Journal of Food Composition and Analysis 50 (2016) 108–113

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Evaluation of fatty acid and mineral content of Tanzanian seeds and oils

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ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form 24 May 2016
Accepted 31 May 2016
Available online 2 June 2016

Keywords:
Food analysis
Food composition
Fatty acid
Tanzania
Pumpkin
Linoleic
Korie
Palm oil
Seed
Minerals

ABSTRACT

Fatty acids (FA) and micronutrients are required for normal growth and development. Deficiency in FA and micronutrients is prevalent in several African countries. The objective of this study was to determine mineral and FA composition of seeds and oils available to residents of Rudewa-Mbuyuni village in Tanzania. Samples were analyzed for FA and mineral composition by gas chromatography mass spectrometry (GC/MS) and, inductively coupled plasma (ICP) emission spectroscopy, respectively. Linoleic acid (LA) and alpha-linolenic acid (ALA) were higher in sunflower (*Helianthus sp)* oil, 252 mg/g and 0.58 mg/g, and pumpkin seeds (*Cucurbita pepo*), 126 mg/g and 0.17 mg/g, respectively. Pumpkin seeds contained 9170 mg/kg of potassium, 115 mg/kg of iron and 62 mg/kg of zinc, which are important cofactors for FA metabolism. Pumpkin seeds and sunflower oil are dietary sources of essential FA (EFA) that could be incorporated into Tanzanian diets, especially where there is a high prevalence of growth stunting, cognitive impairment, and EFA deficiency, such as in Rudewa-Mbuyuni. Since the sunflowers and pumpkin analyzed in this study are widely distributed throughout Africa, these data may be beneficial to various regions where EFA and mineral deficiencies are common.

1. Introduction

Essential fatty acids (EFA) and minerals are dietary constituents crucial for normal growth, development, and maintaining homeostasis. Fat is major dietary energy source and dietary fat intake is low in African diets (Yang and Huffman, 2013). Tanzanians typically consume diets with only about 10–15% of total calories from fat, which is less than half the 25–35% of energy from fat recommended by FAO/WHO (Elmadfa and Kornsteiner, 2009). Tanzanian dietary staples contain low amounts of fat (ranging from 1.5–4%), and generally consist of carbohydrate-rich staples, such as maize, sorghum, millets, and rice (FAO, 2010). The lack of dietary fat intake in Tanzania contributes to lower intakes of EFA and ultimately EFA deficiency (EFAD) (FAO, 2010; Michaelsen et al., 2011). EFAD is also associated with stunting and cognitive impairments (Huffman et al., 2011); therefore, ensuring adequate intake of dietary fats, in particular EFA, is imperative.

Dietary EFA include linoleic acid (LA), an n–6 FA, and alpha-linolenic acid (ALA), an n–3 FA. These EFA cannot be synthesized in the body and must be supplied through dietary intake (Huffman et al., 2011; Kuipers et al., 2011; Luxwolda et al., 2014). More importantly, EFA are metabolized to very long-chain (VLC) polyunsaturated fatty acids (PUFAs). For instance, LA is converted to VLCPUFA arachidonic acid (AA), and ALA is converted to both VLCPUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both EFA and their VLCPUFA metabolites are important for various body functions, including growth, immune function, and cognitive development. In non-coastal and rural areas, such as Rudewa-Mbuyuni village Morogoro, Tanzania, where intake of fish and marine products is low, VLCPUFAs would mostly be formed from endogenous conversion of EFA to their respective metabolites. Thus, it is critical in remote areas, such as Rudewa-Mbuyuni, that individuals are consuming foods high in EFA to meet the body's requirement. Insufficient supply of EFA leads to EFAD, and

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; AOAC, Association of Analytical Communities; BHT, butylated hydroxytoluene; CCN, coconut; DHA, docosahexaenoic acid; DSQ, dual stage quadrupole; EPA, eicosapentaenoic acid; EFA, essential fatty acid; FAME, fatty acid methyl esters; FA, fatty acids; GC, gas chromatography; GLA, gamma-linolenic acid; GC/MS, gas chromatography mass spectrometry; HPLC, high-performance liquid chromatography; ICP, inductively coupled plasma; KRO, Korie oil; LLOQ, lower limit of quantification; LA, linoleic acid; MUFA, monounsaturated fatty acid; n–3, omega 3; n–6, omega 6; OYN, oysternut; PUFA, polyunsaturated fatty acid; PKSY, pumpkin seeds with shells; PKSN, pumpkin seeds without shells; RPO, red palm oil; RT, room temperature; SIM, selective ion monitoring; SFO, sunflower oil; VLC, very long chain; WHO, World Health Organization.

