1^e$	$1.3±0.0^{bc}$	$171.1±2.0^d$	$126.0±1.0^d$	$5.2±0.1^d$	$0.900±0.004^e$	$1.440±0.010^{ab}$
	Freeze-drying	$2.1±0.0^g$	$1.0±0.0^c$	$170.2±1.7^d$	$139.0±1.0^a$	$4.0±0.0^f$	$0.900±0.003^e$	$1.450±0.000^a$
Ocimum basilicum	Sun-drying	$3.4±0.0^d$	$1.7±0.0^{bc}$	$199.2±2.8^a$	$131.0±0.0^b$	$3.8±0.1^{fg}$	$0.945±0.004^a$	$1.450±0.010^a$
	Freeze drying	$2.3±0.0^f$	$1.1±0.0^c$	$200.0±0.8^a$	$134.0±0.0^b$	$3.1±0.1^h$	$0.945±0.002^a$	$1.450±0.000^a$
Vernonia amygdalina	Sun-drying	$5.1±0.0^b$	$2.5±0.0^a$	$175.3±1.8^c$	$101.0±1.0^i$	$6.2±0.2^b$	$0.938±0.002^b$	$1.433±0.010^b$
	Freeze-drying	$3.4±0.1^d$	$1.7±0.0^{bc}$	$176.1±1.5^c$	$116.0±1.0^f$	$5.0±0.0^e$	$0.935±0.001^{bc}$	$1.437±0.005^b$
Telferia occidentalis	Sun-drying	$5.3±0.1^a$	$2.6±0.1^a$	$197.8±1.8^a$	$.97.0±1.0^j$	$6.8±0.1^a$	$0.931±0.001^{cd}$	$1.432±0.005^b$
	Freeze-drying	$5.0±0.1^b$	$2.5±0.1^a$	$197.8±1.9^a$	$105.0±0.0^h$	$5.4±0.2^c$	$0.932±0.000^{cd}$	$1.433±0.005^b$

TABLE 2 : Physicochemical properties of selected vegetable types.

Name of Vegetable	AV (mg/g)	FFA (%)	SV (mg/g)	IV (mg/g)	PV (mg/g)	SG @20^0c	RI @25^0C
Amarathus hybridus	3.68^c	0.84^{bc}	183.25^b	116.3^b	3.18^e	0.928^d	1.440^{bc}
Ocimum grattiimum	2.31^e	1.16^d	170.65^d	132.4^a	4.62^c	0.900^e	1.445^b
Ocimum basilicum	2.82^d	1.41^{cd}	199.65^a	132.1^a	3.45^d	0.945^a	1.450^a
Vernonia amygdalina	4.21^b	4.21^b	175.70^c	108.2^c	3.60^b	0.937^b	1.435^c
Telferia occidentalis	5.13^a	5.13^a	197.80^a	100.5^d	6.12^a	0.932^c	1.433^c

TABLE 3 : Effect of drying methods on mean physico-chemical properties of selected vegetable types.

Drying method	AV(mg/g)	FFA(%)	SV(mg/g)	IV(mg/g)	PV(mg/g)	SG@20^0c	RI@25^0C
Sun drying	4.06^a	2.03^a	185.42^a	113.5^b	5.13^a	0.928^a	1.442^a
Freeze drying	3.19^b	1.60^b	185.38^a	122.2^a	4.05^b	0.928^a	1.439^a

AV= Acid value, FFA= Free fatty acid, SV= Saponification value, IV=Iodine value, PV= Peroxide value, SG=Specific gravity, RI= Refractive Index. Values in the same column having the same superscript are not significantly different at P≥0.05 level by DMRT.

The specific gravity of both the sun-dried and the freeze-dried samples' oils showed no significant difference. Specific gravity was highest in both sun-dried (0.945±0.004) and freeze-dried (0.945±0.002) *O. basilicum* and lowest in both sun-dried (0.900±0.004) and freeze-dried (0.900±0.003) *O. gratissimum*.

TABLE 4 indicated that two types of fatty acids were present in the vegetable samples namely saturated and unsaturated acids. The results indicated that freeze-dried samples had higher myristic acid (except in *A. hybridus*), oleic acid, linoleic acid and linolenic acid compositions while sun-dried samples had higher arachidic acid, behenic acid (except in *O. gratissimum*) and palmitic acid compositions. TABLE 6 showed that the mean value of total unsaturated acid (76.76%) was significantly higher in freeze-dried samples' oils than in sun-dried samples' oils (70.42%).

TABLE 4 : Fatty acid compostion (%) of selected vegetable types as affected by drying method.

