EFFECTIVENESS OF FORTIFIED SUNFLOWER OIL ON RETINOL STATUS OF UNDERFIVE CHILDREN AND LACTATING MOTHERS IN MANYARA AND SHINYANGA REGIONS, TANZANIA

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2019

EXTENDED ABSTRACT

Vitamin A Deficiency (VAD) continues to be a public health problem in low income countries including Tanzania. It affects the vulnerable social groups namely women of reproductive age (15-45 years) including pregnant women and lactating mothers; and children below five years. Despite the Government's efforts to eliminate VAD among the lactating mothers and children below five years, the prevalence is still very high. Vitamin A deficiency in Tanzania accounts for approximately one-third in children below five years, 39% of pregnant women and 35% for lactating mothers. The main underlying cause of VAD is a diet that is chronically insufficient in vitamin A. One intervention to eliminate vitamin A deficiency in this study was fortification of virgin sunflower oil with vitamin A. Despite the fact that utilization of the fortified cooking oil with vitamin A at household level is increasing rapidly in Tanzania, there is little information on the use of fortified cooking oil with vitamin A as the major source for preventing VAD. Therefore, the present study was designed to fill the gap by evaluating the effectiveness of fortifying virgin sunflower oil with vitamin A on the retinol status of children below five years and lactating mothers in Manyara and Shinyanga regions of Tanzania. Each region involved three intervention districts and one control district. Intervention districts were involved in consumption of fortified virgin sunflower oil with vitamin A in the households. The fortification level of vitamin A in the sunflower oil was 30-40 mg/kg as per East African Standards recommendation. No control was made on the amount of oil to be used in the preparation of any meal in the household. Intervention districts were encouraged to buy fortified sunflower oil from the specified shops where the fortified oil was sold at subsidized rate of 2100 Tshs per liter and use it for preparing food. For mothers who were not able to liters (1 L, 3 L, 5 L and 20 L container) there was fortified sunflower oil with vitamin A sold in small quantities known as coops. Through socio-marketing, this group

was also encouraged to consume vitamin A rich foods and diversify their diets. During intervention period the control groups were not receiving sunflower oil fortified with vitamin A. Instead, they were encouraged to use unfortified sunflower oil for cooking and consume vitamin A rich foods as well as diversifying their diets. During intervention period, mothers were visited at their households by the village health workers and home-based care providers once every week to monitor compliance to the use of the fortified oil. They were encouraged to buy the fortified sunflower oil and use it for food preparation. Control groups started receiving the fortified sunflower oil after the intervention period of 18 months. Assessment of consumption of fortified sunflower oil with vitamin A was carried out in the intervention districts where fortified virgin sunflower oil with vitamin A in the households was practiced. A total of 569 mother-child pair was involved to evaluate the potential use of the fortified virgin sunflower oil in controlling vitamin A deficiency. Retinol in the Dry Blood Samples was determined by Retinol Binding Protein enzyme linked immune assay (ELISA) method while Retinol in the oil was determined by HPLC method. Results revealed that, at baseline about 60% (n=313) of the oil samples from the households had retinol concentration level below 20 mg/kg, out of which majority were from Manyara (n=245, 47%) and only few samples (n=68, 13%) were from Shinyanga. At end-line, majority of households oil samples (89.9%, n=471 of the) had retinol concentration of 20-40 mg/kg, which these values were within the recommendation by East African Standards EAS 269:2017 (TZS 1313:2014) fortified edible oils and fats specification. Baseline mean serum retinol concentrations of the children and lactating mothers in the control districts were 14.63±5.31 and 20.75±7.32 µg/ml, respectively, while in the intervention districts, the mean serum retinol concentrations for the children and lactating mothers were 15.14±5.44 and 20.10 ±7.33 µg/ml, respectively. These values were lower than those recommended by World Health Organization which defines the plasma retinol concentrations of <0.7 µmol/L or <17.325

µg/ml of Retinol Binding Protein for the children and plasma retinol concentrations of <1.05 µmol/L or <26.04 µg/ml of Retinol Binding Protein for the lactating mothers as vitamin A deficiency. End-line mean serum retinol concentrations in the control districts were 14.83 ±5.95 µg/ml, for the children and 21.35±7.87 µg/ml for the lactating mothers, while in the intervention districts mean serum retinol concentrations were 17.59±10.20 µg/ml for the children below five years and 22.83±9.48 µg/ml for the lactating mothers. A quasi-experimental non-equivalent control group design with intention to treat vitamin A deficiency showed that, fortified virgin sunflower oil with vitamin A in children below five years and lactating mothers had positive effects on retinol serum concentration. Also fortified virgin sunflower oil with vitamin A had positive effects on anthropometric status namely, weight for age, height for age and weight for height Z-scores. Children who consumed fortified sunflower oil with vitamin A progressively improved in weight for age from -0.81 to -0.72 SD, height for age -1.36 to -0.81 SD and weight for height -0.21 to -0.06 SD Z-scores (baseline vs. end-line). In the control districts weight for age was -1.08 to -0.93 SD, height for age -1.61 to -1.28 SD and weight for height -0.25 to -0.16 SD Z-scores (baseline vs. end-line). Beside fortified oil, other factors which may account for improved serum retinol concentration and anthropometric status of the children and lactating mothers included vitamin A mega doses supplement (children), dietary diversity and maternal knowledge about consumption of vitamin A rich foods and age (breastfeeding and non-breastfeeding). Also, despite significant decrease in vitamin A levels in the fortified virgin sunflower oil during six months of storage (11%) and in the scooping study (10%) and the slight increase in the quality aspects namely peroxide; anisidine, malondialdehyde and Totox values, the oil was still good for healthy. The major socio-economic and demographic factor which significantly (<0.05) influenced vitamin A status of these socio groups is residence. Living in rural areas decreased like hood to diversify foods compared to urban area. Also seasonal vitamin A rich foods

mainly plant based foods is a great problem in rural areas compared to urban areas. Therefore, to address vitamin A deficiency problem; the use of fortified cooking oils with vitamin A; increasing consumption of vitamin A rich foods and dietary diversification should be encouraged to sustain the prevention and control of this deficiency.

DECLARATION

I, Edna Leonard Ndau, do hereby declare to the Senate of Sokoine University of Agriculture that, this thesis is my own original work done within the period of registration and that it has neither been submitted nor concurrently being submitted in any other institution.

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Prof. T.C.E. Mosha Date

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Prof. H. S. Laswai Date

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ACKNOWLEDGEMENTS

Thanks to Almighty God, who by any means enabled me to undertake this study. I am also grateful to the Government of Canada, Global Affairs Canada (GAC), International Development Research Centre (IDRC) and the Canadian International Food Security Research Fund (CIFSRF) who provided financial support to undertake this study.

I am grateful to all people who contributed to the successful accomplishment of this thesis. Although I may not be able to mention all, my sincere appreciation should go to all who made this thesis reality. Thanks to my supervisors Prof. T.C.E. Mosha and Prof. H. S. Laswai of the Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture for their effective supervision, guidance and constructive ideas during this study. Their support in terms of professional inputs during proposal development and writing of this thesis remains a fundamental asset for writing other scientific reports in future. My sincere thanks should go to Prof. Susan Horton of the School of Public Health and Health Systems, University of Waterloo for her guidance and constructive ideas during this study.

I also acknowledge the staff, Department of Food Technology, Nutrition and Consumer Sciences for their guidance and encouragements. Thanks to Mr. Mufui, G., Mr. Gabriel, G. and Mr. Mwanyika, S. from offered guidance in laboratory analysis of samples. I would further like to express my sincere appreciations to TFNC and TFDA staff, who assisted in sample analysis. Many thanks should go to Dr. Felister Tibamanya, Dr. Davis Chaula, Dr. Wenat, Alex, Ms. Maria Simbowe, Ms. Zena Ramadhani and Ms. Twilumba Kadeha for their advice and support provided to me during the study. I express my heartfelt appreciations to my sisters Sarah, Ladygrace, Margareth, Peace and Christine as well as my beloved brothers Elibariki Jr, Elibariki Sr, Ndau Jr and Mr. and Mrs. Pambe's family who stood by my side during my PhD program.

I am also grateful to the lactating mothers and children below five years from Manyara and Shinyanga regions in Tanzania who set aside part of their valuable time to participate in this study and for providing logistical support.

DEDICATION

To my parents, Ndau Leonard Kingu Ndau and Mary-Magdalena Mkumbo, who in their love, patience, prayers and subtle ways initiated and inspired me to pursue my education.

LIST OF PUBLICATIONS

1. Ndau, L. E., Mosha, T. C. E. and Laswai, S. H. (2019). Effectiveness of mass fortification of sunflower oil on vitamin A status of children below five years and lactating mothers in Manyara and Shinyanga, regions, Tanzania. *International Journal of Sciences* (submitted).

2. Ndau, E. L, Mosha, T. C. E., Horton, S. and Laswai, S. H. (2019). Shelf-stability of vitamin A in fortified sunflower oil during storage and retail sale by scooping in Manyara and Shinyanga, regions. Tanzania. *Journal of Food Science and Nutrition* (submitted).

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LIST OF ABBREVIATIONS AND ACRONYMS

$	Dollar
µg	Microgam
2SD	95% of the values are less than two standard deviation away from the mean value
AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
AV	Anisidine Value
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toluene
CF	Correction Factor
Conc	Concentration
DBS	Dry Blood Sample
DDS	Dietary Diversity Score
EAS	East African Standards
ELISA	Enzyme Linked Immune Assay
FACT	Fortification Assessment Coverage Tool
FAO	Food and Agriculture Organization
FFA	Free Fatty Acids
Fig	Figure
g	gram
GDP	Gross Domestic Product
GMP	Good Manufacturing Practices
GV	Growth Velocity
H/A	Height for Age

HIV	Human Immune Virus
HPLC	High Performance Liquid Chromatography
HRP	Horsedish peroxide
Kg	Kilogram
L	Litre
MDDS	Mothers Dietary Diversity Score
mg	Milligram
ml	Milliliter
NBS	National Bureau of Statistics
NIMR	National Institute for Medical Research
no	Number
ns	not significant
p	probability
PHsam	Total test sample Peak Height
PKU	Phenylketonuria
PV	Peroxide Values
RBP	Retinol Binding Protein
RF	Response Factor
RHC	Reproductive Health Clinics
RI	Refractive Index
ROS	Reactive Oxygen Species
SD	Standard Deviation
SMEs	Small and Medium Enterprisers
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TBA	Thiobarbituric Acid

TFDA	Tanzania Food and Drugs Authority
TFNC	Tanzania Food and Nutrition Center
TMB	Tetramethylbenzidine
TV	Totox Value
TZS	Tanzanian Standards
U.S	United State
UNICEF	United Nations International Children's Emergency Fund
URT	United Republic of Tanzania
USAID	United State Agency for International Development
VAD	Vitamin A Deficiency
VF	Final Volume
W	Weight
W/A	Weight for Age
W/H	Weight for Height
WGV	Weight Growth Velocity
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Micronutrient deficiency is a major problem that the world is facing today (FAO and WHO, 2013). Among the key micronutrient deficiencies is vitamin A (World Bank, 2012). This has been the major nutritional concern especially in low and middle income countries (Aaron *et al.*, 2017). The main underlying cause of VAD is a diet that is chronically insufficient in vitamin A (FAO and WHO, 2013). Vitamin A deficiency is also associated with inadequate conversion of β-carotenes to vitamin A or interference with absorption leading to depleted vitamin A stores (WHO, 2009). Other factors responsible for vitamin A deficiency include; high prevalence of infectious diseases such as measles, diarrhea and respiratory infections and economic constraints, socio-cultural limitations and lack of education (WHO, 2009).

Children below five years and lactating mothers are the most vulnerable social groups for VAD due to their increased physiological demand (WHO, 2011). In children, VAD retards growth and impairs the immune function, thus increases the severity of infectious diseases, such as measles and diarrhea and risk of death (Pignitter *et al.,* 2016). Severe VAD in children also accelerates xerophthalmia, the leading cause of preventable childhood blindness (Bailey *et al.,* 2015). In lactating mothers, vitamin A deficiency affects vision and leads to production of milk with low vitamin A (WHO, 2011). About 190 million children below five years (Imdad *et al.*, 2017) and 19 million pregnant women (Yang *et al.,* 2016) in the world are vitamin A deficient. In Africa, 44.4 % of the children under the age of five years are affected by vitamin A deficiency (Kurabachew, 2015) while 163 million cases of pregnant and lactating women are vitamin A deficient

(FAO and WHO, 2013). According to the 2010 Tanzania Demographic and Health Survey report, 33% of children aged 6-59 months and 37% of women aged 15-49 years, (39% of pregnant women and 35% of lactating mothers) have VAD (NBS, 2011). Vitamin A deficiency in low income countries including Tanzania varies dramatically by geographical location for instance vitamin A deficiency for the people who live in urban areas is lower than those who live in rural areas due to high economic status in urban than in rural settings (NBS, 2011). In rural areas consumption of vitamin A rich foods are seasonal especially plant based foods while animal vitamin A rich foods are lowly consumed (WHO, 2011).

There has been several strategies to address vitamin A deficiency problem (FAO and WHO, 2013). These strategies include Vitamin A supplementation, dietary diversity, fortification and nutrition education (TFNC, 2014), however, fortification of vegetable oils with vitamin A has been, and is the best intervention to improve vitamin A status of the vulnerable social groups (Pignitter *et al.,* 2016). Oil is the best vehicle for fortification due to its ideal matrix for vitamin A and it stabilizes retinol and delays oxidation of the vitamin (Laillou *et al.,* 2013). The impact of fortifying edible oils with vitamin A is well documented in several studies (Sandjaja *et al.,* 2014). Oil fortification enhances health by increasing the concentration of retinol in the blood (WHO, 2011).

Despite the increase in family incomes and growth of the middle income families, micronutrients under-nutrition is still experienced as shown by URT (2013). In Tanzania, fortification of vegetable oils has been made mandatory law and is reinforced by TFDA since 2011 (FACT, 2016). This law worked well for the commercial producers in which beneficiaries are the people who live in urban areas (Bymolt and d’Anjou, 2017). The law did not work to the small and medium level enterprisers which have potential to supply oil to the majority of people who live in rural communities (URT, 2016). Thus, to deal with this problem the Government of Tanzania launched a nationwide food fortification program in 2013 as one of the measures to prevent micronutrient under-nutrition (URT, 2013). As part of National food fortification, there was a component for raising awareness campaigns and adoption of new technologies to enable fortification to occur locally using local crops and researchers to track trends in consumption of fortified foods in nearly real time (URT, 2013).

Recently, the Government of Tanzania is working with development partners to make sure that, fortification of foods including vegetable oils works well to the Small and Medium scale Enterprisers (SMEs) (URT, 2016). The MASAVA project also known as "Promoting use of locally produced fortified sunflower oil using E-Vouvher" worked with three SMEs, one from Manyara and two from Singida to fortify locally produced sunflower oil. MASAVA means Mafuta Asili ya Alizeti yenye Vitamini A (Virgin Sunflower oil fortified with vitamin A). The project aimed at improving the retinol status of children below five years and lactating mothers in Manyara and Shinyanga regions through fortification of locally produced and fortified sunflower oil. It has also been reported that, improving micronutrient deficiencies such as vitamin A among vulnerable social groups significantly reduces the risk of mortality by 25–35% (Tariku *et al.*, 2016). Therefore, as part of the effort to combat VAD, this study was conducted to evaluate the effectiveness of fortifying locally produced sunflower oil with vitamin A on vitamin A status of children below five years and lactating mothers in Manyara and Shinyanga regions of Tanzania.

1.2 Study Justification

Increased risks of morbidity and mortality due to VAD, particularly among children below five years and lactating mothers have been reported to be a public health problem in developing countries (TDHS-MIS, 2016). Continuous consumption of foods with chronically insufficient vitamin A has been a reason for increase in the risks for death and diseases associated with vitamin A deficiency among the children below five years and lactating mothers (Bahreynian *et al.,* 2017). In Tanzania, micronutrient deficiencies including vitamin A cost the country over US$ 518 million a year, which is about 2.65 % of the country's GDP (Noor *et al.,* 2017). Beyond the economic losses, micronutrient and mineral deficiencies significantly contribute to infants and maternal mortality, with over 27 000 infant and 1 600 maternal deaths annually (Noor *et al.,* 2017). If micronutrient and mineral deficiencies deaths could be avoided, then the infant mortality rate in Tanzania could be reduced to 41.5 per 1000 population (URT, 2013).

Despite the Government efforts to eliminate VAD for the past 18 years through provision of mega doses of vitamin A supplements and deworming tablets to all children under the age of five years, it has only decreased child mortality rates slightly, from 126 deaths per 1 000 live births in 2 000 to 68 deaths per 1 000 live births in year 2011 (Noor *et al.,* 2017). While celebrating the success of vitamin A supplementation, no new nutrition initiatives have been taken to scale up the efforts to improve nutrition in the country (World Bank, 2012). It has been reported that, scaling up core micronutrient interventions would cost Tanzania less than US$26 million per year (Word Bank, 2011). Food fortification is a major opportunity missed by the Government of Tanzania (World Bank, 2012). Nutrition education campaigns and social marketing which are important issues to strengthen the commitment of Government and industry in increasing the demand and consumption of the fortified foods were also missing. The combination of food fortification and its consumption, nutrition education and social marketing would have been very important in the management of vitamin A deficiency among the children below five years and lactating mothers. This study was designed to establish the effect of consuming sunflower oil fortified with vitamin A on vitamin A status of children below five years and lactating mothers in Manyara and Shinyanga regions, Tanzania. Sunflower oil is the type of oil most commonly consumed in the study regions of Manyara and Shinyanga (BoT, 2017). These regions also have shown the highest prevalence of VAD in 2010 (47.6 and 41.5% for children under the age of five years and 53.5 and 43.4% for lactating mothers, respectively) (NBS, 2011). Information obtained from this study will be useful to nutrition stakeholders, policy planners and the Government in improving vitamin A status of children below five years and lactating mothers. Results from this study will also serve as a basis for out-scaling the sunflower fortification program to the other regions where sunflower oil is produced.

1.3 Objectives

1.3.1 Overall objective

To evaluate the effectiveness of fortifying sunflower oil with vitamin A in improving vitamin A status of children below five years and lactating mothers in Manyara and Shinyanga regions, Tanzania.

1.3.2 Specific objectives

Specific objectives of the study are to:

i. Determine the vitamin A status of children below five years and lactating mothers consuming the fortified sunflower oil;
ii. Determine shelf-stability of sunflower oil fortified with vitamin A during storage and retail sale by scooping;

iii. Determine vitamin A levels in oil samples collected from SMEs, retail shops and households;

iv. Determine socio-economic and demographic factors influencing vitamin A status of lactating mothers and children below five years in Manyara and Shinyanga regions, Tanzania.

1.4 Null Hypothesis

H_O: Vitamin A-fortified sunflower oil improves on the retinol status of children below five years and lactating mothers.

1.5 Alternative Hypothesis

H_1: Vitamin A-fortified sunflower oil has no improvement on the retinol status of under-five children and lactating mother.

1.6 Conceptual Framework

The conceptual framework (Fig. 1) presents existing relationship between variables that were used in the study. It focuses on the association between consumption of vitamin A-fortified sunflower oil with retinol status of lactating mothers and children below five years and socio-economic and demographic factors involved.

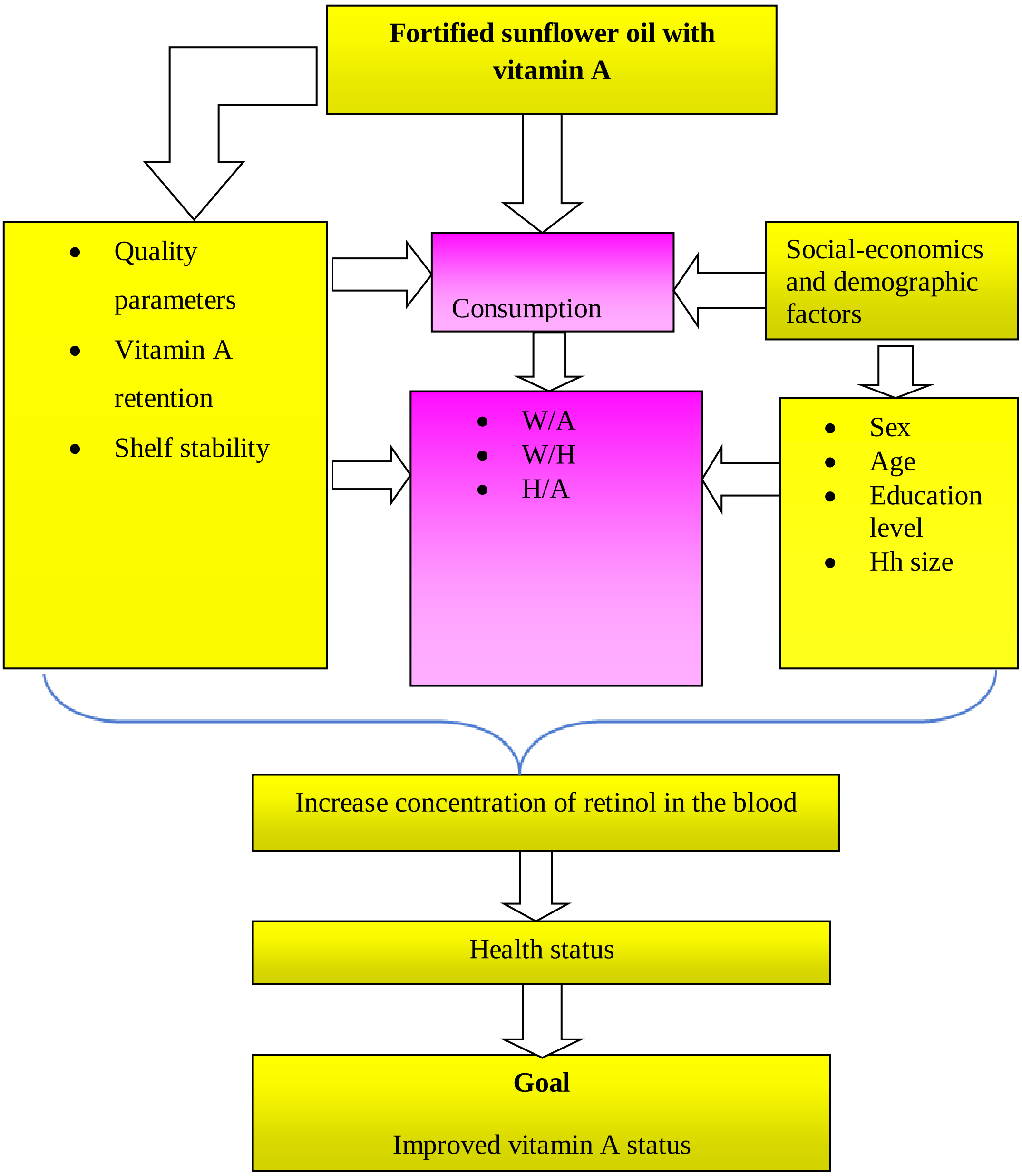

Figure 1: Conceptual framework showing the association between consumption of vitamin A-fortified sunflower oil with retinol status of lactating mothers and children below five years and socio-economic and demographic factors

1.7 Summary of the Conceptual Framework

Consumption of sunflower oil leads to the improvement of anthropometric status and concentration of retinol in the blood. At the same time consumption of fortified sunflower oil is influenced by socio-economic and demographic factors (these include sex, age education level, household family size and income level). Also the quality parameters and vitamin retention (Its shelf stability is important) of fortified sunflower oil is important for the healthy. Meanwhile consumption of fortified sunflower oil affects positively the anthropometric status and concentration of vitamin A. All these parameters which are related to fortified sunflower oil with vitamin A, aimed at improving vitamin A status associated with VAD.