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http://dx.doi.org/10.1016/j.jfca.2016.05.016
0889-1575/

EFAD may result in impairment of growth, impaired immune function, and poor cognitive development.

Aside from EFAD, micronutrient deficiency is associated with growth stunting and cognitive impairment (Ackatia-Armah et al., 2015). Although the mineral profiles of most seeds are characterized, the mineral content of seeds and nuts can differ based on environmental factors such as soil type and mineral profile (Glew et al., 2006; Michaelsen et al., 2011). Therefore, different geographical regions can contain lower amounts of minerals, and since certain minerals are cofactors for EFA metabolism, a decreased intake of these minerals could also impair VLCPUFA production. For instance, zinc is a cofactor for EFA metabolism, and zinc deficiency can limit the conversion of LA to AA, and ALA to EPA and DHA (Harris et al., 2009). However, the mineral content of foods locally available to these remote areas, such as Rudewa-Mbuyuni in Tanzania, is not well characterized.

The Tanzania food composition table provides estimates of nutrient content, such as protein, carbohydrates, total fat and other vitamins and minerals. However, no information is given into individual EFA concentrations of foods listed. Furthermore, the mineral data in the table were calculated based on information obtained from different countries, and it is known there are geographical differences in the composition of nutrients in seeds grown in different areas (Murkovic et al., 1996; Tangolar et al., 2009). Since EFAD and mineral deficiency impact growth and development, there is a need to quantify the EFA and minerals of local foods. In many areas of Africa, such as Rudewa-Mbuyuni village, Morogoro, Tanzania, where there is a high prevalence of growth stunting, identifying local foods available to villagers high in EFA and minerals could allow for dietary recommendations to address stunting. Therefore, in this study we determined the concentration of FA, in particular EFA and VLCPUFAs, and minerals in a variety of local seeds and oils available to villagers living near Morogoro, Tanzania.

2. Methods

2.1. Procurement and preparation of local Tanzanian oils, seeds, and nuts

All seeds, nuts, and oils analyzed in this study are described in Table 1 and were purchased from a local market in Rudewa-Mbuyuni village. The oils were packaged in amber containers to prevent the FA from being oxidized, and seeds and nuts were finely crushed and freeze dried. The freeze-dried samples were stored no longer than 14 days after arriving in the US. All freeze-dried seed samples and food-grade oils were shipped to a Michigan State University laboratory where they were purged with high-purity nitrogen and stored at −20 °C until analysis of FA and mineral content. Due to the shipping and power challenges in this region, freeze drying of all solid products was chosen to maintain product integrity. Freeze drying methods are known to give similar extraction yields when compared to air drying methods (Dulf, 2012), and ALA and LA from freeze-dried samples are reported to be richer compared to air drying methods (Gutiérrez et al., 2008). For pumpkin seeds, samples were analyzed as both whole seed including the outer shell, identified herein as PKSY, and the de-shelled seed without the outer shells, identified herein as PKSN (Table 1). De-shelled pumpkin seeds were included because it is common in some areas to consume the whole seed with the shell, while in other areas the shells are removed.