Name of Vegetable	Drying Method	Myristic Acid	Stearic Acid	Arachidic Acid	Behenic Acid	Ligncceric Acid	Palmitic Acid	Total Saturated Acid	Palmitoleic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Total Unsaturated Acid
Amaranthus hybridus	Sun drying	$O.31^{bc}$	5.12^{de}	0.65^{bc}	1.43^a	0.46^a	22.43^b	31.09^{cd}	0.07^{ab}	22.72^b	45.38^b	0.74^c	68.91^{cd}
	Freeze-drying	0.19^c	5.93^{cde}	0.22^{cd}	0.67^{ab}	0.32^a	19.37^d	26.69^d	0.04^{ab}	23.87^b	48.60^b	0.79^c	73.31^c
Ocimum gratissimum	Sun drying	0.11^c	5.30^{cde}	0.01^d	0.00^b	0.00^b	14.06^g	19.48^e	0.05^{ab}	7.19^d	27.98^d	45.30^b	80.52^b
	freeze drying	0.13^c	3.15^{de}	0.01^d	0.00^b	0.00^b	8.45^h	11.74^f	0.06^{ab}	8.44^d	32.46^c	47.30^b	88.26^a
Ocimum basilicum	Sun drying	0.10^c	2.01^e	0.01^d	0.00^b	0.00^b	14.31^f	16.43^{ef}	0.18^{ab}	11.63^{cd}	19.86^e	51.91^a	83.57^{ab}
	Freeze drying	0.12^c	3.16^{de}	0.00^d	0.01^b	0.00^b	9.03^h	12.32^f	0.74^a	12.23^{cd}	21.15^e	53.57^a	87.68^a
Vernonia amygdalina	Sun drying	0.08^c	10.12^{bc}	0.56^{cd}	1.35^a	0.37^a	26.62^a	39.11^{ab}	0.01^b	12.23^{cd}	48.30^b	0.35^c	60.89^{ef}
	Freeze drying	0.14^c	7.94^{cd}	0.37^{cd}	0.94^{ab}	0.37^a	20.65^c	30.42^{cd}	0.05^{ab}	16.44^c	52.67^a	0.42^c	69.54^{cd}
Telferia occidentalis	Sun drying	0.63^{ab}	16.71^a	1.63^a	1.08^a	0.00^b	21.76^b	41.81^a	0.00^b	32.22^a	25.02^d	0.95^c	58.19^f
	Freeze drying	0.85^a	14.48^{ab}	1.21^{ab}	0.99^a	0.00^b	17.48^e	35.01^{bc}	0.00^b	36.55^a	27.43^d	1.02^c	64.99^{de}

TABLE 5 : Mean fatty acid compostion (%) of selected vegetable types as affected by drying method.

Name of vegetable	Myristic acid	Stearic acid	Archidic acid	Behenic acid	Lignoceric acid	Palmitic acid	Total Saturated acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Total Unsaturated acid
Amaranthus hybridus	0.25^{bc}	5.53^c	0.44^b	1.05^a	0.39^a	20.90^b	28.89^b	0.06^a	23.30^b	46.99^b	0.77^c	71.11^b
Ocimum gratissimum	0.12^c	4.23^c	0.01^c	0.00^b	0.00^b	11.26^d	15.61^c	0.06^a	7.82^d	30.22^c	46.30^b	84.39^a
Ocimum basilicum	0.11^c	2.29^c	0.01^c	0.00^b	0.00^b	11.67d	14.38^c	0.46^a	11.93^c	20.51^e	52.74^a	85.63^a
Vernonia amygdalina	0.11^c	9.03^b	0.47^b	1.15^a	0.37^a	32.64^a	34.77^a	0.03^a	14.34^c	50.49^a	0.39^c	65.22^c
Telferia occidentalis	0.74^a	15.60^{ab}	1.42^a	1.04^a	0.00^b	19.62^c	38.41^a	0.00^a	34.39^a	26.23^d	0.99^c	61.59^c

ORIGINAL ARTICLE

TABLE 6 : Effect of drying methods on mean fatty acids composition (%) of selected vegetable types.

Drying method	Myristic acid	Stearic acid	Archidic acid	Behenic acid	Lignoceric acid	Palmitic acid	Total saturated acid	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Total unsaturated acid
Sundrying	0.25[a]	7.85[a]	0.57[a]	0.17[a]	0.17[a]	19.84[a]	29.58[a]	0.06[a]	17.20[b]	33.31[b]	19.85[a]	70.42[b]
Freezedrying	0.29[a]	6.93[a]	0.52[a]	0.52[a]	0.14[a]	15.00[b]	23.24[b]	0.18[a]	19.51[a]	36.46[a]	20.62[a]	76.76[a]

Values in the same column having the same superscript are not significantly different at P≥0.05level by DMRT.