1.8 Organization of the Thesis

This thesis is organized into three chapters. Chapter one provides general introduction of the problem and rationale for the study. This chapter also highlights the objectives of the study. Chapter two includes published and unpublished papers addressing the specific objectives. Paper One address specific objective number i) Paper Two addresses specific objective number ii) Paper Three addresses specific objective number iii) Paper Four, Five and Six addresses specific objective number iv). Chapter three summarizes the general conclusions and recommendations based on the findings of the study. References are presented at the end of each chapter for easy referencing by the reader.

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CHAPTER TWO

PAPER ONE

Effectiveness of mass fortification of sunflower oil on the vitamin A status of children below five years and lactating mothers in Manyara and Shinyanga regions, Tanzania

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(*Submitted to International Journal of Sciences*)

Abstract

Vitamin A Deficiency (VAD) continues to be a public health problem in low income countries including Tanzania. It affects the vulnerable social groups namely lactating mothers and children below five years. A quasi-experimental non-equivalent control group study design was conducted among 569 mother-child pairs to determine the effect of fortifying virgin sunflower oil with vitamin A on the vitamin A status of lactating mothers and children below five years in Manyara and Shinyanga regions of Tanzania. Purposive and random sampling techniques were used to obtain the study samples of 367 mother-child pair in intervention districts and 202 mother-child pair in control districts. IBM SPSS program version 20.0 was used to analyze the data. Intervention group involved consumption of sunflower oil fortified with vitamin at the households. This group was also encouraged to consume vitamin A rich foods and diversify their diets. Fortification level of vitamin A in the sunflower oil was 30-40 mg/kg as per recommendation by the East African Standards (EAS 269:2017) Edible virgin/cold pressed sunflower oil and Tanzania Standard (TZS 50:2014). Intervention period was 18 months. During intervention period (consumption of sunflower oil fortified with vitamin A) the control group was not receiving sunflower oil fortified with vitamin A. Instead, subjects were encouraged to use normal sunflower oil (not fortified) for cooking and consume vitamin A rich foods as well as diversifying their diets. Results revealed that, more than half (59.7%) of the oil samples collected from the household at baseline had retinol concentrations below 20 mg/kg. Out of 59.7% oil samples from the household, 46.7% samples were from Manyara region while 13% were from Shinyanga region. At the end-line, most of the oil samples from the household (89.9%) had retinol concentration ranging from 20-40 mg/kg. Baseline serum retinol concentrations for the children below five years and lactating mothers in the intervention districts were 15.14±5.44 and 20.10±7.33 µg/ml, respectively. After intervention, serum retinol

concentrations in the intervention group were 17.59±10.20 µg/ml (under-five children) and 22.83±9.48 µg/ml (lactating mothers). Baseline mean serum retinol concentrations of the children below five years and lactating mothers in the control districts were 14.63±5.31 and 20.75±7.32 µg/ml, respectively, while in the intervention districts the mean serum retinol concentrations for the children and lactating mothers were 15.14±5.44 and 20.10±7.33 µg/ml, respectively. End-line mean serum retinol concentrations in the control districts were 14.83±5.95 µg/ml, for the children and 21.35±7.87 µg/ml for the lactating mothers, while in the intervention districts mean serum retinol concentrations were 17.59±10.20 µg/ml for the children below five years and 22.83±9.48 µg/ml for the lactating mothers. Anthropometric measurements of the children namely, weight for age, height for age and weight for height at baseline versus end-line in the control districts were -1.08 to -0.93 SD, -1.61 to -1.28 SD and -0.25 to -0.16 SD Z-scores, respectively, while that of intervention districts were -0.81 to -0.72 SD, -1.36 to -0.81 SD and -0.21 to -0.06 SD Z-scores, respectively. At baseline majority of the children (63.4% in control and 58.4% in intervention areas) and mothers (70.1 % in control and 74.4% in intervention areas) had low dietary diversity. While at end-line children with dietary diversity scores were 57.9% in control and 46.8% in intervention areas and mothers were 69.2% in control and 53.4% in intervention areas. Children who received mega doses of vitamin A supplements in the control districts at baseline versus end-line were 39.8 and 42.4 percent, respectively while those in the intervention districts at baseline versus end-line were 38.7 and 61.3 percent, respectively. Also knowledge of the lactating mothers about vitamin A during baseline was low in both control and intervention areas compared to end-line. High knowledge about vitamin A during end-line was due to the socio marketing which was conducted in both control and intervention areas. It was concluded that, consumption of fortified virgin sunflower oil fortified with vitamin A had positive effect ($p<0.05$) on the serum retinol concentration of the children below five years and

lactating mothers. Other factors which may have influenced the increase in serum concentration among children below five years and lactating mothers were mothers' knowledge about vitamin A-rich foods, intake of mega doses of vitamin A and dietary diversification. These factors also improved the anthropometric status of the children. It is recommended based on these results that; consumption of sunflower oil fortified with vitamin A and consumption of vitamin A rich foods and diversification of diet should be encouraged to control vitamin A deficiency.

Key words: Vitamin A, fortified sunflower oil, children below five years and lactating mothers

Introduction

Vitamin A Deficiency (VAD) continues to be a public health problem in many parts of the world today (Bahreynian *et al.,* 2017). About 190 million children below five years (Imdad *et al.,* 2017) and 19 million pregnant women (Yang *et al.,* 2016) in the world are vitamin A deficient. In Africa, 163 million cases of pregnant and lactating mothers are vitamin A deficient (FAO and WHO, 2013). Continuous consumption of foods with persistently insufficient vitamin A is the main cause of vitamin A deficiency to the children under the age of five years and lactating mothers, the most vulnerable groups (Bahreynian *et al.,* 2017). Vitamin A deficiency is not clinically identifiable until late stages, which last for a long period and leads to many consequences (WHO, 2011b). In children, vitamin A deficiency affects growth, immune functions and physical development (WHO, 2011a). Vitamin A deficiency also leads to severe visual impairments and blindness and significantly increases the risk of severe illness like diarrhea and measles and deaths in children (Silalahi *et al.,* 2017). In lactating mothers, vitamin A deficiency affects vision (WHO, 2011b) and leads to production of milk with low vitamin A (WHO, 2011a).

According to the Tanzania Demographic and Health Survey 2010, more than 33% of children aged 6–59 months and 35% of lactating mothers suffer from vitamin A deficiency (NBS, 2011). These rates are still very high among children below five years and lactating mothers despite the government efforts to eliminate the problem for the past two decades (FACT, 2016). Meanwhile, food fortification is described as the most effective strategy to prevent micronutrient under nutrition including vitamin A deficiency (Noor *et al.,* 2017). Evidence from high income countries illustrated the effectiveness of fortified foods with essential vitamins and minerals in reducing of micronutrient deficiencies at a population level; however, evidence from low and middle income countries, especially in Africa is limited (Mildon *et al.*, 2015). In the previous 18 years, the National Governments and global nutrition communities have invested significant efforts to bring the benefits of fortification of staple foods to lower and middle income countries (Noor *et al.*, 2017). Recently, 81 countries have passed legislation for mandatory fortification of oil, wheat, maize and/or rice making vital contribution to reduce micronutrient deficiencies (Mildon *et al.*, 2015). In Tanzania, fortification of vegetable edible oils has been made mandatory by law and several regulations since 2011 (FACT, 2016). According to Tanzania Foods, Drugs and Cosmetics (Food fortification) Regulations (2011), which made it mandatory to fortify edible oil in Tanzania the recommended national fortification level for vitamin A in the oil is mg/kg 20-40 mg/kg at retail and household level (EAS, 2017).

The fortification law of 2011 targeted the commercial producers; exempting small and medium level enterprisers (SMEs) (Bymolt and d'Anjou, 2017). The SMEs cover a large part of oil eaten in the country and are located almost everywhere in rural areas (BoT, 2017). Despite fortification being widely practiced by commercial oil producers in Tanzania, it has not been able to impact majority of the vulnerable populations (FACT, 2016). This could be explained by the reason that, fortified foods do not reach all households (Victor *et al.*, 2014), which may not serve vast majority of poor and rural households (BoT, 2017).

In order to deal with these challenges, the government of Tanzania launched a nationwide food fortification program in 2013 as one of the measures to prevent micronutrient under-nutrition (URT, 2013). The fortification program included raising awareness campaigns and adopting new technologies that enable fortification of oil to occur locally using local crops and researchers to track trends in consumption of the fortified foods in nearly real time (URT, 2013). Recently, the government of Tanzania is working with development partners to ensure fortification of staple foods including sunflower oil, using small and medium scale enterprisers that sell their products to poor people in rural communities (Robinson and Nyagaya, 2014). The net economic benefit of food fortification in Tanzania is considerable. For every one dollar invested in food fortification in the country can results in an economic return of $ 8.22 or an increase in GDP of 0.58%. It is estimated that, almost 6 800 deaths of the vulnerable people would be reduced due to food fortification annually (URT, 2016).

MASAVA project also known as “Promoting Use of Locally Fortified Sunflower Oil Using E-Vouchers” which was implemented through collaboration between Mennonite Economic Development Associates (MEDA) - Tanzania, Sokoine University of Agriculture (SUA) - Tanzania and the University of Waterloo (UW) - Canada worked with three small and medium level enterprisers (SMEs), one from Manyara and two others from Singida regions of Tanzania, to fortify the locally produced sunflower oil with vitamin A. MASAVA project supported the three SMEs in terms of tools, premixes, and training on how to fortify the sunflower oil with vitamin A, good manufacturing practices (GMP) and good hygiene practices (GHP).

The project also supported the SMEs to meet the registration requirements stipulated by the TBS and TFDA. The MASAVA project aimed at improving serum retinol concentration of children below five years and lactating mothers in Manyara and Shinyanga regions of Tanzania, through the consumption of fortified sunflower oil. Sunflower oil which is a good vehicle for carrying vitamin A, is widely consumed and readily available and accessible by families in Manyara and Shinyanga regions (NBS, 2011). It has been reported that, improving micronutrient deficiencies such as vitamin A deficiency among the vulnerable social groups reduces the risk of mortality by 25–35% (Gaurav *et al.*, 2014).) Therefore, as part of the efforts to combat VAD, this study was conducted to evaluate the effectiveness of fortifying locally produced sunflower oil with vitamin A on retinol status of children below five years and lactating mothers in Manyara and Shinyanga regions of Tanzania.

Methodology

Research Area

MASAVA project was conducted in Manyara and Shinyanga regions of Tanzania. The two regions were selected due to high prevalence of vitamin A deficiency among children below five years and lactating mothers (47.6 and 41.5% for children under the age of five years and 53.5 and 43.4% for lactating mothers, respectively) (NBS, 2011). These regions were also used as pilot areas to determine effectiveness of public intervention of vitamin A deficiency using fortified sunflower oil. Shinyanga region is located in the North-west of Tanzania. According to the 2012 National Census (NBS, 2012), Shinyanga region has a population of 1 534 808 people with 750 841 being males and 783 967 being females. The region has an area of 50 781 square kilometres. The average household size in Shinyanga is 6 people. This region consists of five administrative districts namely; Shinyanga Urban, Shinyanga Rural, Kishapu and Kahama. Meanwhile, Manyara region is located in the northern part of Tanzania. Manyara region has a population of 1 425 131 people with 717 085 being males and 708 046 being females, based on the 2012 National Census (NBS, 2012). The region has an area of 46 359 square kilometres. The region's average household size is 5 people. Manyara region consists of six administrative districts namely; Babati Urban, Babati Rural, Hanang, Mbulu, Simanjiro and Kiteto.

Study Design, Study Population, Sampling Technique and Sample Size

The study was a quasi-experimental non-equivalent control group design with intention to treat vitamin A deficiency. It involved three intervention districts in each region (Babati urban, Babati rural, Hanang, Shinyanga urban, Shinyanga rural and Kahama) and one control district in each region (Mbulu and Kishapu). Baseline household survey was conducted in June and July 2015 while end-line survey was conducted in November and December 2016. The study population comprised of children below five years and lactating mothers living in the selected districts of Manyara and Shinyanga regions of Tanzania; However the study enrolled 6-36 months children from the beginning and worked with them for two years. Inclusion criteria were lactating mothers of different age groups, i.e., young mothers <20 years, middle age mothers 20-34 years and elderly mothers at 35-49 years and children below the age of five years both males and females selected from low, middle and high income families (considering their family income earnings) aged 6-59 months living in the respective study districts with mothers who were breastfeeding but not necessarily the index child.

Included also were index children below five years still receiving post-natal monthly health care at Maternal and Reproductive Health facilities (MRH), family having a cellphone (mothers must know how to read and write) and the mother/caregiver who consented to participate in the study. Exclusion criteria included children with measles and/or infections that had lasted for a month prior to the baseline/end-line data collection or mothers (which were identified by the village health workers and home based care). Excluded were also, children with chronic diseases such as type 1 diabetes mellitus, or mothers with type 2 diabetes mellitus, children or mothers with, HIV infections, children with inborn errors of metabolism, e.g., phenylketonuria (PKU), children whose parents did not have cellphones, those who were no longer attending post-natal monthly health care clinics at the Maternal and Reproductive Health facilities (MRH) and children/mothers who had stayed in the study. Assessment of consumption of sunflower oil fortified with vitamin A was carried out in the intervention areas; Babati Urban, Babati Rural and Hanang districts of Manyara region and Shinyanga Urban, Shinyanga Rural and Kahama districts of Shinyanga region, Tanzania. These districts were purposively selected based on the prevalence of vitamin A deficiency (NBS, 2011), cellphone signal receptivity (for operation of the e-vouchers), which was part of the methods to monitor purchase of fortified sunflower oil, infrastructure to access remote villages, availability of good retail network for edible oil and availability of reliable oil supplier to the districts. Through use of cell-phone (for operation of the e-vouchers) mothers had to send a message for buying fortified sunflower oil to MEDA and from there MEDA responds back to the mother to allow her go to the closer shop in their areas which was selling the fortified sunflower oil and show the retailer that message from MEDA and some money because the oil was sold at a subsidized rate of 2100 Tsh (a discount of 27%) and the retailer gives oil to the mother. Then, retailer was paid by MEDA for the amount 27% which was 800 Tsh. Control districts namely Mbulu and Kishapu were chosen using similar criteria. Subjects were randomly selected by using a table of random numbers. A sample size was determined by statistical power analysis using WHO (1991) formula: $N=t^2x\ (p \times q)/d^2$ whereby N= Sample size; t= the risk of error (1.96); p=expected prevalence (0.5); q=1-p (expected non-prevalence) (0.5) and d=level of precision (0.05). A sample size of 569 mother–child pairs was selected for the study (a sample size of 367 mother-child pair in intervention districts and 202 mother-child pair in control districts). The sample size was distributed according to the population in the study areas.

Intervention involved consumption of sunflower oil fortified with vitamin A in the households. The fortification level of vitamin A in the sunflower oil was 30-40 mg/kg in line with the East African Standards (EAS 769:2017) specification. The households purchased the fortified oil and used it for preparation of foods at home the way they did in the traditional. No control was made on the amount of oil to be used in the preparation of any meal at the household. Through social marketing, intervention districts were encouraged to buy fortified sunflower oil from designated shops where the fortified oil was sold at a subsidized rate and used it for preparing foods. Also households were also encouraged to consume vitamin A rich foods and diversify their diets. During intervention period (August 2015 - December 2016) the control groups were not receiving fortified sunflower oil. Instead, they were encouraged to use sunflower oil for cooking (not fortified) and consume vitamin A rich foods as well as diversifying their diets. Also in order for particularly men to appeal there was a social marketing of the fortified sunflower oil at bicycle races and soccer games. Meanwhile children got the fortified sunflower oil with vitamin A directly from meals like porridge and any solid meal prepared using fortified sunflower oil; and indirectly from their mothers (breast milk). Control groups started receiving the fortified sunflower oil after the intervention period of 18 months.

Monitoring Compliance

During intervention period, mothers were visited at their households by the village health workers (VHWs) and home-based care providers (HBCs) once every week to monitor compliance to the use of the fortified sunflower oil and encouraged them to buy the fortified sunflower oil and use it for food preparation.

Data Collection

Household survey used an adopted questionnaire from GAIN with 6 modules. Household roster: information on the household composition age/education of members including mother and index child, dietary diversity module for mother and index child, health and anthropometric module for index child, oil consumption survey and vitamin A knowledge, attitudes and practices (KAP) survey. The questionnaire was validated and pretested among 10 lactating mothers in Morogoro Urban and Rural districts, Tanzania. Villages selected for pre-testing the questionnaires were Bigwa, Kichangani and Mazimbu in Morogoro urban and Mgeta and Mlali in Morogoro rural districts. Necessary adjustments were made on the questionnaire after pretesting.

Surveys

Baseline survey included 367 mother-child pairs from intervention districts (Babati Urban, Babati Rural, Hanang, Shinyanga Urban, Shinyanga Rural and Kahama) and 202 mother-child pairs from control districts (Mbulu and Kishapu). A total of 126 mothers and 124 children were lost to follow-up before endline survey, leaving 443 eligible mothers and 445 eligible children (Fig.1). The reasons for the drop out were relocation to farms in other areas, travelling to visit relatives in other villages/towns, break-up of families with mothers moving away with young children and refusal to allow their blood samples to be collected. Even for the eligible participants, only 414 mothers and 423 children below five years were compared with the baseline because some of the participants were excluded from the data analysis because they did not have sufficient blood samples for analysis (Fig. 1).

Blood Sample Collection and Handling

Blood samples were collected from the children below five years and lactating mothers at baseline and end-line. Using safety lancets whole blood (serum) was obtained by finger prick. About 50 µL of the blood was taken by a micropipette and dispensed into a protein saver papers (903 Protein Saver Card, Whatman International and UK) and left to dry for few minutes. After drying, the protein saver papers with blood samples were packaged in airtight aluminum bags in which three pieces of desiccators (Silica gel, Merk, NJ, USA) were added to keep the blood spots dry (DBS). DBS were stored under ambient conditions before transportation to the laboratory of the Tanzania Food and Nutrition Centre for analysis.

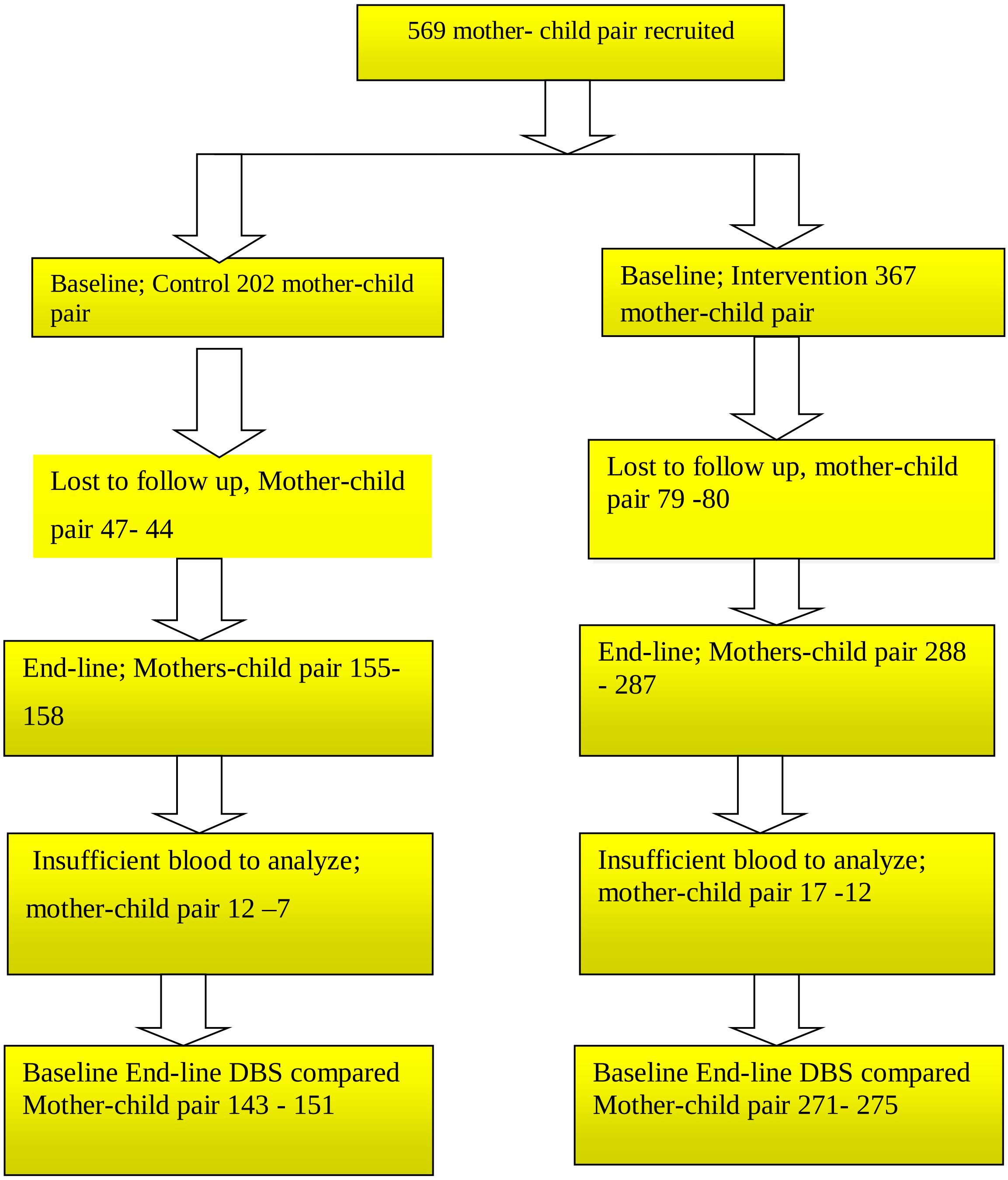

Figure 1: Flow chart of mother-child pairs who participated in the study

Determination of Retinol in DBS

Retinol in the DBS was determined by enzyme linked immune assay (ELISA) method (NBS, 2011). This assay was carried out by the following procedures; the specimen and calibrator sera were diluted in assay buffer and added to individual wells. A monoclonal, anti-RBP antibody conjugated to horsedish peroxide (HRP) enzyme was diluted and then immediately added to the wells.