2.2. Crude seed oil extraction

All glassware used in this analysis was thoroughly cleaned using an acid bath followed by a series of high-performance liquid chromatography (HPLC)-grade organic solvents, to remove any FA contaminants. Lipid extraction from total seed material was performed as previously described, but modified as specified (Cequier-Sanchez et al., 2008). In brief, the samples received were coned and quartered to obtain 400 mg freeze-dried seed material, which was transferred to an individual 16 × 150 mm Teflon-lined screw-capped glass tube. Next, total seed material was incubated at room temperature (RT) with 10 mL 2:1 *v/v* HPLC-grade chloroform (Avantor Performance Materials, Inc., Center Valley, PA)/HPLC-grade methanol containing 100 μg butylated hydroxytoluene (BHT) per mL (Sigma Aldrich, St. Louis, MO). Samples were placed in a sample rack, and mixed on a low setting for 2 h on a titer plate shaker (Lab-Line Instruments, Tripunithura, Kochi, India) under dim lighting. Seeds were gravity filtered using lipid-free filters (GE Healthcare UK Limited, Welwyn Garden City, UK) into new 16 × 100 mm Teflon-lined screw-capped glass tubes, which contained 2.5 mL of HPLC-grade 0.88% *v/v* aqueous KCl (J.T. Baker, Phillipsburg, NJ). This solution was vortexed for 30 s, followed by centrifugation at 3000*g* at 10 °C for 10 min. After centrifugation, the lower organic phase was transferred to a clean glass tube, and the aqueous upper phase was washed with 3 mL HPLC-grade chloroform, then centrifuged at 3000*g* at 10 °C for 10 min. The lower chloroform organic phase was removed and combined with the 2:1 organic phase; then the combined organic phases were evaporated at RT under high-purity nitrogen. After drying, the total crude seed oil was weighed and calculated.

2.3. Methylation of oils to FAMEs, neutralization, and FAME isolation

Aliquots of crude seed oils and food-grade oils (80 mg) were weighed into individual clean 16 × 100 mm glass tubes. Both crude seed oil and food-grade oil samples were re-suspended in HPLC-grade chloroform/methanol (2:1 *v/v*, 100 μg BHT/mL) to obtain a final total lipid concentration of 20 mg/mL. Resuspended oils were prepared for methylation as previously described by Cequier-Sanchez et al. (Cequier-Sanchez et al., 2008). In brief, an aliquot of 100 μL total lipid extract solution was transferred to a clean 16 × 100 mm Teflon-lined screw-capped glass tubes. The internal standard nonadecanoic acid (150 μg; Sigma Aldrich, St. Louis, MO) in HPLC-chloroform was prepared and added to each sample. The

Table 1
List of foods and oils analysed.

food	abbreviation	family	description
coconut	CCN	*Cocos sp*	a tall palm tree fruit; flakes were freeze dried and used in analyses
pumpkin seed (whole)	PKSY	*cucurbita sp*	vigorous perennial vine; flattened seeds, freeze dried
pumpkin seed (de-shelled)	PKSN		
oysternut	OYN	*Telforia sp*	perennial climbing vine; seeds were ground and freeze dried
sunflower oil	SFO	*Helianthus sp*	crude oil processed locally from sunflower seeds
korie oil	KRO	palm olein[a]	commercially refined oil made from crude polyolefin
red palm oil	RPO	*Elaeis sp*	crude oil processed locally, red-orange, high β-carotene

[a] Imported palm olein from Malaysia and Latin America.

Table 2
Saturated fatty acid composition of local Tanzanian oils and seeds[a].

sample ID	myristic (C14:0)	palmitic (C16:0)	stearic (C18:0)	arachidic (C20:0)	lignoceric (C24:0)	total saturated
coconut	21.5	6.97	1.96	0.10	0.01	30.5
pumpkin seed (whole)	0.10	8.76	3.28	0.41	0.02	12.6
pumpkin seed (de-shelled)	0.11	22.9	17.2	0.40	0.30	40.9
oysternut	0.05	56.6	18.0	0.57	0.02	75.2
sunflower oil	0.30	14.0	5.98	1.65	0.45	22.4
korie oil	3.80	302	19.7	1.00	0.04	327
red palm oil	5.38	290	23.7	1.28	0.04	320

[a] *Note:* values expressed in mg FA/g food-grade oil or mg FA/g seed. FA concentrations are representative of a pooled food-grade oil ($n = 1$) or pooled seed ($n = 1$) sample, obtained from a local market in Rudewa-Mbuyuni village.

samples were then dried under high-purity nitrogen at RT. Methylation was performed as previously described by Agren et. al., and modified as previously described by Pickens et al. (Pickens et al., 2015). FAMEs were neutralized and isolated as previously described (Gurzell et al., 2014).

