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.