The mixture was incubated at room temperature for 15 minutes and then washed with buffered (phosphate-buffered saline). Tetramethylbenzidine (TMB) enzyme substrate containing hydrogen peroxide was added, incubated for 10 minutes and the reaction was stopped with Sulphuric acid. The mixture was immediately read at 450 nm with a plate reader and the results were calculated based on values obtained from the calibrator sera. The test results were available 35 to 40 minutes after the start of the assay. All samples including calibrators were run in duplicate. In this study, children with <0.7 µmol/L plasma retinol concentrations or (<17.325 µg/ml of RBP) and lactating mothers with <1.05 µmol/L plasma retinol concentrations or (<26.04 µg/ml of RBP) were defined as vitamin A deficiency (WHO, 2008).

Oil Sample Collection and Handling

Oil samples (capacity 5 ml) were collected from the households and stabilized with two antioxidants, Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) and stored in airtight sample bottles without head space. Sample bottles with oil were stored in ice boxes and transported under cold conditions to the laboratories of the Tanzania Food and Drugs Authority (TFDA), Dar es Salaam for analysis of retinol.

Determination of Retinol in Oil Samples

Vitamin A concentrations in the oil sample were determined by using reverse phase HPLC method 86-06 (De Viries and Silvera, 2002). The concentration of vitamin A in the oil samples was calculated from the peak heights using the response factor (RF std) of retinyl palmitate for vitamin A.

$$\text{Vitamin A (retinol) (mg/kg)} = \frac{(RFA \times PHsam \times 100)}{W}$$

Where RFA was the response factor for vitamin A,

PHsam was the total test sample peak height or area of all Trans and 13-cis retinol,

100 was the dilution volume of the test portion (ml)

W was the weight of the test portion (g)

The cut off points of the retinol concentration in the edible oils were judged based on the harmonized East African Standards (EAS) 269:2017 and Tanzanian Standards (TZS) 50:2014. According to EAS 269:2017 and TZS 50:2014, the recommended concentration of retinol in edible oil at the household level is 20-40 mg/kg in which concentration of 20 mg/kg and 40 mg/kg are the low and upper cut-off points, respectively.

Anthropometric Measurements

Weight: Weight was taken by using digital scale (Seca 876, Hamburg, Germany) equipped with a mother-child function. The weight of children below 24 month was taken after mothers or caregivers stood on the scale set to zero and thereafter the mother/caregiver was given the child. The weight of the child was recorded to the nearest 0.1 kg. Children aged 24 to 36 months were weighed while standing on the digital scale and the weights were recorded to the nearest 0.1 kg. Children were weighted with minimum clothing and without shoes on.

Height: Height was measured using a length board (UNICEF, Rome). Children younger than 24 months were measured while lying down in recumbent position. The child was laid on his back at the center of the length board with shoulders and buttocks lying flat against the measuring surface and the child’s eyes looking straight up. Both legs were fully extended with the toes pointing upwards with the feet lying flat against the foot-piece. The heights of children aged 24 months or more were measured while standing. The child stood against the length board without shoes, with legs straight, arms at sides, shoulders relaxed and head looking straight. The headpiece was then slid down to touch the crown of the head. The length or height was then read parallel with the head piece and recorded to the nearest 0.1 cm. Weights and heights were used to calculate weight for age, weight for height and height for age Z-scores that were used to evaluate the anthropometric status of the under-five children. Weight for age, weight for height and height for weight Z- scores of $-2 < SD < +2$ were considered as normal underweight, stunting and wasting, respectively (WHO, 2006). Edema: During anthropometric measurements, children were also observed for presence of pitting edema. Thumb pressure was applied to the child's feet for three seconds and any pitting was considered to be edema.

Dietary Intake

Dietary intake was assessed by using a 24 hour recall method. Mothers were requested to list all the food items that the mother/caregiver and child had consumed both at home and out of their home in the previous 24 hours.

Dietary Diversity Score

Dietary diversity score (DDS) of children was calculated based on consumption of seven food groups namely, staples (cereals/grains, roots and tubers); legumes nuts and seeds;

dairy products; Meat and fish; vitamin A-rich fruits and vegetables; other fruits and vegetables and eggs. Consumption of ≤3 food groups per day was considered as low dietary diversity, while intake of four food groups was considered medium while consumption of ≥5 food groups was considered as high dietary diversity (WHO, 2008).

Mothers dietary diversity score was calculated based on consumption of nine food groups namely, starch staple; legumes, nuts and seeds; milk and milk products; meat and fish; dark green leafy vegetables; other vitamin A rich fruits and vegetables; eggs; other fruits and vegetables and organ meat. Consumption of ≤3 food groups was classified as low dietary diversity, four food groups was classified as medium dietary diversity score while consumption of ≥5 food groups was classified as high dietary diversity (FAO and FHI, 2016).

Mothers Knowledge about Vitamin A

Mother's knowledge about vitamin A was assessed using a five point Likert Scale. There were twelve statements asked about vitamin A. The first six statements were about beneficial impact of vitamin A i) Vitamin A Helps with child growth ii) Vitamin A Strengthens immunity to illness iii) Vitamin A improves vision iv) Vitamin A improves health/reduces illness v) Vitamin A avoids night blindness vi) Vitamin A reduces mortality; Then, sources of vitamin A vii) Foods rich in Vitamin A (animal sources) viii) Foods rich in Vitamin A (plant sources); and the last four statements were about ways to avoid vitamin A deficiency ix) Balanced diet x) Foods rich in vitamin A xi) Vitamin A Supplement xii) Foods fortified with vitamin A. Mothers were required to indicate whether they strongly agree, agree, undecided, disagree or strongly disagree for each statement. Strongly agree carried the weight of 5, agree 4, undecided 3, disagree 2 and strongly disagree carried the weight of 1. By using a five- point rating responses

(5 strongly agree, 4 agree, 3 undecided, 2 disagree or 1 strongly disagree), means and standard deviation values were determined (Table 6). For the purposes of data to be interpreted, the means of each item that were ranging from 2 and above were characterized as knowledgeable while for the values which were below the mean of 2 were considered low knowledge.

Data analysis

Data were coded, entered in a computer, cleaned and analyzed by using IBM SPSS program version 20. Descriptive analysis was done to obtain means, frequency and percentages of the study groups. Means were compared by t-tests to obtain the differences between baseline and end-line data. A $p<0.05$ was considered statistically significant. Anthropometric indices of height-for age, weight-for-age and weight-for-height were expressed as z-scores using the ENA for SMART program.

Ethical consideration

This study was approved by the National Institute for Medical Research (NIMR) (Tanzania) and the ethics committees for research on human subjects (Canada). Permission was also obtained from the regional and district health authorities to conduct the study in the selected districts. All mothers/caregivers who agreed to participate in this study signed an informed consent form to affirm their willingness to allow their children to participate in the study. Respondents had the liberty to decline participation in the study at any time.

Results

Characteristics of the household participants at baseline in the intervention and control districts are presented in Table 1. The number of children in the intervention and control districts was not statistically different (p>0.05).

Table 1: Characteristics of the households in the control and intervention districts at baseline

Parameter	Control (n=202)		Intervention (n=367)		Total (n=569)		p-value
	n	%	N	%	n	%	
Sex of the child							0.162^{ns}
Male	116	57.4	197	53.7	313	55.0	
Female	86	42.6	170	46.3	256	45.0	
Total	**202**	**100.0**	**367**	**100.0**	**569**	**100.0**	
Age (years)							0.000**
15 - 19	9	4.5	16	4.4	21	3.7	
20- 34	138	68.3	245	66.8	387	68.0	
35 - 49	55	27.2	106	28.8	161	28.3	
Total	**202**	**100.0**	**367**	**100.0**	**569**	**100.0**	
Education level							0.229^{ns}
Informal	18	8.9	34	9.3	52	9.1	
Primary	163	80.7	288	78.5	451	79.3	
Secondary	21	10.4	45	12.2	66	11.6	
Total	**202**	**100.0**	**367**	**100.0**	**569**	**100.0**	
Marital status							0.457^{ns}
Single	15	7.4	14	3.8	29	5.1	
Married	173	85.6	333	90.7	506	88.9	
Divorced	14	7.0	20	5.5	34	6.0	
Total	**202**	**100.0**	**367**	**100.0**	**569**	**100.0**	
HH size (Persons)							0.034**
≤4	43	21.3	99	27.0	142	25.0	
>4	159	78.7	268	73.0	427	75.0	
Total	**202**	**100.0**	**367**	**100.0**	**569**	**100.0**	
Occupation							0.000**
Housewife	150	74.3	250	68.1	400	70.3	
Petty business	25	12.4	40	10.9	65	11.4	
Employed for wage	17	8.4	9	2.5	26	4.6	
Self employed	10	4.9	68	18.5	78	13.7	
Total	**202**	**100.0**	**367**	**100.0**	**569**	**100.0**	
Children's mean age	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	$\mathbf{0.158^{ns}}$
Months	18.22	8.93	19.20	9.90	18.85	9.57	

**Mean significant at p<0.05; ns = not significant

The number of males was almost similar to that of females. The proportion of males was 53.7% while that of females was 46.3%. Most of the lactating mothers in this study (70%) were in the age range of 20-34 years. The age range of lactating mothers in the control and the intervention districts was slightly different ($p<0.05$). Regarding education level, almost all mothers (79.3%) had attained primary school education and there was no difference in maternal education between the mothers in the intervention and the control districts ($p>0.05$). Majority of lactating mothers in this study (88.9%) were married and were also housewives (70.3%). Average household size was significantly larger in the control groups (>4 people) compared to the intervention group (≤4 people). The mean age of the children in the intervention (19.20 months) and control districts (18.22 months) were almost similar ($p>0.05$). Vitamin A concentration in the fortified sunflower oil samples collected during baseline and end-line are shown in Table 2. Vitamin A concentrations in the intervention districts was significantly higher compared to those in the control districts during end-line ($p<0.05$). Also vitamin A concentration in both regions (Manyara and Shinyanga) was significantly high ($p<0.05$) during end-line.

Table 2: Distribution of retinol concentrations in the oil samples collected at the households at baseline versus end-line (n=423)

Group	**Retinol conc. (mg/kg) at baseline**				**Retinol conc. (mg/kg) at end-line**				**p-value**
	< 20		**20-40**		**< 20**		**20-40**		
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Manyara	170	63.8	85	36.2	23	9.8	212	90.2	0.000**
Shinyanga	40	21.3	148	78.7	9	4.8	179	95.2	0.000**
Control	61	33.3	122	66.7	66	36.1	117	63.9	0.680^{ns}
Intervention	183	76.2	57	23.8	31	12.9	209	87.1	0.000**
All	305	72.1	108	27.9	49	11.6	374	90.6	0.000**

**Means values of retinol concentration significant at $p<0.05$; ns = not significant

Table 3 shows the mean serum retinol concentrations of the respondents. There was improvement of the mean serum retinol concentration of the mothers and children between baseline and end-line in both control and intervention districts. Serum retinol concentration in the intervention group at baseline was 20.10±7.33 ug/ml for the lactating mothers and 15.14±5.44 ug/ml for the children while at end-line the concentration was 22.83±9.48 ug/ml for the lactating mothers and 17.59±10.20 ug/ml for the children. In the control districts, the mean serum retinol concentration at baseline was 20.75±7.33 ug/ml for the lactating mothers and 14.63±5.31 ug/ml for the children. Serum retinol concentration at end-line was 21.35±7.87 ug/ml for the lactating mothers and 14.83±5.95 ug/ml for the children. Improvement in serum retinol concentration between the baseline and end-line for the mothers and children in the control groups was not significant. Besides, prevalence of vitamin A deficiency in lactating mothers and children decreased in the intervention group after intervention with fortified sunflower oil. In the control districts, 70.4% of the children and 80% of lactating mothers were vitamin A deficient at baseline while at end-line the prevalence decreased to 68.5 % of children and 60.7% for the lactating mothers. In the intervention districts, 67.9% of the children and 81.9 of the lactating mothers were vitamin A deficient at baseline but the prevalence decreased to 21.4% for the children and 12.3% for the lactating mothers (Table 4). Thus, prevalence of vitamin A deficiency declined among the children below five years and lactating mothers after intervention with vitamin A fortified sunflower oil.

Table 3: Effect of fortified sunflower oil on the mean serum retinol concentration of lactating mothers and children at baseline and end-line

Group	Baseline		End-line		p-value
	Mean	SD	Mean	SD	
Control					
Serum retinol of mothers (µg/ml)	20.75	7.32	21.35	7.87	0.438^{ns}
Serum retinol of children (µg/ml)	14.63	5.31	14.83	5.95	0.465^{ns}
Intervention					
Serum retinol of mothers (µg/ml)	20.10	7.33	22.83	9.48	0.000**
Serum retinol of children (µg/ml)	15.14	5.44	17.59	10.20	0.000**

** Means values at p<0.05; ns =not significant

Table 4: Prevalence of vitamin A deficiency (VAD) among lactating mothers and children below five years at baseline versus end-line

Areas	Baseline				End-line				p-value
	VAD		Normal		VAD		Normal		
	n	%	n	%	n	%	n	%	
Children									
Manyara	188	69.1	84	30.9	76	27.9	196	72.1	0.000**
Shinyanga	103	68.2	48	31.8	84	55.6	67	44.4	0.000**
Control	107	70.4	45	29.6	102	68.5	50	32.8	0.560^{ns}
Intervention	184	67.9	87	32.1	58	21.4	213	78.9	0.000**
Mothers									
Manyara	219	82.6	46	17.4	81	30.6	184	69.4	0.000**
Shinyanga	117	78.5	32	21.5	62	41.6	87	58.4	0.000**
Control	116	80	29	20	110	60.7	35	39.3	0.490^{ns}
Intervention	220	81.9	49	18.2	33	12.3	236	87.7	0.000**

VAD = Vitamin A Deficiency; ** Mean values significant at p<0.05; ns = not significant

Factors that may have contributed to the improvement in the vitamin A status of lactating mothers and children below five years after 18 months of intervention. These factors included intake of a mega dose of vitamin A supplement that was given to children twice every year (Table 5a), dietary diversification (Table 5b) and mother's knowledge about vitamin A (Table 5c) and Mothers knowledge about vitamin A had a strong (p<0.05) effect on the serum retinol concentration of the lactating mothers and children below five years in both the intervention and control groups. Intake of mega doses of vitamin A also had strong (p<0.05) effect on the serum retinol status of the children below five years in the both control and intervention group. Significant influence of dietary diversity was observed in the vitamin A status of lactating mothers and children below five years in both the intervention and control groups.

Table 5a: Children who received vitamin A Mega doses supplements during baseline vs. end-line

Group	**Baseline**		**End-line**		**t-value**	**p-value**
	Received VAS	**Not received VAS**	**Received VAS**	**Not received VAS**		
	%	**%**	**%**	**%**		
Control	39.8	60.2	42.4	57.6	-7.63	0.000**
Intervention	38.7	60.2	61.3	39.8	-11.52	0.000**

** Mean values significant at p<0.05; ns= not significant; VAS= Vitamin A Supplements

Table 5b: Dietary diversity scores of the children and lactating mothers at baseline vs. end-line

Group	**baseline**			**End-line**			**t-value**	**p-value**
	With Low	**% With Medium**	**With High**	**With Low**	**With Medium**	**With High**		
	%	**%**	**%**	**%**	**%**	**%**		
Children								
Control	57.9	29.1	13.0	63.4	21.5	12.7	-3.126	0.002
Intervention	46.8	34.1	16.5	58.4	25.1	16.5	-4.473	0.001
Mothers								
Control	70.1	15.3	14.6	69.2	17.6	13.2	-2.612	0.009
Intervention	74.4	12.8	12.8	53.4	32.2	14.4	2.692	0.008

** Mean values significant at p<0.05; ns not significant

Table 5c: Respondents score on their Knowledge using mean and standard deviation about vitamin A

Statement	Baseline		End-line		p-value
	Mean	SD	Mean	SD	
Control districts					
Vitamin A Helps with child growth (benefit of VA)	1.85	0.47	2.07	0.18	0.00**
Vitamin A Strengthens immunity to illness (benefit of VA)	1.67	0.63	2.16	0.56	0.00**
Vitamin A improves vision (benefit of VA)	1.51	0.64	2.79	0.78	0.00**
Vitamin A improves health/reduces illness (benefit of VA)	1.34	0.64	1.9	0.21	0.00**
Vitamin A avoids night blindness (benefit of VA)	1.34	0.68	2.65	0.43	0.00**
Vitamin A reduces mortality (benefit of VA)	0.94	0.36	1.08	0.61	0.00**
Foods rich in Vitamin A (animal sources)	1.79	0.44	2.21	0.06	0.00**
Foods rich in Vitamin A (plant sources)	1.55	0.56	2.56	0.19	0.00**
Consumption of balanced diet (way to avoid VAD)	1.87	0.55	1.90	0.24	0.00**
Consumption of foods rich in vitamin A (way to avoid VAD)	1.85	0.54	2.76	0.68	0.00**
Consumption of vitamin A Supplement (way to avoid VAD)	1.87	0.34	2.58	0.77	0.00**
Consumption of foods fortified with vitamin A (way to avoid VAD)	1.65	0.68	1.59	0.11	0.00**
Intervention districts					
Vitamin A Helps with child growth (benefit of VA)	1.58	0.45	2.39	0.54	0.00**
Vitamin A Strengthens immunity to illness (benefit of VA)	1.79	0.44	2.08	0.43	0.00**
Vitamin A improves vision (benefit of VA)	1.83	0.52	2.75	0.26	0.00**
Vitamin A improves health/reduces illness (benefit of VA)	1.74	0.42	2.39	0.55	0.00**
Vitamin A avoids night blindness (benefit of VA)	1.61	0.52	2.60	0.22	0.00**
Vitamin A reduces mortality (benefit of VA)	1.32	0.64	1.89	0.45	0.00**
Foods rich in Vitamin A (animal sources)	1.18	0.28	2.33	0.65	0.00**
Foods rich in Vitamin A (plant sources)	1.23	0.61	2.63	0.23	0.00**
Consumption of balanced diet (way to avoid VAD)	1.42	0.62	1.88	0.17	0.00**
Consumption of foods rich in vitamin A (way to avoid VAD)	1.56	0.56	2.84	0.47	0.00**
Consumption of vitamin A Supplement (way to avoid VAD)	1.25	0.48	2.28	0.54	0.00**
Consumption of foods fortified with vitamin A (way to avoid VAD)	1.23	0.61	2.01	0.33	0.00**

VAD= Vitamin A deficiency; VA= Vitamin A

Anthropometric indices - W/A, W/H, H/A Z-scores for the under-five children in the intervention and control districts at baseline and end-line are presented in Table 7. Improvement was observed in all anthropometric indices although greater improvement was observed in linear growth of the children below five years in the intervention areas. The mean height for age was -1.36 SD Z-scores at baseline and -0.81 SD Z-scores at end-line. While there was a significant change in the mean height for age observed in the intervention groups such changes were not observed in the control groups between the baseline and end-line. No changes in the mean weight for height and weight for age were observed between baseline and end-line.

Table 6: Anthropometric status of children below five years in the intervention and control districts at baseline versus end-line

Group	Baseline		End-line		P-value
	Mean	SD	Mean	SD	
Control districts					
Weight for age Z-score	-1.08	0.98	-0.93	1.40	0.268^{ns}
Height for age Z-score	-1.61	1.24	-1.28	1.81	0.055^{ns}
Weight for height Z-sore	-0.25	1.05	-0.16	1.03	0.385^{ns}
Intervention districts					
Weight for age Z-score	-0.81	1.02	-0.72	1.47	0.369
Height for age Z-score	-1.36	1.44	-0.81	2.18	0.000**
Weight for height Z-sore	-0.21	1.46	-0.06	1.20	0.156^{ns}

** Mean values significant at $p < 0.05$; ns = not significant; SD= Standard Deviation

Discussion

This study was conducted to evaluate the effectiveness of using sunflower oil fortified with vitamin A on the vitamin A status of lactating mothers and children below five years. Results in this study revealed that, fortification significantly improved the concentrations of vitamin A in the sunflower oil consumed at the households and only few samples had retinol concentrations below the recommended concentration of 20 mg/kg (Table 2). Results in this study revealed further that, fortification of sunflower oil with vitamin A improved the retinol status of lactating mothers and children below five years in both study regions of Manyara and Shinyanga (Table 3). Fortification of sunflower oil with vitamin A therefore reduced the prevalence of vitamin A in both children and lactating mothers (Table 4). Most of the children and lactating mothers had good serum vitamin A status after consuming fortified sunflower oil with vitamin A. Studies by Sandjaja *et al.* (2015) in Indonesia and Engle-Stone *et al.* (2014) in Cameroon observed improvement in serum retinol concentrations in lactating mothers and children who consumed vitamin A fortified edible oil. Other studies by Huo *et al.* (2014) in China and Perignons *et al.* (2016) in Cambodia also showed that, vitamin A fortified oil was very effective in improving vitamin A status of children. Engle-Stone *et al*. (2013) in Cameroon also reported that, fortification of edible oil with vitamin A improved the vitamin A status of mothers while Soekirman *et al.* (2012) in Indonesia observed improvement of vitamin A status in children who consumed cooking oil fortified with vitamin A. Fortification of edible oil with vitamin A therefore had beneficial effect to the public health since it reduced the prevalence of vitamin A deficiency in lactating mothers and children below five years.

Beside fortified oil, other factors which may have contributed to the improvement in the serum retinol concentration included intake of the mega doses of vitamin A supplement (Table 5a), dietary diversity scores (Table 5b) and Knowledge about vitamin A (Table 5c). Children who received mega doses of vitamin A supplement especially in the intervention districts had significantly improved in serum retinol concentration at the end-line. Also, maternal knowledge about vitamin A rich foods might have contributed to the improvement in the serum retinol concentration at the end-line. Knowledgeable mothers were able to select foods for their families that were rich in vitamin A. Results of this study were in agreement with those of Silva *et al.* (2015) in Brazil who reported that, maternal knowledge was positively correlated with the concentration of serum retinol. The study by Fujita *et al.* (2012) in Kenya also reported that, diversifying diets improved vitamin A status of children and lactating mothers. A review by Wirth *et al.* (2017) observed that, mega doses of vitamin A supplement had the beneficial effect of reducing the mortality associated with measles, diarrhea and other illnesses by improving their vitamin A status in children below five years.