2.4. FAME identification, analysis, and data processing

Resuspended FAMEs were transferred to GC vials with glass inserts for analysis. The injection order of resuspended FAME samples was randomized prior to analysis. FAME analysis was performed on a DSQII quadrupole GC–MS (Thermo Scientific, Waltham, MA,) equipped with a 30-m DB-23 column (0.25 mm id; Agilent Technologies, Santa Clara, CA,) using helium as a carrier gas. GC temperature profile is as follows: 40 °C 1 min; ramp 1, 100 °C/min to 160 °C; ramp 2, 2.8 °C/min to 192 °C; ramp 3, 0.5 °C/min to 201 °C; ramp 4, 50 °C/min to 250 °C and held for 1 min. Selective ion monitoring (SIM) was employed for enhanced sensitivity. Standard FAME mixture (Part# CRM47885; Lot# LC06601 V; Supelco, Bellefonte, PA) was used to identify and quantify individual FAMEs. The certified range of each FAME measured is as follows: 600 μg/mL for palmitic acid; 400 μg/mL for myristic, stearic, (*cis*-9) oleic, arachidic, behenic, and lignoceric acid; 200 μg/mL for (*cis*-7) palmitoleic, (*cis*-11) eicosenoic, (*cis*-9,12) LA, (*cis*-6,9.12) gamma-linolenic acid (GLA), (*cis*-11,14) eicosadienoic, (*cis*-5,8,11,14) AA, (*cis*-13,16) docosadienoic, (*cis*-15) nervonic, (*cis*-11,14,17) ALA, (*cis*-5,8,11,14,17) EPA, and (*cis*-4,7,10,13,16,19) DHA. Standard curves were created from the certified ranges using 5-fold serial dilutions to produce a 5-point standard curve. FAME concentrations below the lower limit of quantification (LLOQ) are defined for each FA in Tables 3–5. Nervonic acid, AA, EPA, DHA, docosadienoic acid were below the LLOQ in all samples analyzed and, thus, were excluded from the tables. Some resuspended FAME samples contained FAME concentrations above the highest standard curve, so these samples were diluted 1:10 and reanalyzed on the same standard curve as undiluted samples. FAME peak integration and quantification was performed using TargetLynx V4.1 (Waters, Milford, MA) based on the FAME standard's retention time and SIM ion abundances. The concentrations of resuspended FAMEs were normalized to the amount of food-grade oil (i.e., sunflower oil), or crude seed oil and total seed material for seed samples (i.e., pumpkin seeds).

2.5. Minerals analysis

Freeze-dried samples of PKSY, PKSN, CCN, and OYN were shipped under dry ice to a third party contractor for mineral analysis. Mineral content of each sample was determined by ICP emission spectroscopy (ICP_S: 28) and the modified official method of analysis of AOAC International no. 984.27, 985.01, and 2011.14 were followed (AOAC, 2010). The amounts of minerals in these samples are expressed as mg/kg.

2.6. Statistical analyses

FA concentrations are representative of a pooled food-grade oil ($n = 1$) or pooled seed ($n = 1$) sample, obtained from a local market in Rudewa-Mbuyuni village. Figures were made in GraphPad Prism version 4 (GraphPad Software, Inc. La Jolla, CA).

3. Results

The FA composition of seeds, nuts, and food-grade oils are presented as the concentration of each FA as mg/g, respectively (Tables 2–5). Several seeds and food-grade oil samples were found to contain varying levels of several FA, as shown in the following data.

3.1. Saturated fats

Several seeds and oils were abundant in saturated FA (Table 2). All samples contained saturated FA, and KRO and RPO had high amounts of saturated FA, 327 mg/g and 320 mg/g respectively. In particular, palmitic acid (16:0) was a highly abundant saturated FA in KRO (302 mg/g) and RPO (290 mg/g).