DISCUSSION

The acid valve (AV) and the free fatty acid (FFA) for the sun-dried samples, oil were higher than that of the freeze-dried samples, oil. According to Ihekoronye and Ngoddy[16] the FFA value and AV of any lipid are measure of hydrolytic rancidity; the higher the values of FFA and AVs of lipid, the higher the degree of hydrolytic rancidity. Therefore, hydrolytic rancidity of sun-dried samples, oil was higher than that of freeze-dried samples, oil. It has been shown that it is desirable to ensure that the free fatty acid content of cooking oil lies within the limit 0.0 to 3.0%[6]. Therefore, all the oils investigated were good edible oils that might be stored for long time with minimum spoilage via oxidative rancidity and they might be of very low cholesterol.

All the vegetable oils had high saponification values. Saponification value which measures the amount of alkali that is required to combine with fatty acids and hydrolyzed esters using a solution of potassium hydroxide in alcohol was found to have no significant difference in sun-dried and freeze-dried samples. However, both the freeze-dried and the sun-dried oil samples exhibited high saponification values. These values were within the range of value reported by Ayo *et al.*,[3] for seed of *khaya senegalensis* oil (195.58mg/g). The results compete favourably with the saponifaction values of palm oil (196-205mg/g), olive (185-196mg/g), soy oil (193mg/g), cotton seed (193-195mg/g) and linseed oil (93-195mg/g) as reported by Pearson[24]. The higher the saponification values the better are the oils in making soap. Therefore, the high saponifcation values recorded for all the oil samples in TABLE 1 make the oils very suitable for soap making and in the manufacture of lather shaving cream[11,15]. These values were lower than the 52.00mg/g for palm oil[12].

The relatively high iodine value of all the oils extracted is an indication of the presence of many unsaturated bonds. The result showed that the degree of unsaturation of freeze-dried samples' oils was higher than that of the sun-dried samples' oils. This suggests that the oils from the freeze-dried vegetable leaves may be consumed by patients with heart problems since consumption of unsaturated oil is accompanied by low risk of atherosclerosis[20].

Peroxide value is an indication of deterioration. Both groups of oils had low peroxide values. Fresh oils had been shown to have peroxide value ranged 20.00mg/g to 40.00mg/g[24]. Therefore, freeze-dried samples' and sun-dried samples' oils investigated were stable due to their relatively lower peroxide values. The low peroxide values of these oils indicated that they were less liable to oxidative rancidity at room temperature[8].

Drying methods had no significant effect on the refractive index of the vegetable samples' oils. The refractive index of both the sun-dried and the freeze-dried samples' oil fell within the range for edible oils. Since edible oil has a refractive index of about 1.470[25,27]. Refractive index is a useful property in the preliminary examination of oil. It shows quickly the degree of unsaturation and whether the oil has unusual components such as hydroxyl groups or conjugated acids[24]. The specific gravity of both the sun-dried and the freeze-dried samples' oils showed no significant difference. The specific gravity values of these oils were in the range of sunflower oil (0.921), cotton seed oil (0.917) and soy bean (0.919)[4].

The mean fatty acid composition in TABLE 5 showed that the major component of scent leaf (*O. gratissimum*) and basil leaf (*O. basilicum*) was linolenic acid while linoleic acid was the major component of the three other samples: green amaranth leaf (*A. hybridus*), bitter leaf (*V. amygdalina*) and fluted pumpkin leaf (*T. occidentalis*). These two essential fatty acids are required for growth, physiological functions and body maintenance. All the samples had higher percentage of unsaturation which was responsible for their liquid nature at room temperature. Comparatively, sun-dried samples had significantly lower percentage of unsaturation than freeze-dried. It had been established that relative to carbohydrate content, the saturated fatty acid increases serum cholesterol in body[17]. Based on this, the freeze-dried samples had lower tendency of cholesterol accumulation.

Furthermore, myristic (14:0) acid had been established as the most important of the dietary risk factors in cholesterol density[7] this was present at a very low percentage in all the samples. It is most likely for fluted pumpkin oil to have the highest cholesterol based on its highest myristic acid content for both sun-dried and freeze-dried samples. Three types of lipoprotein (protein–lipid) in the blood are known. These are low–density lipoprotein (LDL) in which 46% of molecules are cholesterol, high–density lipoprotein (HDL) which includes 20% as cholesterol and very low-density lipoprotein (VLDL) which has 8% cholesterol. High level of total blood cholesterol is associated with the incidence of cholesterol density (CHD) as well as high interest of saturated fatty acid[7]. A direct comparison of myristic and palmitic acids showed that both raised LDL cholesterol relative to oleic acid but that myristic acid is slightly more powerful, while stearic acid (18.0) may not be a hyper cholestrolemic as the other saturated fatty acid (apparently because it is converted to oleic acid) Zock[28].

CONCLUSION

The study indicate that oils extracted from freeze dried leaves had lower iodine value when compared with sun- dried while the acid value and free fatty acid are higher in sun dried. Saponification value and peroxide had no significant difference in sun and freeze dried samples. The results further showed that the freeze dried had higher percentage of unsaturation judging from their fatty acid composition.

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ORIGINAL ARTICLE

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