Results of this study also showed improvement in the anthropometric measurements of the studied children. Greater improvement was observed in the linear growth (height for age) of the children at the end-line (Table 6). This could be due to the fact that, vitamin A plays a great role in growth and development and decreases morbidity in children (WHO, 2011b). These findings were in line with those of Dabone and Delisle (2013) in Burkina Faso who reported positive effects on anthropometric status of children after consuming vitamin A fortified oils for six months. In Sri Lanka, Marasinghe *et al.* (2015) also observed improvement in the vitamin A status and anthropometric measurements of children who were consuming oil fortified with vitamin A.

The findings further revealed that, there was an increase in mothers knowledge regarding vitamin A at the end-line compared to the knowledge they had at baseline in both control and intervention groups (Table 6c). An increase of mothers knowledge about vitamin A could be due to the socio marketing which was conducted in the study areas during intervention period in both intervention and control areas. This was an important aspect that enhanced improvement in the anthropometric status of children since mothers were the major providers of care that their children needed during their first five years of life (WHO, 2011c). Maternal knowledge about diets rich in vitamin A is essential for good health and overall improvement in the nutritional status, especially when resources were scarce (Rahman and Sapkota (2014). These findings were in agreement with those reported by Saaka (2014) in Northern Ghana who observed that, increase in maternal childcare knowledge contributes significantly to the improvement in the child's anthropometric status.

Conclusions and Recommendations

It was concluded from this study that, consumption of sunflower oil fortified with vitamin A, significantly improved the serum retinol concentrations of both lactating mothers and children. Also administration of mega doses of vitamin A supplement contributed to the improvement in the serum retinol concentration of children below five years in intervention districts. Likewise, maternal knowledge about vitamin A rich foods and dietary diversity improved vitamin A status of both lactating mothers and children. Edible oil fortified with vitamin A, mega doses of vitamin A supplements (in intervention groups) and maternal knowledge about vitamin A rich foods and dietary diversification significantly improved the linear growth (height for age) of the children. To address the problem of vitamin A deficiency, it is therefore recommended to promote fortification of edible oil with vitamin A, increase consumption of vitamin A rich foods such as green leafy and yellow vegetables and increase dietary diversification.

Acknowledgements

The authors are grateful to the Government of Canada that through the Global Affairs Canada (GAC) (#107790), International Development Research Centre (IDRC and Canadian International Food Security Research Fund (CIFSRF) Grant, Phase 2 provided financial support to undertake this study.

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PAPER TWO

Shelf-stability of vitamin A in fortified sunflower oil during storage and retail sale by scooping in Manyara and Shinyanga, regions. Tanzania

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(*Journal of Food Science and Nutrition)*

Abstract

Background/objectives: Fortification of edible oil with vitamin A is the most effective strategy for prevention and controlling of vitamin A deficiency among children below five years lactating mothers. However, stability of vitamin A in oil during storage and sale at the retail level remains a limiting factor. This study was conducted to determine shelf stability of vitamin A in fortified sunflower oil during storage and retail sale by scooping. Fortified virgin sunflower oil samples which were locally produced in Manyara and Shinyanga regions of Tanzania were collected from the retail shops and Small and Medium level oil Enterprisers (SMEs). **Materials and methods:** In a storage stability study, samples of fortified sunflower oil from the three SMEs were determined at intervals of one month for a total period of 6 months. Also in a scooping study, samples of the fortified sunflower oil drawn from the selected retail shops in the intervention districts; were determined from the date of opening the container to the date of being emptied (day 1 to 16). Vitamin A in the oil was determined by using HPLC. Quality parameters of the oil were also determined by using AOAC methods. Descriptive analysis was calculated to obtain means, frequency and percentages of the retinol concentrations. Statistical significant was determined at $p<0.05$. **Results:** More than 89% of vitamin A in the fortified virgin sunflower oil was retained during 6 months of storage at ambient conditions. For the oil that was sold by scooping, more than 80% of vitamin A in the fortified sunflower oil was retained. For the shelf stability study, the average monthly degradation rates of vitamin A in the sunflower oil were 0.477 mg/kg (for SME 1), 0.385 mg/kg (for SME 2) and 0.171 mg/kg (for SME 3). For the fortified oil that was sold by scooping, the daily average rates of vitamin A degradation were 0.421 mg/kg (SME 3) and 0.343 mg/kg (SME 2) and 0.238 mg/kg (SME 1). Quality aspects of the fortified virgin sunflower oil namely peroxide, anisidine, thiobarbituric acid (malondialdehyde) and Totox values, during shelf life storage and sale by scooping significantly increased with time. **Conclusions:** It is concluded that vitamin A was stable in fortified virgin sunflower oil during six months storage and sale by scooping. All quality aspects in a storage shelf life study and scooping study value were within the recommended standards.

Key words: Sunflower oil, fortification, shelf stability, Vitamin A, cooping and quality

Introduction

Vitamin A (chemically known as retinol) is an essential micronutrient required for vision, growth, cell differentiation, reproduction (spermatogenesis, oogenesis, placental development and embryonic growth), and the regulation of the immune function (WHO, 2009). This micronutrient is also an important antioxidant which protects the body against oxidative damage caused by free radicals generated in several metabolic processes in the human body (Silalahi *et al.,* 2017). Vitamin A deficiency remains a public health problem in many parts of the world (FAO and WHO 2013) but more prevalent in Sub-Saharan Africa (Silalahi *et al.,* 2017). Globally, children below five years and women of reproductive age (particularly pregnant women and lactating mothers) are the groups at high risk of developing vitamin A deficiency and its adverse health consequences (FAO and WHO, 2013). Persistence of low dietary intakes of vitamin A-rich foods (eggs, milk, liver, deep orange fruits and dark green leafy vegetables) is the major cause of vitamin A deficiency (Silalahi *et al.,* 2017). Fortification of edible oil with vitamin A is the most effective mid-term strategy for prevention and controlling vitamin A deficiency (Engle-Stone *et al.,* 2017).

Studies by Silalahi *et al.* (2017) and Andarwulan *et al.* (2014) have indicated that, fortification of vegetable oil is a suitable food vehicle to deliver fat-soluble vitamins such as vitamin A in large populations including children below five years and lactating mothers. This approach can have good impact on child health by directly increasing vitamin A intake and indirectly by raising levels of vitamin A obtained by children from breast milk (Silalahi *et al.*, 2017). Vitamin A in fortified oil is greater and stable than any other food vehicle such as flour or sugar (Souganidis *et al.,* 2013), because oil is an ideal matrix for vitamin A, which stabilizes retinol and delays oxidation of the vitamin (Oluwalana *et al.,* 2015).

Stability of vitamin A in edible oil depends much on processing and proper storage conditions (Oluwalana *et al.,* 2015), however, the quality of oil matters a lot (Neufeld *et al.,* 2017). Oil quality is characterized by the amount of peroxides and free fatty acids (Popa *et al.,* 2017). The amount of peroxide values prior to fortification is the most important criteria for ensuring stability of vitamin A in the oil (Andarwulan *et al.*, 2014). Vitamin A in fortified oil oxidizes fast if peroxides and free fatty acids are present in large quantity (Selinger *et al.,* 2017). The oxidation level greatly interacts with the stability of vitamin A which causes vitamin A to oxidize faster and loses its vitamin bio-potency (Pignitter *et al.,* 2016). Degradation of vitamin A in oil is further facilitated by presence of pro-oxidants (light, air and transition metals) (Vrbikova *et al.,* 2014). Vitamin A in oil decreases with storage time when the oil is stored at high, ambient temperatures and humidity (Kostik *et al.*, 2014).

In Tanzania, fortification of edible oils has been mandatory since 2011 (Tanzania Foods Drugs and Cosmetics Regulations, 2011). From 2013 to date, the Government of Tanzania adopted the harmonized East African Standard which stipulates that, vitamin A concentration in edible oil should be 20-40 mg/kg at household and market level, and 30-40 mg/kg of vitamin A at the factory level (EAC, 2017). With this fortification regulation, large scale oil processors are doing well although compliance has been the major challenge (FACT, 2016). For the Small and Medium level Enterprisers (SMEs), the regulation is still working slowly (BoT, 2017). Currently, the Government of Tanzania is working with the development partners to ensure that, fortification of edible oil works well with the SMEs (URT, 2016).

The MASAVA project is one of the efforts aimed to build the capacity of the SMEs in Manyara and Shinyanga regions of Tanzania to fortify the locally produced sunflower oil with vitamin A. The project aimed at improving serum retinol status of children below five years and lactating mothers in Manyara and Shinyanga regions, through the consumption of sunflower oil fortified with vitamin A. In Tanzania, locally produced sunflower oil contributes about 40% of the national demand for cooking oil. About 60% of the edible oil used in the country is imported (BoT, 2017). Small and Medium level enterprisers (SMEs) are the main producers of the edible oils since they are located almost everywhere in the country and they can reach the poorest people in the remote areas (URT, 2016). With the SMEs being the major distributors of edible oils to poor the communities where large scale processors cannot extend; there rises a question whether the quality of the oils from these SMEs may have negative influence on the shelf stability of vitamin A.

To satisfy the customer's needs, most of the retailers in poor communities sell cooking oil in small quantities known as scoops (Henry, 2016). This practice involves exposing the open oil containers direct to the sunlight, high temperatures, air and dust for a long period of the time (Silalahi *et al.*, 2017). The extent to which the oil is exposed to the sunlight, high temperatures, air and dust affects the stability of vitamin A in the oil. With time degradation of vitamin A may take place to the extent that the oil becomes rancid. The scooping practice therefore may have a negative effect in the oil when the oil is opened and the oil is sold out in small quantities for a number of days. This study aimed at determining the shelf stability of vitamin A in fortified sunflower oils collected from three SMEs (SME 1, SME 2 and SME 3). The study also aimed at determining the effect of scooping practice on the retention of vitamin A in fortified sunflower oil. In a storage stability study, oil samples were collected from the three oil processors (SME 1, SME 2 and SME 3). The storage stability was carried out for six months. In the scooping study, oil samples were collected from the markets by scooping oil samples from the day the oil contained was opened to the day of the oil was sold out.

Materials and Methods

MASAVA project worked with three SMEs in Manyara (one) and Singida (two) regions, Tanzania. The three SMEs fortified sunflower oil with vitamin A (Retinyl palmitate) and then the oil were distributed through retailers in Shinyanga and Manyara regions where the study participants (lactating mothers) were selected. The two regions were selected due to high prevalence of vitamin A deficiency among children below five years and lactating mothers (NBS, 2011). Also the project was a pilot to assess the feasibility and nutritional impact of fortifying unrefined sunflower oil at small and medium enterprises outside of major cities. Each region had three intervention districts and one control district. Intervention districts were Babati Urban, Babati Rural, Hanang districts of Manyara and Shinyanga Urban, Shinyanga Rural and Kahama districts of Shinyanga region. Control districts were Mbulu (Manyara) and Kishapu (Shinyanga). The project aimed at improving the vitamin A status of lactating mothers and children below five years children. These regions were used as pilots to determine the effectiveness of public intervention of vitamin A deficiency using fortified sunflower oil.

The shelf-stability and scooping study

In a shelf-stability study, samples of oil were collected from each of the three SMEs namely SME 1, SME 2 and SME 3. These oil samples were analyzed for vitamin A concentration at intervals of one month for a total period of 6 months. The fortified oil was kept at ambient conditions in the laboratories of the DFTNCS, at Sokoine University of Agriculture. Oil samples were drawn at 9 pm every 15^{th} day of the month for a total of 6 consecutive months. A total number of 36 fortified sunflower oil samples were collected for evaluation. In this case 2 samples of the fortified sunflower oil were drawn from the three oil brands for a total of 6 months.

In the scooping study, a total of 96 fortified sunflower oil samples were collected for evaluation in which 2 samples were drawn from each of the three containers every day for the total of 16 days. The oil samples were drawn randomly from the selected retail shops (in the intervention districts) in Babati Urban, Babati Rural, Hanang, Shinyanga Urban, Shinyanga Rural and Kahama districts. Samples were not drawn from control districts of Mbulu and Kishapu. Three fortified sunflower oil brands (SME 1, SME 2 and SME 3) which were randomly consumed in the districts were collected for analysis of vitamin A concentration. One sample (scoop) was drawn at 4pm daily for 1-16 consecutive days.

Handling of oil samples

Oils collected from the SMEs were transported to Sokoine University of Agriculture and stored at ambient temperatures (30 ± 3^0C) for shelf stability study. Oil samples collected monthly from the stored lot and those collected by scooping from the pilot districts were stabilized with a combination of two antioxidants namely, Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) and stored in airtight sample bottles (capacity 5 ml) without head space. Scooped oil samples in bottles were stored in ice boxes and transported under cold conditions to the laboratories of the Tanzania Food and Drugs Authority (TFDA), Dar es Salaam for analysis.

Sample analysis

Vitamin A concentration in the sunflower oil samples was determined by using HPLC method 86-06 (De Viries and Silvera, 2002). The standards and samples were saponified in basic ethanol-water solution, neutralized, and diluted. The concentration of vitamin A (mg/kg) in the oil samples was calculated from the peak heights using the response factor (RF std) of retinyl palmitate for vitamin A.

$$\text{Vitamin A (retinol) (mg/kg)} = \frac{(RFA \times PHsam \times 100)}{W}$$

where RFA was the response factor for vitamin A,

PHsam was the total test sample peak height or area of all Trans and 13-cis retinol,

100 was the dilution volume of the test portion (ml)

W was the weight of the test portion (g)

Percent vitamin A loss from the scooped oil samples was calculated on the basis of the vitamin A concentration from day one to day sixteen and from the first month to the sixth month of the storage under ambient condition.

Quality attributes

Quality attributes determined in the oil samples included peroxide values (PV), anisidine values (AV), totox values (TV) and thiobarbituric acid (TBA).

Peroxide values (meq/kg- oil): Peroxide values of the fortified sunflower oil were determined by measuring iodine released from potassium iodide using AOAC (1995) method 965.33. Peroxide values were expressed as milli- equivalent of peroxide per 1000/g of sample.

Anisidine value: Anisidine values of the fortified oil samples were determined by using a method by Labrinea *et al.* (2001).

Calculations were made from the following formula;

$$\text{Anisidine value} = \frac{25(1.2 \times Eb - Ea)}{W}$$

Ea was the net absorbance of the oil solution

Eb was the net absorbance of the oil anisidine solution

W was the weight of the sample (g)

Totox values: Totox values were calculated as follows.

Totox = 2PV + AV

Where

PV was peroxide value

AV was anisidine value

Malondialdehyde (TBA) values: Malondialdehyde values of the fortified oil were determined spectrophotometrically using a method by Dropper and Hadley (1990). The malondialdehyde concentrations of the oil samples were calculated as µM/g using the following formula;

$$\text{Malondialdehyde } (\mu M/g) = \frac{(Ac \times V)}{W}$$

Ac was the amount of MDA (µM) determined from the calibration curve

V was the volume in ml

W was the weight of the sample taken (g)

Statistical analysis

Statistical analysis was done by IBM SPSS program version 20. Descriptive analysis was calculated to obtain means, frequency and percentages of the retinol means. The retinol means were compared by t-tests to obtain the differences between the start and after six months of storage and between day 1 and day 16 of the scooping study. A $p<0.05$ was considered statistically significant.

Results

The oil samples collected during scooping practice and those from the SMEs were fortified to comply with the harmonized East African Standards EAS 269:2017 for - Edible virgin/cold pressed sunflower oil and the Tanzania Standard TZS 50:2014. According to the harmonized East African Standards EAS 269:2017 (TZS 1313:2014) for fortified edible oils and fats specification, the recommended vitamin A concentration in edible oil (as retinyl palmitate) at the factory level is 30-40 mg/kg while the concentration of vitamin A at the retail and household levels should be 20-40 mg/kg. Therefore, adequacy of vitamin A in the stored and scooped oil was judged based on the recommended concentration of 20-40 mg/kg at the retail and household levels. The common volume of oil purchased for cooking at the participating households in Manyara and Shinyanga regions are shown in Fig. 1.

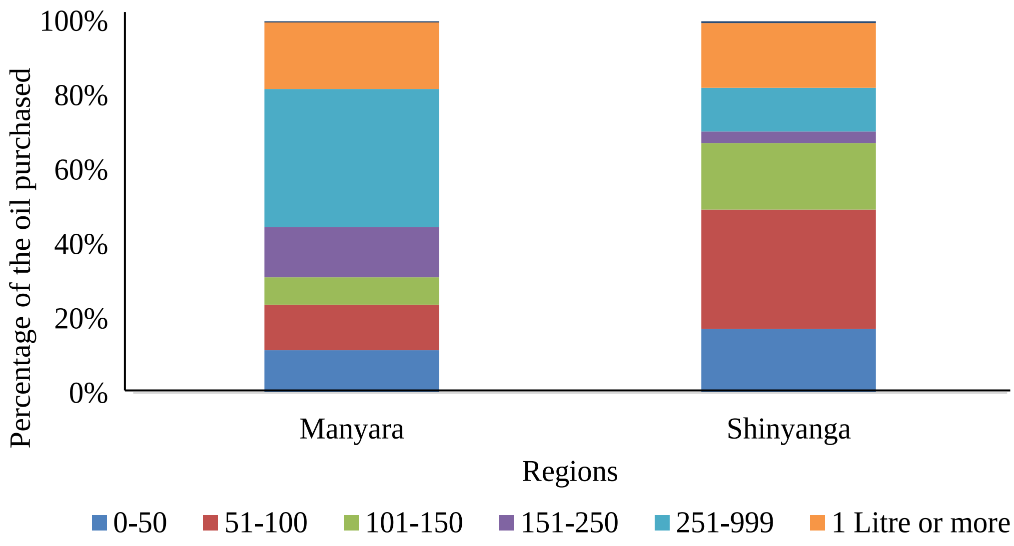

Figure 1: Average amount of oil (ml) purchased by scoops in Manyara and Shinyanga

Most common purchased volume in Manyara: 250 ml; 500 ml; 1 Litre or multiples of 1 Litres
Most common purchased volume in Shinyanga: 30, 60, 90 or 150 ml; 1 Litre or multiples thereof.

Most of the retailers (95%, n=76) reported to sell out their 20L oil container in 16 days with only few reporting to empty their containers beyond 16 days (Fig. 2). Even after 16 days of scooping, all oil samples still retained vitamin A concentrations of 20-40 mg/kg (Fig. 3). The daily average rate of vitamin A degradation in the sunflower oil was 0.421 mg/kg (SME 3) and 0.343 mg/kg (SME 2) and 0.238 mg/kg (SME 1) (Fig. 4).

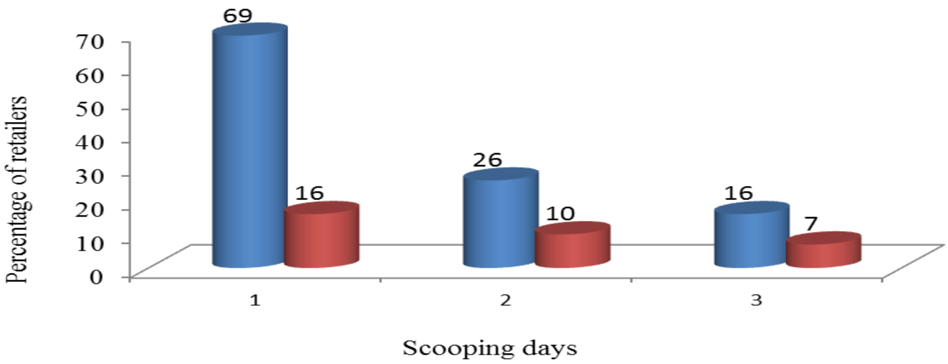

Figure 2: Proportion of the retailers practicing scooping for various numbers of days

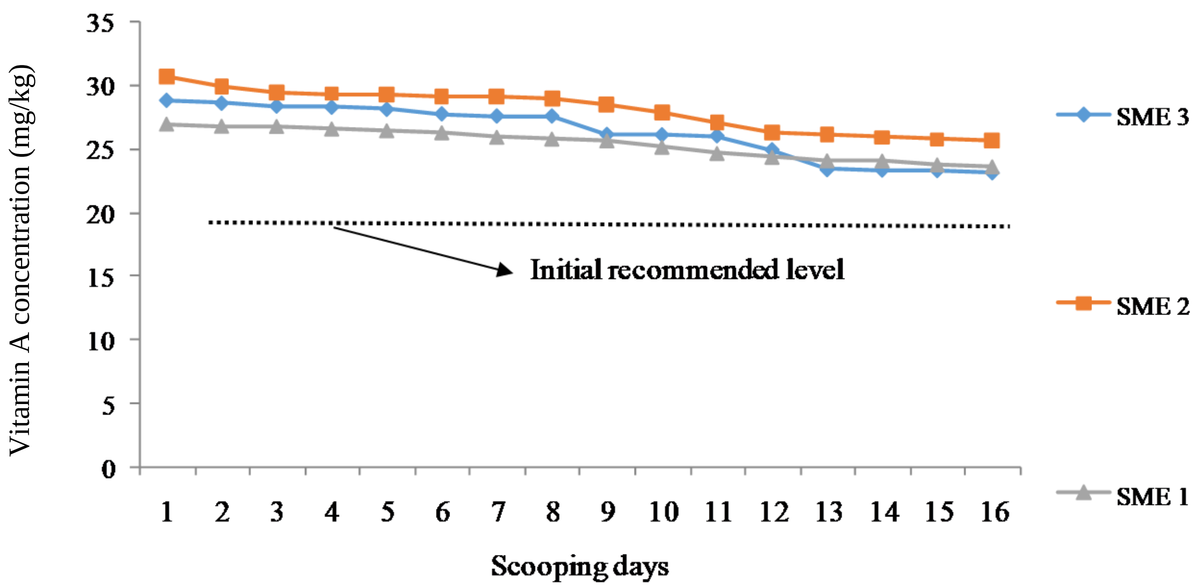

Figure 3: Residual levels of vitamin A (mg/kg) during the scooping days versus the recommended concentration of vitamin A (20-40 mg/kg) at a retail level

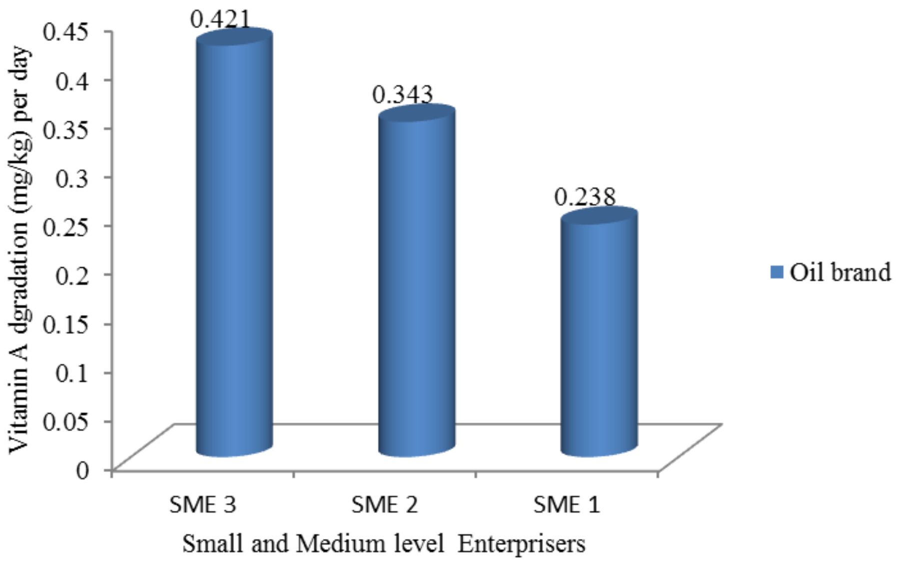

Figure 4: The average rate at which vitamin A was degrading (mg/kg) per day for the scooped oil

Table 1 shows the Quality attributes- peroxide value, anisidine value, thiobarbituric acid and totox value of the fortified sunflower oil sold by scooping for the first day when the container was opened and the last day (day 16) when the 20L container was sold out. There was a significant increase (at $p<0.05$) in the quality attributes of the oil. However, these quality parameters were below the recommended upper limits by Tanzania and East African Standards (TZS 50:2014 and EAS 269:2017).