Table 3
Monounsaturated fatty acid composition of local Tanzanian oils and seeds[a].

sample ID	oleic (C18:1)	eicosenoic (C20:1)	total MUFA
coconut	5.11	<LLOQ	5.11
pumpkin seed (whole)	14.5	0.07	14.6
pumpkin seed (de-shelled)	35.5	1.20	36.7
oysternut	20.5	<LLOQ	20.5
sunflower oil	93.8	0.75	94.6
korie oil	426	1.97	428
red palm oil	296	<LLOQ	296

[a] *Note:* values expressed in mg FA/g food-grade oil or mg FA/g seed. FA concentrations are representative of a pooled food-grade oil ($n = 1$) or pooled seed ($n = 1$) sample, obtained from a local market in Rudewa-Mbuyuni village. The LLOQ for eicosenoic was: 1) 0.107 mg/g in coconut, 2) 0.146 mg/g in oysternut, and 3) 0.35 mg/g in red palm oil. LLOQ, Lower limit of quantification; MUFA, monounsaturated fatty acids.

Table 4
Total n–3 fatty acid composition of local Tanzanian oils and seeds[a].

sample ID	ALA (C18:3)	Total n–3
coconut	<LLOQ	<LLOQ
pumpkin seed (whole)	0.17	0.17
pumpkin seed (de-shelled)	0.05	0.05
oysternut	<LLOQ	<LLOQ
sunflower oil	0.58	0.58
korie oil	0.53	0.53
red palm oil	1.38	1.38

[a] *Note:* values expressed in mg FA/g food-grade oil or mg FA/g seed. FA concentrations are representative of a pooled food-grade oil ($n = 1$) or pooled seed ($n = 1$) sample, obtained from a local market in Rudewa-Mbuyuni village. The LLOQ for ALA was: 1) 0.26 mg/g in coconut, and 2) 0.35 mg/g in oysternut. ALA, α-linolenic acid; LLOQ, lower limit of quantification.

Table 5
Total n–6 fatty acid composition of local Tanzanian oils and seeds[a].

sample ID	LA (C18:2)	GLA (C18:3)	eicosadienoic (C20:2)	Total n–6
coconut	1.21	0.82	<LLOQ	2.03
pumpkin seed (whole)	41.3	0.53	0.10	41.9
pumpkin seed (de-shelled)	126	0.75	<LLOQ	127
oysternut	106	1.13	<LLOQ	107
sunflower oil	252	2.88	<LLOQ	255
korie oil	102	11.8	<LLOQ	114
red palm oil	87.3	<LLOQ	<LLOQ	87.3

[a] *Note:* values expressed in mg FA/g food-grade oil or mg FA/g seed. FA concentrations are representative of a pooled food-grade oil (n = 1) or pooled seed (n = 1) sample, obtained from a local market in Rudewa-Mbuyuni village. The LLOQ for GLA was: 0.020 mg/g in red palm oil. The LLOQ for eicosadienoic was: 1) 0.260 mg/g in coconut, 2) 0.256 mg/g in pumpkin seeds (de-shelled), 3) 0.354 mg/g in oysternut, and 4) 0.850 mg/g in sunflower, korie, and red palm oil. LLOQ, lower limit of quantification; LA, linoleic acid; GLA, γ-linoleic acid.

3.2. Monounsaturated fatty acids

MUFA contents varied greatly across the seed and oil samples analyzed (Table 3). KRO had the highest concentration of oleic acid (426 mg/g), followed by RPO (296 mg/g). The concentration of eicosenoic acid was low in these samples. Food-grade oils had considerably higher concentrations of total MUFA compared to seeds and nuts.

3.3. n–3 fatty acids

In general, all seeds and oils contained low concentrations of n–3 FA (Table 4), which included ALA (18:3n-3). As mentioned, EPA and DHA were <LLOQ in all samples analyzed in this study. SFO and whole pumpkin seeds (i.e., PKSY) had 0.58 mg/g and 0.17 mg/g total n–3 amounts respectively. RPO had 1.38 mg/g and KRO had 0.53 mg/g total n–3 FA.

3.4. n–6 fatty acids

n–6 FA were more abundant than n–3 FA in the foods analyzed (Table 5). LA was the most abundant n–6 fatty acid in all samples. LA was most abundant in SFO and pumpkin seeds without shells (PKSN). Coconut had very low levels of LA and contained some small amounts of AA which was not present in the other samples.

3.5. Minerals

PKSY and PKSN were most abundant with macro and micro-elements except for copper (Fig. 1). Whole pumpkin seeds (i.e., PKSY) contained 429 mg/kg of calcium compared to seeds without shells (i.e., PKSN), which contained 267 mg/kg of calcium (Fig. 1B). Coconut was less abundant in all the minerals analyzed except for manganese and potassium. Sodium levels for these samples were all below 200 mg/kg. Pumpkin seeds contained more iron compared to other seeds (Fig. 1A). PKSY contained 115 mg/kg of iron while PKSN contained 80 mg/kg of iron. Additionally, zinc levels were high in the pumpkin seeds samples, and PKSY contained 50.4 mg/kg compared to 62.4 mg/kg in PKSN.

4. Discussion

The purpose of this study was to identify sources high in EFA and micronutrients, in a variety of local oils and seeds available to residents of Rudewa-Mbuyuni village in Tanzania. Here we show SFO, KRO, and pumpkin seeds, whole (i.e., PKSY) and de-shelled (i.e., PKSN), were the most abundant in EFA (i.e., LA and ALA) compared to other foods analyzed in this study (Table 3 and 4). In addition, SFO were the least abundant in total saturated FA and MUFAs compared to the other oils. Despite having high levels of n–6 FA, pumpkin seed also had lower levels of saturated FA as well as MUFAs (Table 2 and 3). In general, pumpkin seeds contained the highest amounts of fold changes of micronutrients observed (Fig. 1). As mentioned, the Tanzania food composition table (Lukmanji et al., 2008) provides the estimate of nutrient content, such as protein, carbohydrates, total fat and other vitamins and minerals. However, no information is given on the individual EFA concentrations of foods listed. Furthermore, dietary deficiencies in EFA are associated with growth, consistent with results from other studies conducted in both developed and developing countries

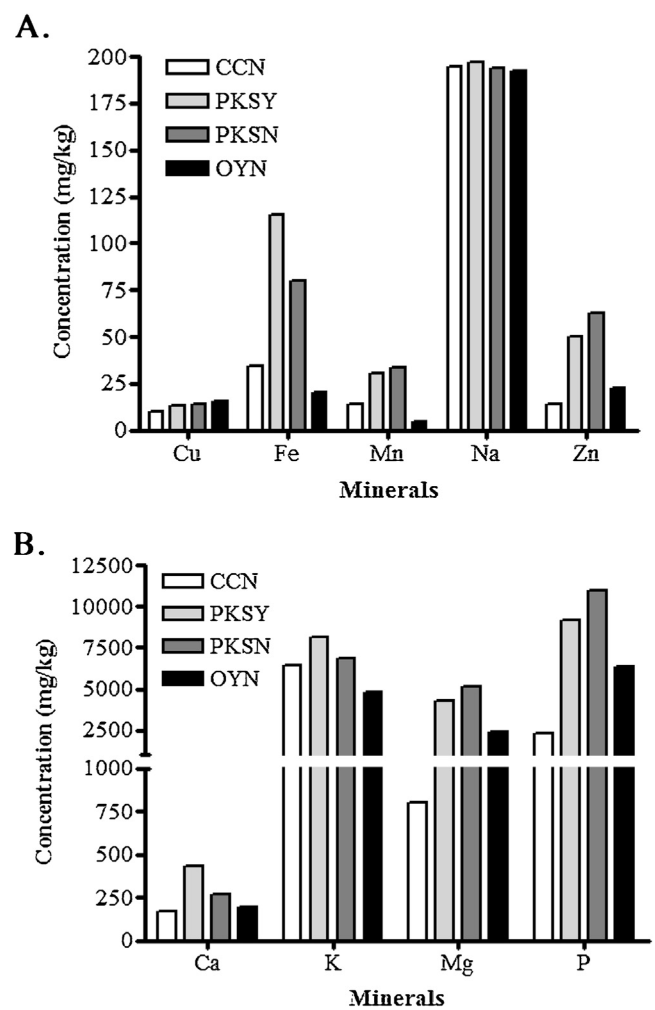

Fig 1. Concentrations of minerals expressed in mg/kg, A) indicates micro minerals, B) indicates values for macro minerals. Ca, calcium; CCN, coconut; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; P, phosphorus; PKSY, pumpkin seed (whole); PKSN, pumpkin seed (de-shelled); OYN, oysternut; Zn, zinc;.