Figure 5 shows the residual levels of vitamin A (mg/kg) during six months shelf-stability study. At the factory level, sunflower oil was fortified at the concentration of 24.84 mg/kg (SME 1), 24.77 mg/kg (SME 2) and 25.9 mg/kg (SME 3). After the six months of storage at ambient conditions the vitamin A concentration decreased to 23.81 mg/kg (SME 1), 22.46 mg/kg (SME 2) and 23.04 mg/kg (SME 3).

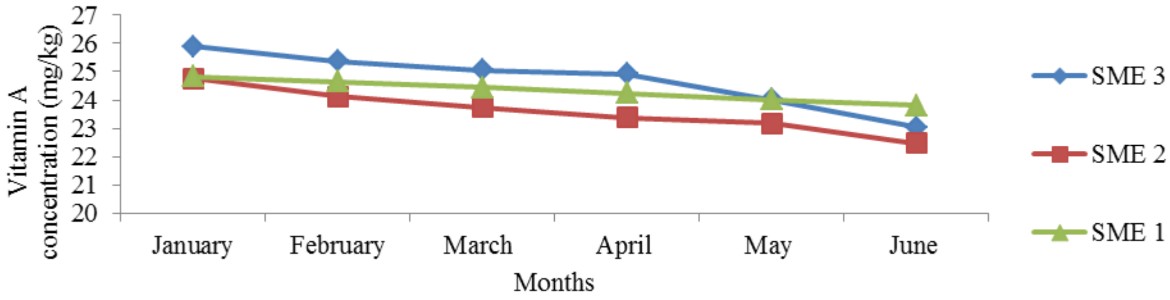

Figure 5: Residual levels of vitamin A (mg/kg) during six months of storage versus the recommended concentration of vitamin A (30-40 mg/kg) at a factory level

Table 1: Quality parameters of the fortified sunflower oils sold by scooping at retail shops

Quality attributes	**Oil brand**												**p-value**
	Day 1						**Day 16**						
	SME 1		**SME 2**		**SME 3**		**SME 1**		**SME 2**		**SME 3**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Peroxide (meq/kg)	2.19	0.02	2.00	0.01	2.02	0.01	2.99	0.02	2.50	0.03	2.52	0.02	0.00**
Anisidine (AV Units)	34.75	0.01	30.91	0.01	31.99	0.04	36.20	0.01	32.30	0.03	32.90	0.03	0.00**
Totox (meq/kg)	39.13	0.05	34.19	0.01	36.03	0.03	40.55	0.03	35.01	0.03	41.42	0.01	0.00**
TBA (mg/kg)	1.48	0.04	1.04	0.01	1.47	0.01	1.58	0.01	1.12	0.01	1.32	0.02	0.00**

Ref: TZS 50:2014 and 299:2013; Acceptable values for peroxide = < 10 meq/kg, anisidine = < 30 AV Units, Totox = < 50 meq/kg
and TBA= < 2 μM/g; ** mean values significant at $p<0.05$; SD=standard deviation SME= small and medium enterprisers, TBA= Thiobarbituric acid

The average monthly degradation rate of sunflower oil was 0.477 mg/kg (SME 3), 0.385 (SME 2) and 0.171 (SME 1) (Fig. 6).

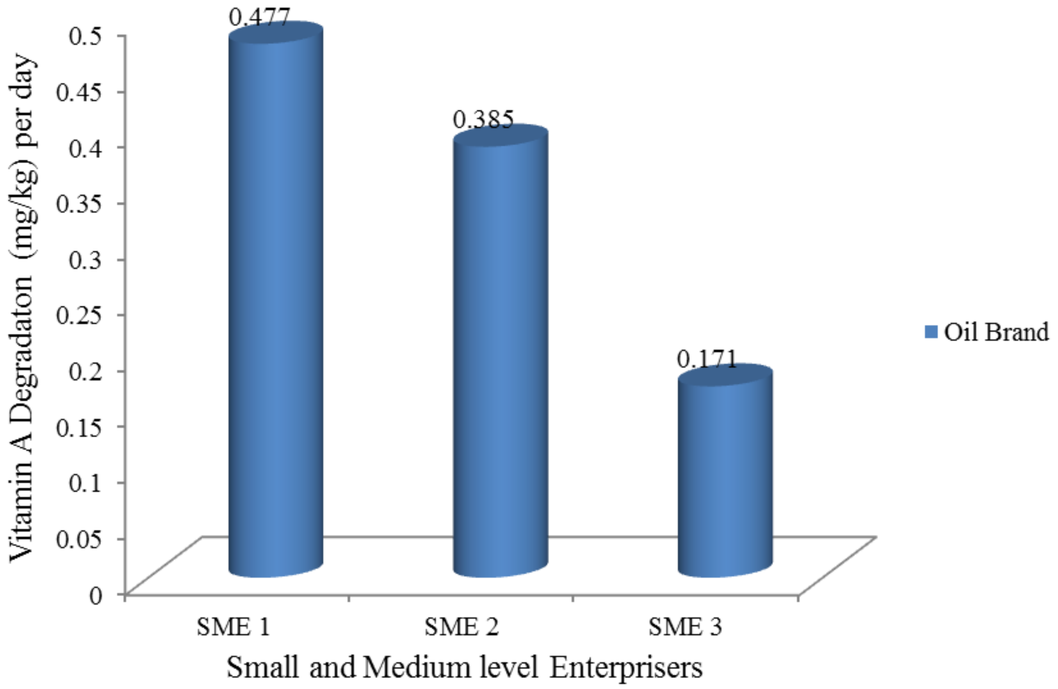

Figure 6: The average rate at which vitamin A (mg/kg) was degrading per month during the six months of storage

Table 2 presents the quality parameters of the fortified sunflower oil at the beginning and after six months of storage. All quality parameters of the oil at the beginning and after six months of storage were within the acceptable value

Table 2: Quality parameters of the fortified sunflower oil at the start (baseline) and after six months of storage (end-line)

Quality attributes	**Oil brand**												**p-value**
	At baseline						**After six months of storage**						
	SME 1		**SME 2**		**SME 3**		**SME 1**		**SME 2**		**SME 3**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Peroxide (meq/kg	2.01	0.11	2.00	0.01	2.09	0.06	3.9	0.04	3.81	0.09	3.62	0.02	0.00**
Anisidine (AV Units)	28.01	0.15	27.09	0.01	26.99	0.01	29.09	0.08	28.10	0.12	28.00	0.20	0.00**
Totox (meq/kg)	33.37	0.03	30.45	0.02	31.23	0.01	36.23	0.11	34.9	0.03	37.08	0.16	0.00**
TBA (mg/kg)	1.05	0.03	1.00	0.01	1.01	0.01	1.24	0.09	1.23	0.075	1.13	0.13	0.00**

Ref: TZS 50:2014 and 299:2013; Acceptable values for peroxide = < 10 meg/kg, anisidine = < 30 AV Units, Totox = < 50 meq/kg and TBA= < 2 μM/g; ** Mean values significant at $p<0.05$; SD=standard deviation SME= small and medium enterprisers, TBA= Thiobarbituric acid

Discussion

Results in this study revealed that, six months storage of fortified sunflower oil at ambient conditions (Fig. 5) resulted into minimal vitamin A losses. The levels of vitamin A remaining in all sunflower oil brands were within the levels of 20-40 mg/kg recommended by the harmonized East African Standards (2017). More than 89% of vitamin A concentration in the fortified sunflower oil brands was retained during the six months of storage (Fig. 4). Laillou and collogues, (2012) highlighted that, 80.3 % of vitamin A was retained when fortified sunflower oil was stored at room temperature for three months. Also, Silalahi *et al.* (2017) reported from their study that vitamin A in fortified oil was stable for up to 12 months when stored at room temperature while Andarwulan *et al.* (2014) documented that, vitamin A in fortified sunflower oil kept at room temperature was stable for 3 months. Results by Rajaram and Nagarajan (2015) further showed that, vitamin A in fortified sunflower oil remained stable when stored for five months at room temperature.

All quality attributes of the fortified sunflower oil namely peroxide values, anisidine value, thiobarbituric acid and totox value (Table 2) were lower than the upper limits stated by the Tanzania Standard (TZS 50:2014) and the harmonized East African Standards (EAS 269:2017). The oil samples were therefore of good quality and thus suitable for human consumption. Similar results have been reported by Moigradean *et al.* (2012) who found that, there was a slight increase in the oil quality attributes during storage time (12 months of storage). Good quality oil is crucial for human healthy while low quality oil may have high reactive oxygen species (ROS) and other end products of lipid peroxidation (Nita and Grzybowski, 2016), that have been associated with occurrence of many chronic health problems such as, cardiovascular diseases, inflammatory diseases, cataracts and cancers (Gashaw and Getachew, 2014).

In a scooping study, more than 80% of vitamin A concentration in fortified sunflower oil was retained (Fig. 6). Vitamin A in fortified sunflower oil was stable from the date of opening the 20L container to the date of selling the oil out (Fig. 3). Vitamin A concentrations in the oils were within the accepted range of 20 - 40 mg/kg recommended by the harmonized East African Standards (2017). The rate of vitamin A degradation per day was minimal for all the sunflower oil brands. This might be due to the fact that, the rate at which vitamin A in the oil degraded was highly dependent on the levels of quality attributes (peroxide values, anisidine values, totox values and thiobarbituric acid) (Table 1). The levels of these quality parameters also reflected good manufacturing practices (GMP) and good hygienic practices (GHP) when the oil was processed at the factory (WFP, 2011). Exposure to air and light during storage may have contributed to the degradation of vitamin A in the oil samples (Piscopo and Poiana, 2012).

Conclusions and recommendations

In this study, vitamin A was stable in fortified sunflower oil during six months of storage and during retail by scooping. Retention of vitamin A in the oil samples was high with minimal degradation of the vitamin with increase in the storage time. Furthermore the quality attributes of the fortified sunflower oil sold at retail by scooping and those of the stored oil remained within the acceptable levels. It is therefore recommended that, oil processors should abide to the fortification specifications stipulated in the regulations to avoid fortifying the oils with vitamin A below the recommended levels.

Also, oil processors should maintain good manufacturing and hygiene practices to ensure high quality oil. Retailers should observe good handling practices of fortified sunflower oil so as to maintain the quality of the oil and ensure high retention of vitamin A in the oil. Retailers must also keep the oil under good storage conditions by avoiding direct light, exposure to air, dust and metals which accelerate oxidation thus reducing the quality of the oil.

Acknowledgements

The authors are grateful to the Government of Canada that through the Global Affairs Canada (GAC) (#107790), International Development Research Centre (IDRC) and Canadian International Food Security Research Fund (CIFSRF) Grant, Phase 2 that, provided financial support to undertake this study.

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PAPER THREE

Vitamin A levels in fortified sunflower oil along the market value in Manyara and Shinyanga, regions, Tanzania

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(Submitted to International Journal of Sciences, Basic and Applied Research)

Abstract

Vitamin A deficiency is the major problem in low and middle income countries. Fortification of edible oils is the best strategy to control vitamin A deficiency in the populations at risk. However, low compliance to fortification may reduce the expected nutritional impact to the consumers. This study was conducted to determine vitamin A levels in fortified sunflower oil along the value chain in Manyara and Shinyanga regions of Tanzania. Samples of locally produced and fortified sunflower oil were collected from the market value chain- factories (n=3), retail shops (n=117) and households (n=445). Retinol concentrations of these oil samples were determined by HPLC. Results revealed that, sunflower oil samples collected along the market value chain had variable concentrations of vitamin A. Oil samples collected at the factories were under fortified with vitamin A concentration below the recommended 30-40 mg/kg. The mean vitamin A concentration in the oil samples from both regions was within the recommended values of 20-40 mg/kg. Most of the oils collected from the households (n=381, 85.6%) and retail shops (n=110, 94%) however, had vitamin A concentrations within the recommended levels (20-40 mg/kg). Only few oil samples collected at the market (retail shops) (n=7, 6%) and households (n=64, 14.4%) had vitamin A concentration below the recommended levels of 20-40 mg/kg. Results revealed further that, all quality attributes of the sunflower oil samples namely refractive index, free fatty acid, acid, peroxide, anisidine, TBA values, collected from the factories, retail shops and households were within the limits recommended by Tanzanian Bureau of standards. It was concluded based on this study that, vitamin A concentrations in the fortified sunflower oil samples collected from the SMEs had vitamin A concentration lower than the recommended fortification levels. Most of the sunflower oil collected from the retail shops and households were within the recommended fortification levels. All the fortified sunflower oil samples collected from

the factory (SMEs), retail shops and households met the limits for the recommended quality attributes. It is therefore recommended on the basis of this study that, producers should adhere to the National Fortification guidelines. Also oil producers should adhere to Good Manufacturing Practices and Good Hygiene Practices including adherence to the Hazard Analysis Critical Control Point program to ensure high quality fortified oil.

Introduction

Vitamin A is among the most important micronutrients needed for several vital functions in the body (Pignitter *et al.,* 2016). Vitamin A helps to maintain the function of epithelia, mucous membranes, visual system, growth, reproduction and immune function (Laillou *et al.,* 2013). Vitamin A is naturally obtained from vitamin A rich foods since human bodies cannot synthesize it (Maigari *et al.*, 2012). It is available in two forms; pre-formed vitamin A (retinol) and pro-vitamin A (e.g. Betacarotene) (Souganidis *et al.,* 2013). Preformed vitamin A is found in animal sources only for e.g. liver, egg yolk, butter and whole milk (WHO, 2011) while pro-vitamin A is found in plant sources e.g. in dark green leafy vegetables, yellow vegetables and yellow and orange fruits (Jemberu *et al.,* 2017).

Inadequate consumption of vitamin A rich foods leads to deficiency of vitamin A, which causes high risk for morbidity and mortality among the vulnerable social groups namely lactating mothers and children below five years (Silalahi *et al.,* 2017). In low and middle income countries consumption of foods with pre-formed vitamin A is minimal due to low economic status of the people (WHO, 2011). When preformed vitamin A is consumed, it is highly bioavailable compared to provitamin A (Souganidis *et al.,* 2013).

Despite poor bioavailability of provitamin A in the body, consumption of provitamin A rich plant foods is the main source of vitamin A consumed in low and middle income countries (Laillou *et al.,* 2013). In these countries, diets are monotonous and mainly based on cereals and legumes that are poor sources of vitamin A (Chimimba *et al.*, 2016).

There has been several strategies to address vitamin A deficiency problem (FAO and WHO, 2013). Fortification of vegetable oils with vitamin A has been and is the best intervention strategy used to improve vitamin A status of the vulnerable social groups (Pignitter, *et al.,* 2016). Oil is the best vehicle for vitamin A due to its ideal matrix for vitamin A because it stabilizes retinol and delays oxidation of the vitamin (Pignitter *et al.*, 2016). The impact of fortifying edible oils with vitamin A is well demonstrated by several studies (Souganidis *et al.,* 2013; Laillou *et al.,* 2014; Sandjaja *et al.*, 2014). Oil fortification, enhances health by increasing blood retinol concentration (FAO and WHO, 2013).

In Tanzania, fortification of vegetable edible oils has been made mandatory by law and reinforced by several regulations since 2011 (URT, 2013), however, compliance to Tanzania National Fortification Regulation by the small and medium level oil producers is very low (FACT, 2016). That's the reason for, most of the oils available in the market are inadequately fortified in line with the recommended standards (Aaron *et al.*, 2017) and some edible oils in the market are not fortified at all or over fortified (FACT, 2016). Nationally, the proportion of households consuming fortified oils is 53.6%, but 16.3% of the oil consumed is adequately fortified (FACT, 2016). It has also been reported by Aaron *et al*. (2016) that, 90% of edible oil consumed at the household level in India that was classified as fortifiable, only 24% of the oil was adequately fortified. Low compliance reduces nutritional impact of the fortified oil to the vulnerable consumers (Luthringer *et al.,* 2015). In Manyara and Shinyanga regions of Tanzania, compliance to fortification by local SMEs has not been evaluated. Evaluating compliance is important in the efforts to eradicate vitamin A deficiency among the vulnerable social groups namely lactating mothers and under five children (Aaron *et al.,* 2016). As part of the efforts to combat Vitamin A deficiency, this study was conducted to determine compliance of the SMEs to fortified sunflower oil with vitamin A by determining vitamin A concentration in the oil obtained from the market value chain from the factory (SMEs), retail and household levels. According to National Food Fortification regulation, the concentration of vitamin A in edible oil should be 30-40 mg/kg at the factory level and 20-40 mg/kg at the retail and household level (EAS, 2017).

Methods and Materials

This study was conducted in Manyara and Shinyanga regions of Tanzania. These regions were selected due to high prevalence of vitamin A deficiency among women of reproductive age (15-45 years) and children under the age of five years (NBS, 2011). These regions were used as pilot study areas to determine public intervention of vitamin A deficiency using fortified sunflower oil. Samples of sunflower oil fortified by the SMEs were collected from the market chain value - at the factories, retail shops and at the household levels. The retinol concentration of the oil samples was determined by using HPLC.

Sample handling

Oil samples collected from the SMEs (n=3) immediately after processing and those from retail shops (n=117) and households (n=445) were stabilized by a mixture of antioxidants - Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA). The stabilized oil samples were stored in airtight sample bottles (ca 5 mL) filled without leaving a head space. Sample bottles were stored in cool ice boxes and transported under cold conditions to the laboratories of the Tanzania Food and Drug Authority (TFDA), Dar es Salaam.

Sample analysis

Vitamin A concentration in the sunflower oil samples was determined by using reverse phase High Performance Liquid Chromatography (HPLC) method 86-06 (De Viries and Silvera, 2002). A sample of 5g of oil was used. Vitamin A concentration was calculated using the following formula;

$$\text{Vitamin A (retinol) (mg/kg)} = \frac{(RFA \times PHsam \times 100)}{W}$$

Where RFA was the response factor for vitamin A,

PHsam was the total test sample peak height or area of all Trans and 13-cis retinol,

100 was the dilution volume of the test portion (ml) and

W was the weight of the test portion (g)

Quality aspects of the fortified sunflower oil samples

Refractive indices - Refractive indices of the fortified oil samples were measured using AOAC (1995) method 920.14

Acid value (mg KOH/g) - Acid values of the fortified sunflower oil were determined by a standard titration method (AOAC, 1995).

Peroxide value (Meq/kg- oil) - Peroxide values of the fortified sunflower oil collected from the value chain were determined by measuring iodide released from potassium iodide (AOAC, 1995) using method 965.33. Peroxide values were expressed as milli-equivalents of peroxide per 1000/g of the sample.

Anisidine value - Anisidine values of the fortified sunflower oil samples collected from the market value chain were determined by using a method by Labrineaa *et al.* (2001). Calculations were made from the following formula;

$$\text{Anisidine value} = 25\,\frac{(1.2 \times Eb - Ea)}{W}$$

Whereby,

Ea was the net absorbance of the oil solution

Eb was the net absorbance of the oil anisidine solution

W was weight of sample in gram

Malondialdehyde (MDA) values - Malondialdehyde values of the fortified sunflower oil collected from the market value chain were determined spectrophotometrically using a method by Draper and Hardley (1990). The malondialdehyde concentrations of the sunflower oil samples were calculated as µM/g using the following formula;

$$\text{Malodialdihyde } (\mu M/g) = \frac{(Ac \times V)}{W}$$

Whereby,

Ac was the amount of MDA (µM) determined from the calibration curve

V was the volume in mL

W was the weight of the sample taken in gram

Statistical analysis

Statistical analysis was done by IBM SPSS program version 20. Descriptive analysis was calculated to obtain means, frequency and percentages of the retinol means. The retinol means were compared by t-tests to obtain the differences between the regions. A $p<0.05$ was considered statistically significant.

Results

Figure 1 shows the mean concentration of vitamin A (mg/kg) in the fortified sunflower oil samples from the production sites (SMEs). The oil samples from the SMEs were fortified to comply with the Eat African Standards (EAS)-299:2013 for Edible virgin/cold pressed sunflower oil. This EAS 299:2017 is similar to the Tanzania Standard (TZS) 50:2014. According to the EAS 769:2017 (TZS 1313:2014) specifications, the recommended levels of fortification of vitamin A in edible oils and fats (as retinyl palmitate) at the factory level should be 30-40 mg/kg while the concentration of vitamin A at the retail and household levels should be 20-40 mg/kg.

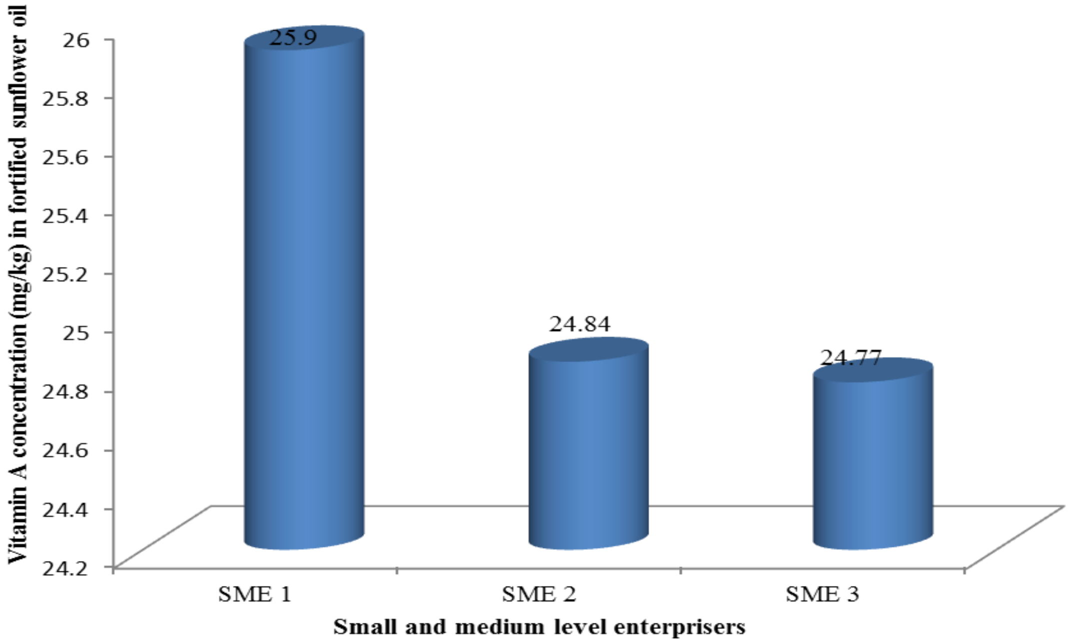

Figure 1: Concentrations of vitamin A in fortified sunflower oil collected from the factories (SMEs)

In this study, majority of the sunflower oil samples collected from the households and retail shops in both regions, had vitamin A concentrations within the recommended concentrations of 20-40 mg/kg. There were only few oil samples with vitamin A concentrations below the recommended lower limit of less than 20 mg/kg (Table 1).

Table 1: Distribution of vitamin A levels (mg/kg) in the sunflower oil samples collected from the households (n=445) and retailers (n=117)

Group	Retinol concentrations				P- value
	Less than 20 mg/kg		20-40 mg/kg		
	n	%	n	%	
Households					
Manyara	39	16.1	203	83.9	0.000*
Shinyanga	25	12.3	178	87.7	
Both regions	64	14.4	381	85.6	
Retail shops					
Manyara	7	11.1	56	88.9	0.000*
Shinyanga	1	1.9	53	98.1	
Both regions	8	6.0	110	94.0	

Table 2 presents the quality attributes of the fortified sunflower oil samples from the production sites (SMEs) through the market chain (retail shops) to the consumers (household level). All quality attributes of the oil samples were within the recommended levels by WHO/FAO (1994), East African Standards (2013) and Tanzania Bureau of Standards (2014). The recommended quality attributes in the sunflower oil are Refractive Index (1.464-1.480); Free fatty acid (4), acid value (14 mg KOH/g), Peroxide (<10 meq/kg); Anisidine Value (<30 AV Units); Totox (50 meq/kg) and Thiobarbituric acid (<2 (μM/g)). The fortified sunflower oil collected at the retail shops and household level had quality attributes at the acceptable levels and thus was healthy and suitable for human consumption.

Table 2: Quality attributes of the fortified sunflower oil samples collected from the SMEs (n = 30), retail shops (n = 117) and households (n = 445)

Stage	RI		FFA		Acid (mg KOH/g)		Peroxide (meq/kg)		Anisidine (AV Units)		Totox (meq/kg)		TBA ((μM/g)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
SMEs														
SME 1	1.35	0.01	2.0	0.22	3.00	0.14	1.50	0.00	24.2	0.23	33.13	0.03	1.30	0.00
SME 2	1.38	0.02	2.3	0.44	3.32	0.02	2.10	0.01	24.5	0.26	34.09	0.01	1.33	0.00
SME 3	1.39	0.02	2.4	0.11	3.40	0.07	2.11	0.01	25.9	0.00	34.33	0.01	1.19	0.10
Retail shops														
Manyara	1.455	0.000	2.312	0.005	3.361	0.005	2.712	0.008	25.951	0.004	34.744	0.007	1.351	0.007
Shinyanga	1.458	0.000	2.113	0.011	3.442	0.011	2.451	0.007	26.133	0.013	34.923	0.015	1.371	0.004
Both regions	1.457	0.002	2.210	0.141	3.400	0.057	2.580	0.184	26.040	0.128	34.830	0.127	1.360	0.014
Households														
Manyara	1.4567	0.003	2.730	0.002	3.860	0.002	2.740	0.002	26.101	0.002	35.050	0.002	1.401	0.003
Shinyanga	1.4605	0.002	2.810	0.002	4.081	0.003	2.861	0.003	26.331	0.002	35.151	0.004	1.452	0.009
Both regions	1.4580	0.003	2.770	0.056	3.970	0.155	2.800	0.085	26.215	0.163	35.100	0.070	1.425	0.035

Ref. cut-off values by TZS50:2014 EAS299:2013; Refractive Index (1.464-1.480); Free fatty acid (4), acid value (14 mg KOH/g); Peroxide (<10 meq/kg); Anisidine Value (<30 AV Units); Totox (50 meq/kg); TBA (<2 (μM/g)). SME= small and medium enterprisers; SD=standard deviation; RI= Refractive Index; FFA= Free fatty acids; TBA= Thiobarbituric acid

Discussion

The retinol concentrations in the fortified sunflower oil collected from the SMEs were lower than the concentrations (30-40 mg/kg) recommended at the factory level but were within the acceptable range of vitamin A concentrations at retail and household levels (20-40 mg/kg). A study by Aaron *et al.* (2017) reported under-fortification of edible oils from commercial factories in 8 middle and low income countries including Tanzania. Study by FACT (2016), also reported under-fortification edible oils in Tanzania. Low compliance to the recommended fortification levels reduces vitamin A availability and intake of vitamin A in fortified products (BoT, 2017). This potentially reduces the nutritional benefit that the vulnerable social groups namely lactating mothers and children below five years would get (Luthringer *et al.,* 2015).

Despite the few oil samples collected from retail shops and households having vitamin A concentrations below the recommended levels, the mean vitamin A concentration in the oil samples collected from the two regions, at retail shops and household levels were within the recommended range of 20-40 mg/kg (Table 1). Oil samples with low vitamin A concentrations in this study, could be due to the fact that, the oils were under-fortified at the factory level (Chimimba *et al.,* 2016). Inadequate fortification of edible oil limits the intended nutritional benefit to the consumers (Aaron *et al.,* 2016). The findings of this study are in agreement with those reported by Ogunmoyela *et al.* (2013). In their study, they reported that, fortification levels of vitamin A in some of their edible oil samples from the market had vitamin A levels below the recommended fortification levels.

A study by Aaron *et al.* (2016) also reported that, some of the edible oils consumed at the households in India, classified as fortifiable, were neither fortified nor adequately fortified. Most of the low and middle income countries do not adhere to the recommended fortification standards (Neufeld *et al.*, 2017). Reasons for not adhering to the recommended fortification standards include poor quality oil which does not meet the fortification criteria, poor processing procedures, lack of awareness of the fortification standards and purposeful under fortification at factory level to reduce the production costs (Luthringer *et al.*, 2015). Also, the length of the market chain affects the holding time of the oil before reaching the end user (consumers) because vitamin A levels decrease at different stages of supply chain including during oil transportation from production sites to the wholesale and retail market (Bagriansky and Ranum, 1998).

According to Chimimba *et al.* (2016), vitamin A levels decrease at various stages of the supply chain with the least retention in the market when the oils are sold while exposed to high temperature, air and dust. These factors accelerate oxidation of the vitamin A in the oil, thus affecting the stability of vitamin A and quality of the oils that enhance vitamin A degradation (Vrbikova *et al.*, 2014). Therefore, in order to achieve improvements in the intake of vitamin A in foods for the target social groups in the population, good manufacturing practices (GMP) and good hygienic practices (GHP) must be strictly observed during processing. Likewise the quality of the oil to be fortified in terms of concentrations of peroxides, free fatty acids and other compounds must be within the recommended levels in order to preserve vitamin A. All quality attributes of the sunflower oil samples collected from the SMEs, retail shops and households were lower than the cut off levels recommended by Tanzania Bureau of Standards and East African Standards for edible oils (Table 2). Good quality of the oil samples from these SMEs could be due to adherence to the specifications of the East African Standard 769:2013(TZS 1313:2014) regarding fortification of edible oils and fats on good manufacturing practices (GMP) and good hygienic practices (GHP) (WFP, 2011). Also the quality attributes of the oil in this study, impacted on the stability of the vitamin A in the fortified sunflower oil positively since they were within the acceptable range by Tanzania Bureau of Standards and East African Standards for edible oils (Laillou *et al.*, 2013). Vitamin A in fortified oil oxidizes fast if peroxides and free fatty acids are present in large (Pignitter *et al.*, 2016). The oxidation level greatly interacts with the stability of vitamin A which causes vitamin A to oxidize faster and loses its vitamin bio-potency (Sandjaja *et al.*, 2014).

Conclusion and Recommendations

It was concluded from this study that, some sunflower oil samples collected from the Small and Medium scale oil Enterprisers (SMEs) had lower concentration of vitamin A than the recommended fortification levels. Majority of the oils collected from the households and retail shops, however, had vitamin A concentrations within the recommended levels. Only few oil samples collected from retail shops and households contained vitamin A concentrations below the recommended levels. Results from this study further revealed that, all the sunflower oil samples collected from the retail shops and households met the limits for the recommended quality attributes. It is therefore recommended that, producers should adhere to the National Fortification guidelines. Also, oil producers should adhere to Good Manufacturing Practices and Good hygiene Practices including Hazard Analysis Critical Control Point program to ensure high quality fortified oil.

Acknowledgement

The authors are grateful to the Government of Canada, Global Affairs Canada (GAC) and IDRC-CIFSRF for providing financial support to undertake this study.

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PAPER FOUR

Influence of dietary diversity on the anthropometric status and of 6-23 months-old children Manyara and Shinyanga, regions, Tanzania

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(Accepted to African Journal of Food, Agriculture, Nutrition and Development)

Abstract

Lack of dietary diversity is a severe problem among poor populations in the developing world. It affects all age groups especially infants and young children who need high energy and nutrient-dense foods to support their optimal growth and development. Diversifying diets ensures good quality nutrient intake and hence supports growth and development in young children. This study was conducted to determine the influence of dietary diversity on the anthropometric status of 6-23 months children in Manyara and Shinyanga regions, Tanzania. Purposive and random sampling techniques were used to obtain a sample of 370 children aged 6-23 months living in the study areas. Data were collected using a questionnaire and analyzed using SPSS computer program version 20.0 and ENA for SMART. The mean age of the children was 13.41±5.03 months and the mean dietary diversity score of the children was 3.25±1.20 food groups. More than half of the children (57.3%, n=219) had low dietary diversity (≤3 food groups). Almost all the children (95.4%, n=353) consumed cereal staple foods in the previous 24 hours. Fruits and vegetables were consumed by 70% (n=259) of the children while vitamin A-rich fruits and vegetables were consumed by 55.4% (n=205) of the children. Prevalence of underweight, stunting and wasting among the children was 32.4, 57.3 and 8.9 percent, respectively. Prevalence of underweight, stunting and wasting for the children with low dietary diversity was 19.2, 38 and 6 percent, respectively. Low diversity was strongly associated with weight for age, and length for age. Also strong association existed between low dietary diversity and family size and rural/urban residence. Living in urban areas increased the likelihood of consuming diets with high diversity. These findings suggest that; there is a need for interventions to combat under-nutrition by promoting consumption of a variety food groups that are locally available and affordable.

Key words: Dietary diversity, anthropometry, growth velocity, 6-23 months children and under-nutrition.

INTRODUCTION

Dietary diversity is defined as the number of different food groups consumed over a given reference period [1]. It is very important because it ensures adequate intake of essential nutrients by individuals [2]. Lack of dietary diversity is the foremost problem in poor populations of the developing world [3]. It affects all age groups but it is worse-off in infants and young children aged 6-23 months [4]. This group needs high energy and nutrient-dense foods to support their fast growth and development [1]. The age of 6-23 months is known to be a transition period from exclusive breastfeeding to solid and semi-solid foods [5]. It is a very vulnerable period in which under-nutrition starts in many infants and young children, contributing to high prevalence of stunting, underweight and wasting [6]. In developing countries, under-nutrition among infants and young children is caused by low quality foods associated with lack of dietary diversity [7]. Poor nutritional status resulting from lack of dietary diversity has been documented by several authors [8, 9, 10] because nutritional status at this critical age of 6-23 months depends much on the diversity of food varieties consumed [11, 12]. Dietary diversity affects nutrient adequacy in individuals [13, 14, and 15] thus children consuming more diverse diets are more likely to meet their nutrient needs than those who consume minimally diverse diets [1]. Minimally diverse diets mean proportion of children aged 6–23 months who receive foods less than 4 food groups daily [16]. Consumption of food varieties increases nutrient intake, hence improves nutritional status of the infants and young children [17].

Despite improvements in many health indicators in the past few years and the well-established role of nutrition in physical growth, the rate of under-nutrition among children under the age of five years in Tanzania remains very high [18]. Current prevalence rates of under-nutrition among under-five children in Tanzania are 14% for underweight, 34% for stunting and 5% for wasting [19]. High rates of under-nutrition are due to poor diet and lack of dietary diversity [20]. Intake of predominantly starchy staples with few or no animal products with only seasonal availability of fruits and vegetables are major causes of under-nutrition [21]. Such diets do not meet the nutrient requirements of the infants and young children [8], which make these children more susceptible to diseases and poor nutritional status [12]. According to the 2015-2016 Tanzania Demographic and Health and Malaria Indicator Survey report, only 9% of children in the age 6-23 months are fed according to the minimum acceptable dietary standards [19]. It has been shown that; moving from a monotonous diet to a diet containing diverse food items increases energy and micronutrients intake [9] therefore diversifying diets improves nutritional adequacy and the overall health of the child [5]. This study was designed to determine the influence of dietary diversity on the anthropometric status of children aged 6-23 months in Shinyanga and Manyara regions, Tanzania.

Methodology

Description of the Study Area

A cross sectional study design among children below five years was conducted in June 2015 to July in eight districts, four in Manyara and four others in Shinyanga regions. The districts involved were Babati Urban, Babati Rural, Hanang and Mbulu districts (Manyara region) and Shinyanga urban, Shinyanga rural, Kahama and Kishapu districts (Shinyanga region) Tanzania. The regions were purposively selected for this study because of high prevalence rate of under-nutrition among below five years [23, 24].

Sampling Technique and Sample Size

The study population involved children aged 6-23 months (males and females) living in the selected districts of Manyara and Shinyanga regions, Tanzania. Children aged 6-23 months from low, middle and high income families and attending post-natal monthly health care clinics at the Maternal and Reproductive Health facilities (MRH) were eligible for inclusion into the study. Included also were mothers/caregivers who consented to participate in this study. Children with measles and/or infections that have lasted for a month prior to the baseline data collection, children with chronic diseases such as type 1 diabetes mellitus, HIV, inborn errors of metabolism, e.g., phenylketonuria (PKU), those who were no longer attending MRH and those whose mothers/caregivers refused to participate in the study as well as children who have stayed in the study areas for less than three months at the time of the study were excluded. Respondents were randomly selected by assigning numbers and drawing at random from each social group from a qualified group. Sample size was determined by using a statistical power analysis using a formula $n=t^2x\ (p \times q)/d^2$ [23]. Where n=Sample size (370), t=the risk of error (1.96), p=expected prevalence (0.60), q=1-p (expected non-prevalence) (0.40) and d=level of precision (0.05). A total of 370 children aged 6-23 mo. were selected for the study.

Data Collection

An adopted household survey questionnaire from GAIN was used to collect socio-economic and demographic information of the households, dietary diversity, health and anthropometric information of the children. The questionnaire was pretested among ten lactating mothers/caregivers in Morogoro urban and rural districts of Tanzania. The villages in which the questionnaires were pre-tested were Bigwa, Kichangani and Mazimbu in Morogoro town and Mgeta and Mlali in Morogoro rural districts. Necessary adjustments were made on the questionnaire accordingly. Ten enumerators were trained before administration of the questionnaire. They were taught on how to ask questions, expected answers, probing to reaffirm the responses, how to record the answers and also how to take anthropometric measurement. Administration of the question was done by home visit during the morning hours of the day. Respondents were interviewed face to face and the enumerators recorded the responses from the subjects.

Weight measurements were obtained using UNICEF SECA scale (Model 874 Hamburg-Germany). Children were weighed after having their parent/caregiver stand on the scale, reset the scale to zero, and then have the /caregiver hold the child. The child was weighed wearing light clothes. Weight was recorded to the nearest 0.1 kg.

Measurements of length were carried out using a UNICEF length board. Children were measured while lying down flat on the center of the length board with shoulders and buttocks touching against the measuring surface. The measurement was read to the nearest 0.1 cm. Weights and lengths were used to calculate weight for age (WA), weight for height (WH) and height for age (HA) Z-scores. Based on WHO recommendation, children with $-2 < SD < +2$ values were classified as normal W/A, W/H and H/A Z-scores [26].

Edema: Clinical observation was made to check presence of pitting edema in the feet, face, arms and abdomen. Thumb pressure was applied to the child's feet, face or any other swollen part for about 3 seconds. If the pit did not recover, it was a confirmation of edema.

The children’s dietary intake in the previous 24 hours was collected from their mothers/caregivers. Mothers were requested to list all the food items that were consumed by the child, both at home and out the home. Dietary Diversity Score (DDS) was calculated based on consumption of seven food groups namely (1) staples (cereals/grains, roots and tubers); (2) legumes and nuts; (3) dairy products; (4) animal/flesh foods; (5)

vitamin A-rich fruits and vegetables; and (6) other fruits and vegetables (7) eggs. According to WHO recommendation [27] consumption of ≤3 food groups was classified as low dietary diversity, while consumption of 4 food groups was regarded as the average and consumption of ≥5 food groups was classified as high dietary diversity.

Data were analyzed by SPSS program version 20. Descriptive analysis was used to calculate frequencies, percentages, means and standard deviations. The association between dietary diversity scores and the anthropometric indicators (Weight for Age, Height for Age and Weight for Height) was determined by using binary logistic regression analysis. Anthropometric indicators were taken as dependent variables, which were influenced by dietary diversity as an independent variable. ENA for SMART program was used to calculate Z-scores. Z-scores were then used to classify children into the following levels; stunting, wasting and underweight.

Ethical approval was obtained from the Ethics Committee of the National Institute for Medical Research (NIMR) and the University of Waterloo (Canada) ethics committee for research on human subjects. Informed consent was obtained from the parents/caregivers to allow their children to participate in the study. Confidentiality of the data collected was ensured and all respondents were identified by numbers instead of their actual names.

Results and Discussion

Table 1 summarizes the socio-economic and demographic characteristics of the studied children. The mean age, weight and recumbent length of the children at baseline was 19.96±10.34 months, 9.26±1.56 kg and 74.30±6.77 cm, respectively. Majority (70.3%) of the children were from families with ≥4 people. Out of 370 children, 61.9% were males and 38.1% were females. Most (80.5%) of the children were living in rural areas. Almost all mothers/caregivers (98.9%) were married, 88.6% were housewives and 68.4% had primary education level.

In this study, consumption of ≤3 food groups was considered as low dietary diversity, while consumption of 4 food groups was regarded as medium and consumption of ≥5 food groups was considered as high dietary diversity. More than half (57.3%) of the children in this study had low dietary diversity (≤3) food groups (Fig.1). Low dietary diversity scores among the children could be due to low economic status, low maternal education and/or seasonal variation of foods in their areas and geographical location [5]. The findings in this study are comparable to those reported by [3] in Ghana [3] and by [28, 29] in Ethiopia, in which more than half of the study children aged 6-23 months scored low dietary diversity (≤3 food groups per day). Similar studies conducted in Bangladesh, Vietnam and Ethiopia [17] and Tobago [1] also support these findings. High dietary diversity is necessary for this young population to meet their requirements for essential nutrients because they need energy and nutrient-dense foods to grow and develop both physically and mentally [4].

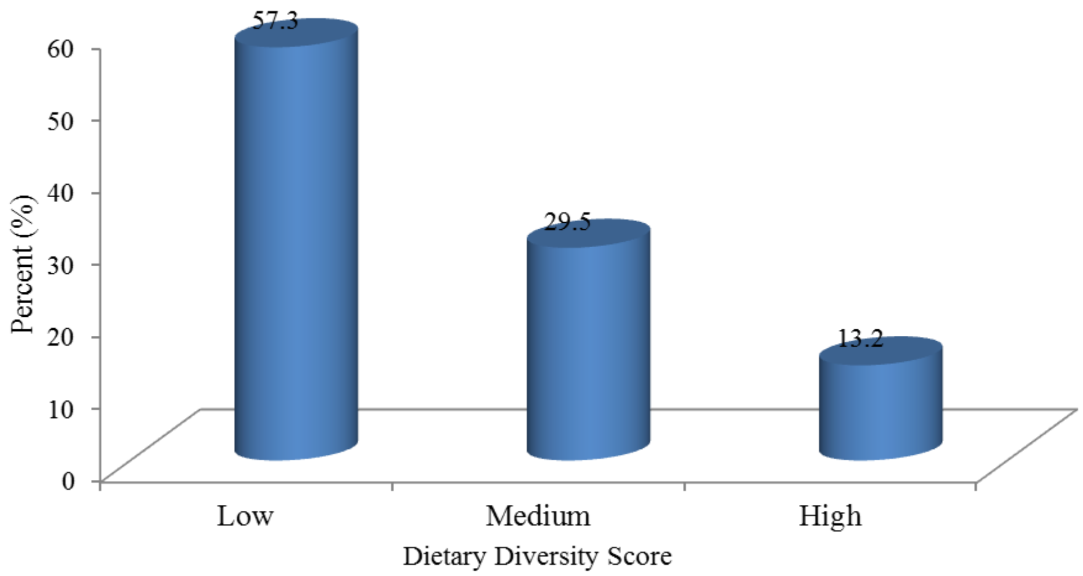

Figure 1: Proportion of children aged 6-23 months with low, medium and high dietary diversity scores

Almost all of the children (95.4%, n=353) consumed starchy staple foods. Children who consumed fruits and vegetables were 70% (n=259) while those who consumed vitamin A-rich fruits and vegetables were 55.4% (n=205). Other food groups were rarely consumed by the study children (Figure 2). All the study children were still being breastfed at the time of the study. Although children seemed to consume high amount of fruits and vegetables, these foods were seasonal because the baseline survey took place during the rainy season, when fruits and vegetables were plenty. Intake of fruits and vegetables is important because they provide various vitamins, minerals and phytochemicals that are essential for healthy [30]. While some nutrients are commonly found in many fruits and vegetables, e.g. pro-vitamin A, vitamin C and potassium, there can be vast differences in their overall nutrient content [31]. Consumption of a variety of fruits and vegetables increases the likelihood of children getting large amount of nutrients.