(Huffman et al., 2011; Rombaldi Bernardi et al., 2012; Simopoulos, 1991). In our current study, these data suggest that SFO, KRO, and pumpkin seeds are good sources of EFA, and incorporation of these foods into local diets may help combat deficiency and growth problems.

The oils analyzed in this study were readily available for purchase in Rudewa Mbuyuni, village in Morogoro, Tanzania. Here we show KRO and RPO contained more saturated FAs (i.e., palmitic acid) and MUFAs (i.e., oleic acid) compared to SFO (Tables 2 and 3). Oleic acid was the most abundant MUFA present in these oils (Table 3) and our results are similar to those reports by Rui et al. (Rui et al., 2007). Overall SFO was the most abundant in LA and ALA compared to KRO and RPO (Table 4 and 5). The LA concentrations of SFO reported in this study are consistent with other findings (Michaelsen et al., 2011; Rui et al., 2007). In general, the ALA concentrations were low in the food-grade oil samples analyzed. However, our findings are consistent with other studies (Fernandez et al., 2002; Glew et al., 2006). The SFO and RPO samples analyzed in this study were locally processed and would be considered more "crude-like" compared to KRO. KRO is commercially produced oil that is widely used by many Tanzanians, and interestingly, the FA content of KRO is not listed on the label or in the Tanzanian food composition table. Although sunflowers are locally cultivated in Rudewa, they are mainly used as cash crops and most families in rural villages save sunflower seeds for household consumption. SFO is therefore more expensive compared to KRO and RPO, and thus, a majority of households in this village use KRO and RPO more frequently than SFO because of cost. Both KRO and RPO are high in saturated fat and MUFA and also contain LA; however, the amount of LA in these oils was lower than that found in SFO.

In Rudewa Tanzania, it is common to consume pumpkin seeds with their outer shell because of convenience. We report whole pumpkin seeds (i.e., PKSY) had low concentrations of all FAs except ALA and eicosadienoic acid, compared to de-shelled (i.e., PKSN) pumpkin seeds (Tables 2–5). We expected the PKSY sample to contain lower concentrations of FA compared to PKSN, since shells contributed to the weight of total seed material, and we normalized our FA concentrations to the total seed material used in lipid extractions. Therefore, the more abundant ALA and eicosadienoic acid in the PKSY sample compared to the PKSN sample may reflect a high concentration of ALA and eicosadienoic acid in the pumpkin seed shell (Table 4). Overall, pumpkin seeds contained the highest concentrations of LA and ALA (Table 4 and 5). Coconuts contained mostly saturated fat (Table 2) and very low levels of EFA (Table 4 and 5). Coconuts are widely used by this population despite their low FA content. OYN had high LA content, as well as other FA, however, OYN is not widely found in this area, and therefore, is not widely consumed compared to other seeds and nuts analyzed in this study (Table 4 and 5). Pumpkin seeds and OYN could be incorporated more into local diets to increase dietary LA intake, but pumpkin seeds contained highest quantities of both EFA.

The metabolism of EFA requires minerals (i.e., zinc) as enzymatic cofactors (Harris et al., 2009). As mentioned, although the mineral profiles of most seeds have been already characterized, the mineral content of seeds and nuts can differ based on the geographical and environment factors, such as soil (Glew et al., 2006; Michaelsen et al., 2011). Also, the mineral contents listed in the Tanzanian food composition table are based on calculated values and not through direct analysis of the foods. Pumpkin seeds were more abundant in several minerals including calcium, iron, zinc, manganese, magnesium, phosphorus, and potassium compared to the other nuts and seeds analyzed (Fig. 1). The mineral concentrations of these pumpkin seeds from this region are also consistent with other findings in African countries (Fernandez et al., 2002; Glew et al., 2006). Several of these minerals are known to be important for growth and are also cofactors for both FA and protein metabolism (Coleman, 1992; Harris et al., 2009). In general, the analysis of FA and minerals in these samples has shown that pumpkin seeds contained sufficient amounts of LA as well as minerals and these results are consistent with those reported by other studies (Dzisiak, 2004; Glew et al., 2006; Murkovic et al., 1996; Tarek and Khaled, 2001).