High consumption of starchy staples and fruits and vegetables might be due to easy availability and affordability of these food groups during that season [15]. Studies conducted in Cambodia [32] and Ghana [3] also reported high consumption of cereals/grains and fruits and vegetables among children of 6-23 months old. Animal products were rarely consumed by children involved in this study. Similarly, results from Ethiopia [19] and Kenya [21] showed that, children of 6-23 months old consumed animal products rarely. Animal based foods are good sources of nutrients that are required for growth, and also of micronutrients that support the immune system [33]. In most of the developing countries including Tanzania, animal foods are rarely consumed [30]. The reasons might be due to lack of knowledge, low economic income and high cost of these products because even the livestock-keepers who raised the animals sold them out and did not consume the animals [21].

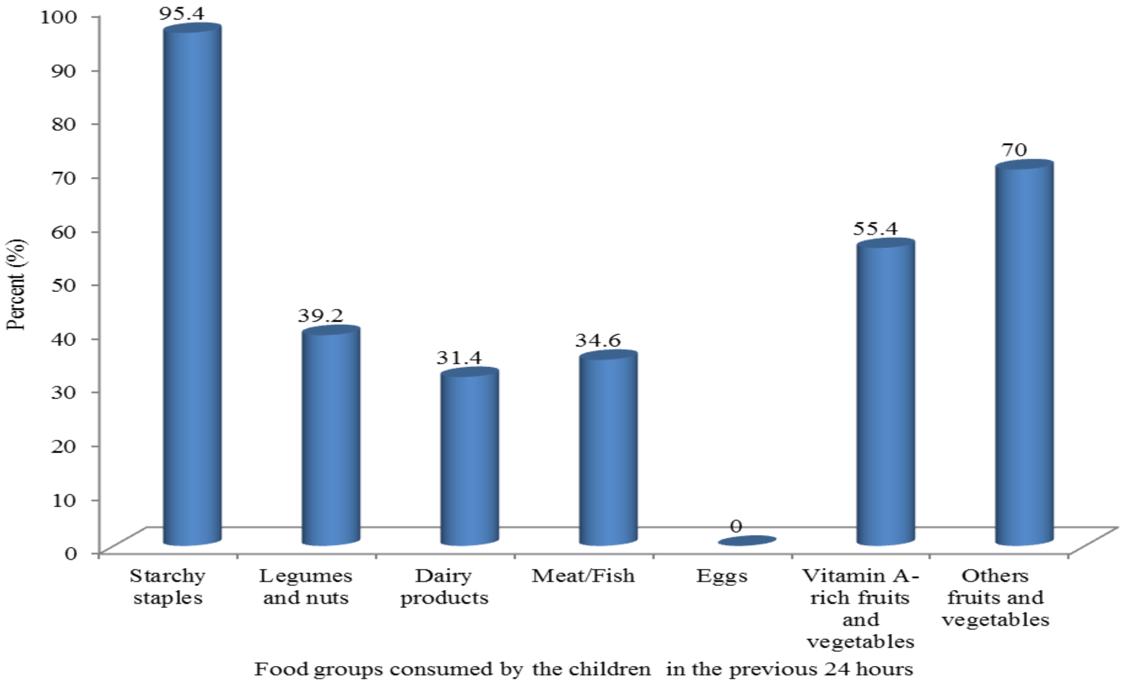

Figure 2: Proportion of children aged 6-23 months who consumed different food groups in the previous 24-hrs

Table 2 shows the mean Z-scores for dietary diversity, weight for age, height for age, weight for height. The mean dietary diversity scores of the children were 3.25 ± 1.2 groups per day and mean z-scores were -0.86±1.24 SD (weight for age), -1.58±1.500 SD (height for age) and 0.03±1.14 SD (weight for height). Statistical significance ($p<0.05$) was observed between the mean weight and the mean age in males and females. Prevalence of malnutrition among the children aged 6-23 months was high. Underweight was 32.4, stunting was 57.3 and wasting 8.9 percent (Table 3). Underweight and stunting was significantly high ($p<0.05$) among children. These prevalence rates were higher than those reported in the 2015-2016 Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) and Tanzania National Nutrition Survey of 2014 [19, 34]. High rate of malnutrition (underweight, stunting and wasting) among children might be due to poor nutrition, which could be attributed to lack of dietary diversity [12].

Prevalence of underweight, stunting and wasting among the children with respect to dietary diversity is presented in Table 4. Most children with low dietary diversity scores (≤3 food groups) were at high risk of becoming malnourished compared to those with medium (4 food groups) and high (≥5 food groups) dietary diversity scores. These results concurred with those reported in Iran and India, and Ethiopia in which children who were fed with non-diversified diet were more likely to have poor anthropometric status than those who were fed diversified diet by [2, 28]. This is because diversified food intake reflects higher dietary quality and greater possibility of meeting daily energy and nutrient requirements of young children [9].

The association of low dietary diversity and the anthropometric attributes is summarized in Table 5. Low dietary diversity among the 6-23 months children was strongly associated with stunting and underweight. A study conducted in Nigeria showed similar findings

[35]. Previous studies in Ethiopia and Indonesia found that, low dietary diversity was associated with stunting [7, 36] while a study from Kenya South Ethiopia and Indonesia revealed that, low dietary diversity was associated with underweight and stunting among young children [7, 21 and 37]. This is because diversifying diets ensures good quality nutrient intake and hence supporting growth and development of young children [18]. Our study results further revealed strong association between low dietary diversity and the area of the area of geographical location of the children. Similar studies conducted in Philippine, Southern Beninin, Southern Ethiopia and Ghana observed significant association between low diet diversity and geographical location among the young children [5, 9, 12 and 38]. Living in rural areas decreased the likelihood for diversified diets compared to their urban counterparts, due to the fact that, family incomes and education are better in urban than rural districts [30]. The findings further revealed that; large family size of four children and above could be the cause for low dietary diversity among the children. Previous studies conducted in Ethiopia and Southern Benin also documented similar findings [5, 39]. Family income also influenced dietary diversification among the children. Families with low income are less likely to diversify diets. Thus, children consuming less diverse diets are less likely to meet their nutrient needs than those who consume more diverse diets [1].

Conclusions and Recommendations

Based on findings of this study, majority of the children had low dietary diversity and poor anthropometric status, in which most of them had low weight for age, height for age and weight for height. The most common food group consumed by almost all children was starchy staples. A strong association exists between low dietary diversity and underweight, stunting. Rural residence and family size were the other factors that were strongly associated with low dietary diversity. These findings suggest that; there is a need for interventions to combat under-nutrition particularly by promoting consumption of diverse of food groups that are locally available and affordable.

Acknowledgement

The authors are grateful to the Government of Canada which through the Global Affairs Canada (GAC) (#107790), International Development Research Centre (IDRC) and Canadian International Food Security Research Fund (CIFSRF) Grant; (Phase 2) which provided financial support to undertake this study.

Table 1: Socio-economic and demographic characteristics of the studied households

Parameter	n	%
Sex of the child		
Male	229	61.90
Female	141	38.10
Total	**370**	**100.0**
Residence		
Rural	298	80.50
Urban	72	19.50
Total	**370**	**100.0**
Marital status		
Single	4	1.10
Married	366	98.90
Total	**370**	**100.0**
Family size		
<4	110	29.70
≥4	260	70.30
Total	**370**	**100.0**
Occupation of mother/ Caregiver		
Housewife	328	88.60
Employed for wage	32	8.70
Self employed	10	2.70
Total	**370**	**100.0**
Education level of mother/caregiver		
Informal education	35	9.40
Primary school	253	68.40
Secondary school	67	18.10
College/University	15	4.10
Total	**370**	**100.0**

Table 2: Anthropometric status and dietary diversity score of the children aged 6-23 months

Parameter	Male		Female		Total		p-value
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age (mo)	13.51	4.96	13.99	4.85	13.41	5.03	0.49
Weight (kg)	9.47	1.53	9.06	1.57	9.26	1.56	0.01**
Length (cm)	74.7	6.81	74.02	6.56	74.30	6.77	0.325
W/A	-0.75	1.26	-1.04	1.20	-0.86	1.24	0.01**
L/A	-1.51	1.49	-1.70	1.51	-1.58	1.50	0.12
W/H	0.09	1.16	-0.07	1.09	0.03	1.14	0.16
DDS	3.32	1.25	3.17	1.17	3.25	1.20	0.13

W/A=Weight for age; L/A=Length for age, W/H=Weight for height, DDS=Dietary diversity score; SD=standard deviation ** = Statistical significance at p<0.05

Table 3: Prevalence of stunting, underweight and wasting among the studied children (n=370)

Anthropometric indicators	Z-score	Male		Female		All		p-value
		n	**%**	**n**	**%**	**n**	**%**	
Underweight	-3<Z-score<-2 and Z-score<-3	65	28.38	55	39.01	120	32.43	0.01**
Normal	-2<Z-score<+2	164	71.62	86	60.99	250	67.57	
Total		**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	
Stunting	3<Z-score<-2 and Z-score<-3	126	55.02	86	60.99	212	57.3	0.00**
Normal	-2<Z-score<+ 2	103	44.98	55	39.01	158	42.7	
Total		**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	
Wasting	-3<Z-score<-2 and Z-score<-3	18	7.86	15	10.64	33	8.92	0.33
Normal	-2<Z-score<+ 2	211	92.14	126	89.36	337	91.01	
Total		**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	

** = Significant different at p<0.05

Table 4: Prevalence of wasting, stunting and underweight with low, medium and high dietary diversity among 6-23 months children (n=370)

Anthropo metrics attributes	**Low DDS**						**Medium DDS**						**High DDS**					
	M		**F**		**Total**		**M**		**F**		**Total**		**M**		**F**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Under-weight	40	17.50	31	21.99	71	19.19	17	7.42	18	12.77	35	9.46	8	3.49	6	4.26	14	3.77
Normal	189	82.53	110	78.01	299	80.81	212	92.58	123	87.23	335	90.54	221	96.51	135	95.74	356	96.23
Total	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**
Stunting	76	33.19	46	32.62	122	32.97	37	16.16	29	20.57	66	17.84	13	5.68	11	7.80	24	6.49
Normal	153	66.81	95	67.38	248	67.03	192	83.84	112	79.43	304	82.16	216	94.32	130	92.20	346	93.51
Total	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**
Wasting	15	6.55	7	4.96	22	5.95	2	0.87	5	3.55	7	1.89	1	0.44	3	2.13	4	1.08
Normal	214	93.45	134	95.04	348	94.05	227	99.13	136	96.45	363	98.11	228	99.56	138	97.87	366	98.92
Total	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	**229**	**100.0**	**141**	**100.0**	**370**	**100.0**	**229**	**100.00**	**141**	**100.0**	**370**	**100.0**

M = Male; F = Female; DDS = Dietary diversity score

Table 5: Association between low dietary diversity and anthropometric attributes

Parameter	B	S.E.	Wald	df	p-value	Exp(B)
Education	0.356	0.274	1.691	1	0.193	1.427
Family income	0.711	0.326	4.753	1	0.029**	2.036
Marital	1.150	1.176	0.957	1	0.328	3.159
Family size	-1.743	0.377	21.385	1	0.000**	0.175
WAZ	-0.654	0.309	4.470	1	0.035**	0.520
HAZ	-0.627	0.449	1.947	1	0.033**	0.534
WHZ	-0.045	0.371	0.015	1	0.904	0.956
Geographical location	-1.628	0.313	2.723	1	0.022**	0.517
Constant	-1.011	2.429	0.173	1	0.677	0.364

Dependent variables- anthropometric attributes (WAZ, HAZ, WHZ), family income, education, marital and family size; ** = significance difference at $p<0.05$
Independent variable- Low dietary diversity

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PAPER FIVE

Influence of vitamin A status on the anthropometric attributes of children aged 6-36 months in Manyara and Shinyanga regions, Tanzania

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(*Published in the Tanzania Journal of Agriculture Sciences* (2017) 16(1): 25 – 35.

Abstract

Vitamin A deficiency remains one of the health challenge causing morbidity and mortality among children below five years in Tanzania. This study was conducted to determine the influence of vitamin A status on the anthropometric attributes of the children aged 6-36 months living in Manyara and Shinyanga regions, Tanzania. Purposive and random sampling techniques were used to obtain a sample of 462 children aged 6-36 months. Data were analyzed using IBM SPSS version 20.0 and ENA for SMART. Results showed that, the average prevalence of vitamin A deficiency among 6-36 months old children was 69.5% (n=321). Prevalence of vitamin A deficiency among children aged 6-36 months by regions was 69.3% (n=303) in Manyara and 69.8% (n=159) in Shinyanga. Prevalence of vitamin A deficiency among non-breastfed children was 75% (n=117) while among breastfed children was 66.0% (n=203). Vitamin A deficiency was therefore significantly higher among the non-breastfed children than their breastfed peers. Prevalence of wasting (weight-for-height z-score) among the children aged 6-36 months was 6.9% (n=32), stunting (height-for-age z-score) was 51% (n=236) and underweight (weight for age z-score) was 25.5% (n=118). Vitamin A status has influence on the anthropometric attributes; however statistical significance was observed only on age and stunting. It is concluded from this study that, prevalence of vitamin A deficiency among 6-36 months old children was higher than the national average. It was significantly higher in the non-breastfed children than their breastfed peers. Also, children with VAD suffered from one or more forms of under-nutrition (wasting, stunting and underweight). Stunting and age of the children were the strong predictors of vitamin A status. These findings call for coordinated and sustainable intervention programs to reduce vitamin A deficiency and under-nutrition to optimize growth among children aged 6-36 months.

Key words: Vitamin A Deficiency, anthropometric attributes and children aged 6-36 months,

Introduction

Vitamin A Deficiency (VAD) remains a serious public health problem in many parts of the world today (Jemberu *et al.,* 2017). It is more prevalent in low and middle income countries including Tanzania (Laillou *et al.,* 2013). Vitamin A makes significant contribution to childhood morbidity and mortality (FAO and WHO, 2013). In Tanzania for instance, more than 33% of children aged 6-59 months suffer from vitamin A deficiency (NBS, 2011). More 27 000 infant deaths per annum in Tanzania are due to micronutrients deficiencies including vitamin A (Noor *et al.,* 2017). Demographic and Healthy Survey (2010) report showed that a third of children in Tanzania aged 6-59 months suffer from vitamin A deficiency (NBS, 2011). Causes of VAD are multi-factorial; however, the main underlying cause is poor diet, which is chronically supplies insufficient amount of vitamin A (Ndau *et al.,* 2016). VAD is not clinically identifiable until late stages, which lasts for a long period of time leading to many physiological consequences (WHO, 2009). Deficiency of vitamin A during childhood affects growth, immune competence, physical and cognitive development (Marasinghe *et al.,* 2015).

Before 1996, prevalence rate of stunting, underweight and wasting among children below five years in Tanzania was very high (TDHS-MIS, 2016). However, from 1996-2005 prevalence rates of stunting, underweight and wasting dropped from 50 to 44 percent, 27 to 17 percent and 9 to 3.5 percent, respectively. Between 2005 and 2015 prevalence rates of stunting and underweight continued to drop to 34 and 14 percent, respectively, however the prevalence rate of wasting has increased from 3.5 to 5 percent (TDHS-MIS, 2016). From 1996, to date there has been a great improvement in the anthropometric status of children below five years; however, some children are still suffering from one or more forms of under-nutrition namely stunting, underweight, wasting or specific nutrient deficiencies e.g. iron, iodine and vitamin A deficiencies (FACT, 2016).

Potential interventions to combat under-nutrition and micronutrient deficiencies among children below five years in Tanzania include supplementation, dietary diversity, nutrition education, vaccination, fortification and scaling up of breast feeding (TFNC, 2014). Combination of the various interventions against micronutrient deficiencies would accelerate survival, well-being, growth and development of the children below five years (FAO and WHO, 2013). It has been reported that improving micronutrient status such as reducing vitamin A deficiency among children below five years would significantly reduce the risk of mortality by 25-35% (Gaurav *et al.*, 2014). The prevalence rate of VAD in Manyara region ranged between 31-39% while in Shinyanga region the prevalence ranged between 39-50% (NBS, 2011). Therefore, as part of the effort to combat VAD in Tanzania, this study was designed to evaluate the influence of vitamin A status on the anthropometric attributes of children aged 6-36 months in Manyara and Shinyanga regions, Tanzania.

Materials and Methods

Study Design and Area

A cross sectional design, was conducted in Babati urban, Babati rural, Hanang and Mbulu districts of Manyara region and Shinyanga urban, Shinyanga rural, Kahama and Kishapu districts of Shinyanga region in June to July 2015 and November to December 2016. These regions were selected due to high prevalence of vitamin A deficiency among children below five years and lactating mothers (NBS, 2011). Shinyanga region is located in North-west of Tanzanian at latitude 3.3695^0 S and longitude 34.1532^0 E. According to the 2012 National Population Census (NBS, 2012), Shinyanga region had a population of 1 534 808 people of whom 750 841 were males while 783 967 were females. This region covers an area of 50 781 km^2. Manyara region is located in Northern part of Tanzania at latitude 4.3150^0 S and longitude 36.9541^0 E. According to the 2012 National Population Census (NBS, 2012) Manyara region had a population of 1 425 131 people out of whom 717 085 were males while 708 046 were females. Manyara region covers an area of 46 359 km^2 (NBS, 2012).

Study Population, Sampling Technique and Sample Size

The study population involved children (both males and females) aged 6-36 months old were selected from low, middle and high income families. The children were receiving post-natal monthly health care at Maternal and Reproductive Health clinics (RHC). Respondents were mothers/caregivers who consented to participate in this study. Children with chronic diseases such as type 1 diabetes mellitus, HIV, inborn errors of metabolism, e.g. phenylketonuria (PKU) and those who were sick during the study were excluded from the study. Subjects were selected randomly by using tables of random numbers assigned to mothers/caregivers attending the monthly growth monitoring clinics. Sample size was determined by calculation of statistical power using a WHO (1991) formula $N=t^2 *(p \times q)/d^2$ whereby N was the sample size, p=the expected prevalence rate of VAD among children aged 6-36 months in the study areas (0.378), t=the margin of error (1.96), q=1-p (0.9); d=level of precision (0.05). Using this equation, a sample size of 462 children (225 from Shinyanga and 237 from Manyara region) were recruited for the study. Out of these, 249 (53.9%) were males while 213 (46.1%) were females. The sample size was distributed according to the population in the study areas.

Data Collection

A questionnaire used from GAIN was adopted for use in this study to obtain socio-economic and demographic information of mothers/caregivers of the studied children. The questionnaire was pre-tested among 10 lactating mothers/caregivers in Morogoro urban and rural districts, Tanzania. Villages used for pre-testing the questionnaires were Bigwa, Kichangani and Mazimbu in Morogoro Urban and Mgeta and Mlali in Morogoro rural districts. Necessary adjustments were made on the questionnaire after pre-testing. Ten enumerators were trained before administration of the questionnaire.

They were taught on how to ask questions, expected answers, probing to reaffirm the responses, how to record the answers and also how to take anthropometric measurements and conducting finger prick to collect blood samples and preparation of dry blood samples (DBS). Administration of the question was done by home visit during the morning hours of the day. The questionnaire was administered to the respondents through face-to-face interviews and the enumerators recorded the responses.

Method of collecting blood sample

Determination of serum retinol

Blood samples were drawn from the under-five children by finger prick method using safety lancets. About 25 µL of blood were taken by a micropipette and dispensed into a protein saver papers (903 Protein Saver Card, Whatman International, UK). After drying, the protein saver papers with blood samples were packaged in airtight aluminum bags in which three pieces of desiccators were added to keep the blood sample dry (DBS). DBS were stored under ambient conditions before transportation to Tanzania Food and Nutrition Center (TFNC) laboratory for analysis. Retinol in the DBS was determined by enzyme-linked immunosorbent assay (ELISA) method (WHO, 2011). Children with 0.70

μmol/l or below serum retinol concentration or <17.325 μg/kg Retinol Binding Protein (RBP) were considered to be deficient in vitamin A (WHO, 2011).

Anthropometric (weight and length/height) measurements

Body weights were measured by using SECA electronic scale (Model-SECA 874 Hamburg Germany). The scale was adjusted to zero before taking measurements. Children were weighed bare feet with only light clothes on. Children younger than 24 months were weighed after having the mother/caregiver stand on the scale, reset the scale to zero, then have the mother/caregiver hold the child and the child's weight was read. Measurement was taken three times and the average weight recorded to the nearest 0.1kg. Children aged 24 to 36 months were weighed while standing on the digital scale.

Heights were measured by using a length board (Manufactured by UNICEF). Children younger than 24 months were measured while lying down without shoes on. The child was placed on his/her back at the center of the length board such that the child was lying straight with shoulders and buttocks flat against the measuring surface and child's eyes looking straight up. Both legs were fully extended with the toes pointing upward and the feet kept flat against the foot-piece. Recumbent length of the child was measured three times and the average recorded to the nearest 0.1 cm. For children over 24 months, they were allowed to stand against the length board bare feet, with legs straight, eyes of the subjects looked straight ahead and the line of sight was in level with the surface, hands pointed vertically downwards against the body, while the head, shoulder blades and buttocks touched against the wall. Then the headpiece was slid down to touch the crown of the head. The body height was thereafter read parallel with the headpiece three times and the average height recorded to the nearest 0.1 cm. Z–Scores of weight for age, length for age and weight for length were calculated following WHO (2006) recommendation. Children with -2 < SD < +2 values were classified as normal (WHO, 2006).

Data Analysis

Data were analyzed by STATA program version 13 and SPSS version 20. Means, frequencies and percentages were obtained using descriptive analysis. The mean values of Vitamin A of children in the two regions were compared by using t-tests. The strength of association between VAD and the anthropometric attributes was determined by binary logistic regression analysis in which VAD was taken as a dependent variable that was influenced by various anthropometric attributes (independent variables). Measures of anthropometric status (WAZ, HAZ and WHZ) were analyzed by using ENA for SMART program. Statistical significance was set at p-value of 0.05.