Taken together, our results indicate pumpkin seeds were more abundant in EFAs and minerals compared to the other local foods analyzed in our study. In Rudewa Mbuyuni village, a majority of the villagers are farmers and participate in subsistence farming (Ntwenya et al., 2015). Also a majority of these households rely on plant-based diets to support their nutrient and energy requirements. Taken together, our results indicate SFO and pumpkin seeds are local foods available to villagers near Rudewa Mbuyuni that are higher in EFA. Also pumpkin seeds had a high concentration of the majority of minerals analyzed in this study, and these minerals (i.e., iron and zinc) are important for various functions in the body including growth, immunity, and cognitive development (Adu-Afarwuah et al., 2007, 2008). In this region, pumpkins are found year round and are commonly cultivated in backyards of households. Both the pumpkin and pumpkin leaves are consumed more often, although pumpkin leaves are added to sauces and green leaves during preparation of these meals, the seeds are mostly saved to be used for planting. Additionally, incorporating oils such as SFO and KRO into cooking methods, along with an increased supplementation of pumpkin seeds, may increase dietary intake EFA and mineral.

Our study was conducted on a variety of locally available foods in Rudewa-Mbuyuni Tanzania to identify dietary sources of EFA and minerals. We recognize that the generalizability of these observations is limited. Therefore, studies need to be conducted to assess the feasibility of incorporating our suggested foods to villagers. We acknowledge our analysis did not include all foods available to these villagers; however, the variety of foods we report are most commonly found and consumed by households in this region. The results in this study cannot be generalized to the entire Tanzanian population because foods were purchased from only one area. However, future studies should address this limitation by analyzing foods from various regions in Tanzania. In addition, the FA analysis was conducted on raw foods, and seeds such as the pumpkin seeds and oyster nuts are often not consumed raw. Future studies should investigate the impact of local cooking methods on EFA degradation in these foods, and measure antioxidant vitamins that may protect EFA during cooking and processing. For instance, pumpkin seeds are also a good source of vitamin E (Veronezi and Jorge, 2012) and vitamin E isomers, including γ-tocomonoenol and α-tocomonoenols (Butinar et al., 2011), which may help protect EFA from rancidity during processing or storage. Another limitation of our study is we do not have details on how the seeds, nuts, and oils were stored prior to purchase from the market. We also do not have details regarding the processing methods of food-grade oils in Tanzania.

To our knowledge, no research group has sought to identify the EFA and mineral content of local oils, seeds, and nuts available in this region of Tanzania. Our research suggests pumpkin seeds could be used to increase dietary EFA as well as micronutrient intake in an area where growth stunting and cognitive impairment are prevalent and associated with these nutrient deficiencies.

5. Conclusion

Pumpkin seeds, SFO, and KRO are good sources of EFA available to villagers in Rudewa-Mbuyuni Tanzania. Furthermore, pumpkin seeds contained high levels of EFA and highest amount of minerals (i.e., iron, potassium, calcium, and zinc) compared to other samples analyzed in this study. Since EFAD and mineral deficiencies are associated with growth stunting and cognitive impairment, and there is a high prevalence of these conditions in the village of Rudewa-Mbuyuni, these data suggest that incorporating pumpkin seeds, SFO, and KRO into the diet may be an effective method to increase intake of these nutrients crucial for growth and cognitive development.

Conflicts of interest

The authors declare no conflict of interest to disclose.

Acknowledgements

We thank Dr. Scott Smith for his assistance with GC/MS and MSU's Mass Spectrometry and Metabolomic Core Facility. We would also like to acknowledge Mariana Ngowi for assisting with the collection and shipping of the food-grade oil samples from Rudewa-Mbuyuni village to Michigan State University for analysis. We would also like to acknowledge iAGRI/USAID for funding this study.

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