Ethical Clearance and Research Permit

This study was approved by the National Institute for Medical Research (NIMR) (Tanzania) and the University of Waterloo (Canada) ethics committees for research on human subjects. Permission was also obtained from the regional and district health authorities to conduct the study in the selected districts. All mothers/caregivers who agreed to participate in this study signed an informed consent form to affirm their willingness to allow their children to participate in the study. Respondents had the liberty to decline participation in the study at any time.

Results

Table 1 summarizes the socio-economic and demographic characteristics of the mothers/caregivers of the studied children. Majority of the mothers/caregivers (69.3%, n=320) were housewives while only small proportion of the mothers/caregivers (4.3%, n=20) were employed for wage. Majority of the mothers/caregivers had attained only primary school education level (84.2%, n=389) and only few mothers/caregivers had attained secondary school education level (4.3%, n=20). None of the mothers/caregivers had attained College/University or vocational training level and a handful of the mothers/caregivers (11.5%, n=53) had no formal education. Formal education is essential for mothers/caregivers not only in understanding nutrition information but also in opening up chances for employment and other entrepreneurial skills. Majority of the mothers/caregivers in the study were young (64%), between the ages of 20-34 years. About 24% of the mothers/caregivers were teenagers while few mothers/caregivers (12%) were above the age of 35 years. About 71% of mothers/caregivers in the study had a parity of 1-4 births, while 23% of them had a parity of 5-10 births. The high maternal parity was reflected in the family sizes, in which most households (48%) had family size of 3-4 people. These findings were in agreement with the data reported in the National 2012 Population and Housing census, in which the average family size in Manyara and Shinyanga regions was four (URT, 2012). Majority of the mothers/caregivers involved in the study (77.1%) were married and only a few were single (13%), widowed (6.0%) and divorced (3.9%).

Table 2 shows the characteristics of the studied children. The mean age of the children was 19.5±10.149 months, mean height-for-age Z-score was -0.65±1.26 Standard Deviation (SD), mean height for age was -1.41±1.71 SD, mean weight-for-height was 0.14±1.21 SD while mean serum retinol concentration was 14.87±5.40 µg/ml. Table 3 summarizes the prevalence of VAD among children aged 6-36 months in the study regions. The proportion of children with VAD (69.5%, n=321) was significantly higher (P=0.00) than children without VAD (30.5%, n=141). More than 69.5% (n=321) children had serum retinol binding protein less than the cut-off of 17.3256 µg/ml which implied that these children were vitamin A deficient. Very few children (30.5%, n=141) had adequate vitamin A levels of 17.3256 µg/ml and above. Out of the 69.5% (n=321) children who were vitamin A deficient, 34.1 (n=167) were males while 31.4% (n=154) were females. The proportion of males with VAD in Manyara region (71.5%, n=118) was significantly = higher (p=0.00) than that of their female counterparts (66.7%, n=92). Conversely, more females had VAD in Shinyanga region (76.0%, n=57) compared to their male counterparts (64.3%, n=54) (p>0.05). Overall, more males (n=249) had VAD compared to females (n=213). Prevalence of VAD in Manyara region was 69.3% (n=210) while in Shinyanga region the prevalence was 69.8% (n=111).

Table 4 shows prevalence of VAD among breastfed and non-breastfed children involved in the study. Prevalence of VAD among breastfed children was 66.1% (n=203) while for the non-breastfed children the VAD prevalence was 75.5% (n=117). This implied that, VAD was significantly higher (p=0.00) among the non-breastfed children than among their breastfed peers. Table 5 shows the prevalence of wasting, underweight and stunting among the studied children. Prevalence of underweight among the 6-36 months old children was 25.5% (n=118), stunting was 51.1% (n=236) while prevalence of wasting was 6.9% (n=32). Prevalence of underweight was slightly higher (p>0.05) among males (26.5%, n=66) than females (24.4%, n=52). Conversely, slightly more females (p>0.05) were stunted (51.3%, n=110) compared to their male counterparts (50.6%, n=126). Likewise, slightly more females (p>0.05) were wasted (7.0%, n=15) compared to the males peers (6.8%, n=17). Table 6 shows the prevalence of VAD among underweight, stunted and wasted children (n=462). Most of the children (both males and females) who were underweight, stunted and wasted were also deficient in vitamin A. The proportion of underweight and stunted males who had VAD were significantly higher (p=0.00) compared to that of females, however, the proportion of wasted males with VAD was similar ($p>0.05$) to that of the females. Table 7 indicates the prevalence of VAD among breastfed and non-breastfed children who were also underweight, stunted and wasted (n=462). The proportion of underweight males and females with VAD was significantly higher (p=0.00) among the non-breastfed than the breastfed children. Likewise, the proportion of stunted males and females with VAD was significantly higher (p=0.00) among the non-breastfed children than among the breastfed peers. A similar trend was also observed for the wasted children.

Table 8 data show the Standardized Beta-coefficients and level of significance of anthropometric attributes predicting VAD among children aged 6-36 months (n=462) Among the determinants breastfed/non-breastfed, dietary diversity, geographical location, family income, underweight stunting and wasting (p=0.03, p=0.00, p=0.03, p=0.01, p=0.01 and p=0.02, respectively) were strong predictors of VAD among children aged 6-36 months.

Discussion

The findings in this study revealed that, prevalence of vitamin A deficiency among children aged 6-36 months were very high in Manyara (69.3%) and Shinyanga (69.8%) regions (Table 2). These prevalence rates were higher than the national average of 47.6% in Manyara and 41.5% in Shinyanga region (NBS, 2011). One possible reason could be the declining coverage of vitamin A mega dose supplementation during the survey. According to NBS (2011) and TFNC (2014) coverage of vitamin A mega-dose supplementation was at times very low in remote districts. Coverage of vitamin A mega-dose supplementation in Manyara has been reported at 41.8% while in Shinyanga the coverage was 71.1%. The rate of vitamin A deficiency among children was higher among boys than among girls. The findings in this study are in line with the Tanzania

Demographic and Health Survey (2010) in which high rate of vitamin A deficiency was observed among boys (NBS, 2011). Similar findings were reported by Tariku *et al.* (2016) whereby more boys were affected by vitamin A deficiency than girls. The high prevalence of VAD could be attributed to several factors such as low maternal intake of vitamin A rich foods during pregnancy and lactation (Ulak *et al.,* 2016), poverty (Makaka, 2016), inappropriate feeding behaviors and low dietary diversity (Zhao *et al.,* 2016), lifestyles and increase in age-associated physiological demands for vitamin A especially for boys (Schwinger *et al.,* 2016). The findings further revealed that, vitamin A deficiency in children was significantly higher among non-breastfed than among breastfed children (Table 4).

Therefore, older non-breastfed children were at a higher risk of becoming vitamin A deficient compared to their younger breastfed peers. A study in Iran showed similar results in which prevalence of VAD among non-breastfed children was higher compared to the breastfed groups (Bahreynian *et al.,* 2017). High prevalence of vitamin A deficiency among non-breastfed children could be a result of limited intake of vitamin A from mothers' breast milk (Kuchenbecker *et al.,* 2015). Breastfed children usually get vitamin A from two sources, first from their mothers' milk and second from their normal diets. For this reason, breastfed children are likely to get sufficient amount of vitamin A since human milk is a good source of vitamin A unless the mother has low serum retinol concentration (Ulak *et al.,* 2016). The prevalence rate of underweight and stunting among the children aged 6-36 months was higher than wasting; however, males were more affected than females in all forms of under-nutrition (Table 5). This is probably due to the fact that, this age group is the turning point for increased energy and micronutrient requirements to support their rapid growth and development of males (WHO, 2011). These results are in agreement with the TFNC (2014) which reported low prevalence rate of wasting than stunting and underweight among children, yet more males were wasted compared to females.

Also, the findings in this study are in agreement with Abdelaziz *et al.* (2015) who found high prevalence of stunting and underweight than wasting in children and more males were stunted and underweight compared to the female counterparts. Possible reason for predominant under-nutrition among males than among females could be due to the geographical differences, poverty (Zhao *et al.*, 2016), inappropriate feeding practices and low dietary diversity (Asfaw *et al.*, 2015).

Prevalence of VAD among children with under-nutrition (wasting, underweight and stunting) is shown in Table 5. Most of the children who had VAD also suffered from one or more forms of under-nutrition, however, children who were underweight and stunted were more susceptible to VAD. Prevalence of VAD among breastfed and non-breastfed children with wasting, stunting and underweight is shown in (Table 7). Stunting was significantly higher among the non-breastfed children with VAD.

In this study, children with VAD had a higher risk of being stunted than being underweight or wasted. The findings in this study concurred with results of a study by Marasinghe *et al*. (2015) and Tariku *et al*. (2016) who found that VAD in children was associated with stunting because VAD adversely affects linear growth of children. This connotes that; vitamin A plays a great role in linear growth of children under the age of five years (Tariku *et al*., 2016). Our findings are also comparable with those reported Maslova *et al*. (2009) who found that plasma retinol concentrations were negatively associated with child's age implying that as the child grows the physiological requirements for retinol also increase.

The strength of association between VAD and anthropometric attributes of 6 – 36 month old children was determined by binary logistic regression analysis in which VAD was taken as a dependent variable that was influenced by various anthropometric attributes (independent variables) (Table 8). Breastfed/non-breastfed, dietary diversity, geographical location, family income, underweight stunting and wasting were the main factors which influenced VAD. This could be explained by the reason that, as the child grows, when and under-nutrition conditions (underweight, stunting and wasting) co-exist is also very likely to be vitamin A deficient. As children grow older they also stop breastfeeding and thus no longer receive vitamin A from their mothers. Also, children who do not meet minimally diverse diets per day are more likely to become deficiency in vitamin and other micronutrient deficiencies. Additionally, living in urban areas increases the like hood for diversifying diets compared to those in rural areas. Likewise, children from normal/high income families are more likely to consume vitamin A rich foods and diversify their diets compared to the low income families. Findings of this study were in agreement with those reported by Marasinghe *et al.* (2015) in which VAD in children was strongly associated with stunting because VAD negatively affects change in height (linear growth). A study by Tariku *et al.*(2016) observed positive association between VAD and anthropometric attributes (underweight, stunting and wasting in Ethiopia.

Conclusions and Recommendations

In this study, prevalence of vitamin A deficiency among children was significantly higher than the national average. VAD was significantly higher among non-breastfed than among breastfed children and also higher among males than among females because males expend their vitamin A stores rapidly due to their high physiologic needs compared to the females. Most of the children with vitamin A deficiency also suffered from one or more forms of under-nutrition (underweight, stunting and/or wasting). Vitamin A status was strongly associated with age of the child and stunting condition. Age and height-for-age status of the child were therefore good anthropometric attributes for predicting vitamin A status of children aged 6–36 months. These findings call for coordinated and sustainable intervention programs to reduce under-nutrition and micronutrient deficiencies such as vitamin A deficiency to optimize growth among children age 6–36 months.

Acknowledgements

The authors are grateful to the Government of Canada, Global Affairs Canada (GAC), International Development Research Centre (IDRC) and the Canadian International Food Security Research Fund (CIFSRF) who provided financial support (Grant #107790, Phase 2) to undertake this study.

List of Tables

Table 1: Socio-economic and demographic characteristics of the mothers/caregivers (n=462)

Parameter	No. of Respondents	Percent
Occupation		
Petty traders	62	13.40
Housewives	320	69.30
Employed for wage	20	4.30
Farmers	60	12.98
Educational level		
Uneducated/Informal	53	11.50
Primary	389	84.20
Secondary	20	4.30
College/Vocation	0	0.00
Age (years)		
≤19	111	24.00
20-34	296	64.00
≥35	55	12.00
Maternal parity		
1-4	328	71.00
5-10	106	23.00
>10	28	6.00
Marital Status		
Single	60	13.00
Married	356	77.10
Widowed	28	6.00
Divorced	18	3.90
Family size		
1-2	83	18.00
3-4	222	48.00
5-6	129	28.00
>6	28	6.00

Table 2: Characteristics of the studied children (n=462)

Parameter	Mean	Standard Deviation
Age (months)	19.5	10.15
WAZ Score	-0.65	1.26
HAZ Score	-1.41	1.71
WHZ Score	0.14	1.21
Serum retinol (µg/mL)	14.87	5.40

WAZ = weight for height Z-score; WAZ = weight for age Z-score; HAZ = height for age Z-score

Table 3: Prevalence of VAD among children aged 6-36 months (n=462)

Children in Region	**Vit A Cut-off (µg/mL)**	**Boys**		**Girls**		**Total**		**P - value**
		n	**%**	**n**	**%**	**n**	**%**	
Manyara region								
With VAD	<17.325	118	71.52	92	66.67	210	69.3	0.00**
Without VAD	≥ 17.325	47	28.48	46	33.33	93	30.7	
Total		**165**	**100.0**	**138**	**100.0**	**303**	**100.0**	
Shinyanga region								
With VAD	<17.325	54	64.29	57	76	111	69.8	0.00**
Without VAD	≥ 17.325	30	35.71	18	24	48	30.2	
Total		**84**	**100.0**	**75**	**100.0**	**159**	**100.0**	
All regions								
With VAD	<17.325	167	67.07	154	72.3	321	69.5	0.00**
Without VAD	≥ 17.325	82	32.93	59	27.7	141	30.5	
Total		**249**	**100.0**	**213**	**100.0**	**462**	**100.0**	

VAD - Vitamin A Deficiency; ** Significant difference at p ≤ 0.05

Table 4: Vitamin A deficiency among breastfed and non-breastfed children involved in the study (n=462)

Children	**Vitamin A Cut-off (µg/mL)**	**Male**		**Female**		**All**		**P-value**
		n	**%**	**n**	**%**	**n**	**%**	
BF-VAD	<17.325	103	63.19	100	69.44	203	66.12	0.61
BF- Normal	≥ 17.325	60	36.81	44	30.56	104	33.88	
All BF		**163**	**100.0**	**144**	**100.0**	**307**	**100.0**	
NBF - VAD	<17.325	63	73.26	54	78.26	117	75.48	0.00**
NBF-Normal	≥ 17.325	23	26.74	15	21.74	38	24.52	
All NBF		**86**	**100.0**	**69**	**100.0**	**155**	**100.0**	

BF=breastfed; NBF = non-breastfed; BF-VAD= breastfed vitamin A deficiency; NBF – VAD = non-breastfed vitamin A deficiency, **Significant difference at p ≤ 0.05

Table 5: Prevalence of wasting, underweight and stunting among children aged 6-36 months (n=462)

Anthropometric attribute	**Z-score (SD)**	**Male**		**Female**		**Total**		**p - value**
		n	**%**	**n**	**%**	**n**	**%**	
Underweight (WAZ)	≥-3 < SD < -2	66	26.50	52	24.41	118	25.54	0.00**
Normal (WAZ)	-2 < SD <+2	183	73.50	161	75.59	344	74.46	
Total		**249**	**100.0**	**213**	**100.0**	**462**	**100.0**	
Stunting (HAZ)	≥-3 < SD < -2	126	50.60	110	51.37	236	51.08	0.00**
Normal (HAZ)	-2 < SD < + 2	123	49.40	103	48.63	226	48.92	
Total		**249**	**100.0**	**213**	**100.0**	**462**	**100.0**	
Wasting (WHZ)	≥-3 < SD < -2	17	6.80	15	7.04	32	6.93	0.17
Normal (WHZ)	-2 < SD < + 2	232	93.20	198	92.96	430	93.07	
Total		**249**	**100.0**	**213**	**100.0**	**462**	**100.0**	

** Significant difference at p ≤ 0.05; WAZ = weight for height Z-score; WAZ = weight for age Z-score; HAZ = height for age Z-score; SD = Standard deviation

Table 6: Prevalence of VAD among underweight, stunted and wasted children (n=462)

Anthropometric attributes	**<17.325 µg/mL**				**≥ 17.325 µg/mL**				**Total**		**P-value**
	Male		**Female**		**Male**		**Female**				
	n	%	n	%	n	%	n	%	n	%	
Underweight	47	18.88	37	17.37	19	7.63	15	7.04	118	25.54	0.00**
Normal	202	81.12	176	82.63	230	92.37	198	92.96	344	74.46	
Total	**249**	**100.0**	**213**	**100.0**	**249**	**100.0**	**213**	**100.0**	**462**	**100.0**	
Stunting	83	33.33	74	34.74	43	17.27	36	16.9	236	51.08	0.00**
Normal	166	66.67	139	65.26	206	82.73	177	80.1	226	48.92	
Total	**249**	**100.0**	**213**	**100.0**	**249**	**100.0**	**213**	**100.0**	**462**	**100.0**	
Wasting	11	4.42	11	5.16	6	2.41	4	1.88	32	6.93	0.19
Normal	238	95.58	202	94.84	343	97.59	209	98.12	430	93.07	
Total	**249**	**100.0**	**213**	**100.0**	**249**	**100.0**	**213**	**100.0**	**462**	**100.0**	

With VAD= <17.325 µg/mL; Without VAD ≥ 17.325 µg/mL**= Significant difference at $p<0.05$

Table 7: Prevalence of VAD among breastfed and non-breastfed children with wasting, stunting and underweight (n=307)

Anthropometric attributes	**Breastfed-with VAD**				**Non-Breastfed with VAD**				**Total**		**P - value**
	Male		**Female**		**Male**		**Female**				
	n	%	n	%	n	%	n	%	n	%	
Underweight	28	8.14	26	5.80	7	17.18	4	18.06	65	14.07	0.00**
Normal	135	91.86	118	94.20	79	82.82	65	81.94	397	85.93	
Total	**163**	**100.0**	**144**	**100.0**	**86**	**100.0**	**69**	**100.0**	**462**	**100.0**	
Stunting	49	13.95	41	11.60	12	30.06	8	28.47	110	23.81	0.00**
Normal	114	86.05	103	88.40	74	69.94	61	71.53	352	76.19	
Total	**163**	**100.0**	**144**	**100.0**	**86**	**100.0**	**69**	**100.0**	**462**	**100.0**	
Wasting	9	2.33	9	2.90	2	5.52	2	5.52	22	4.76	0.16
Normal	154	97.67	135	97.10	84	94.48	67	94.48	440	95.24	
Total	**163**	**100.0**	**144**	**100.0**	**86**	**100.0**	**69**	**100.0**	**462**	**100.0**	

VAD = vitamin A deficiency; **Significant difference at $p \leq 0.05$

Table 8: Standardized Beta-coefficients and level of significance of anthropometric attributes predicting VAD among children aged 6-36 months (n=462)

Parameter	**B**	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**
Maternal's education level	0.375	0.265	2.011	1	0.156	1.456
Geographical Location	0.537	0.288	3.487	1	0.032**	1.711
Breastfed/non- breastfed	0.458	0.262	3.049	1	0.031**	1.581
Sex	0.392	0.239	2.693	1	0.101	1.480
WAZ	-.805	0.303	7.058	1	0.008**	0.447
HAZ	0.450	0.181	6.160	1	0.013**	1.569
WHZ	1.296	0.538	5.800	1	0.016**	3.655
DDS	-3.124	0.392	63.582	1	0.000*	0.044
Family size	-0.030	0.276	0.012	1	0.913	0.970
Family income	2.381	0.304	61.194	1	0.000**	10.819
Constant	-4.769	0.786	36.789	1	0.000	0.008

Dependent variable – Vitamin A deficiency
Independent variables - anthropometric attributes - W/A = weight for age, H/A = height for age; W/H - weight for height: Maternal Education level, Geographical Location, breastfed/Non breastfed, Sex, WAZ, HAZ, WHZ, DDS, family income. ** Significant difference at $p \leq 0.05$

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PAPER SIX

Factors influencing vitamin A status of lactating mothers in Manyara and Shinyanga regions, Tanzania

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(Published in the Tanzania Journal of Agricultural Sciences (2016) 15(1): 21-32

Abstract

Vitamin A Deficiency (VAD) is a major public health problem that the world is facing today. It is more prevalent in low income countries including Tanzania. This study was conducted to determine factors influencing Vitamin A status of lactating mothers in selected regions of Manyara and Shinyanga, Tanzania. Purposive and random sampling were used to obtain a sample of 569 lactating mothers categorized in age groups - young age (15-19 years) middle age (20-34 years) and elderly (35-49 years). Knowledge was determined by five points Likert scale. Socio economic and demographic data were analyzed by SPSS program version 20. Results showed that, majority of households (98%) were headed by males. Prevalence of VAD was of public health significance (prevalence >50%) among lactating mothers in all the districts studied. Prevalence of VAD among lactating mothers was 88.5% for young mothers, 84.6% for middle age and 86.3% for elderly mothers. Rural residence had higher prevalence of vitamin A deficiency (63.6%) than urban areas (16.7%). Only 40% of the lactating mothers consumed animal products, 30% consumed yellow/orange fruits while 20% consumed yellow/orange root foods including orange fleshed sweet potatoes. Lactating mothers in this study lacked knowledge about vitamin A. It was concluded that, prevalence of VAD among lactating women was high. Rural residence was the major factor that significantly influenced vitamin A status. To address the vitamin A deficiency problem, nutrition and healthy planners should put more efforts on food fortification especially of edible oil and promote consumption of diverse diets at household level. Also they should educate lactating mothers and the community as a whole on the importance of consuming fortified oil and selection of foods rich in vitamin A.

Key words: Vitamin A status, lactating mothers, socio-economic factors, Tanzania

Introduction

Vitamin A Deficiency (VAD) is a major problem in the world today and highly prevalent in low income countries including Tanzania (Tariku *et al.,* 2016). It is the leading cause of morbidity and mortality in nutritionally vulnerable groups including lactating mothers (FAO and WHO, 2013). Vitamin A is normally found in animal-source foods in preformed form (retinol) (Mbah *et al.,* 2013) and in plant-source foods as pro-vitamin A (Souganidis *et al.,* 2013). The widespread consumption of primarily plant-based diets exacerbate vitamin A deficiency because of the poor bioavailability of pro-vitamin A carotenoids (Mbah *et al.,* 2013). Vitamin A deficiency is mainly caused by consumption of a diet which is persistently insufficient in vitamin A (Aktar *et al.,* 2013).

Lactating mothers are the most susceptible demographic group to vitamin A deficiency, because of the increased requirements for energy and micronutrients during lactation (Henjum *et al.,* 2015). They require enough stores of vitamin A to satisfy their own physiological needs and those of their infants, since infants are born with low vitamin A stores and are dependent on external sources mostly breast milk (WHO, 2011). When lactating mothers become vitamin A deficient the breast-fed infants are also affected by that deficiency (Agne-Djigo *et al.,* 2012).

In Tanzania, micronutrient deficiencies including vitamin A, cost the country over US$ 518 million, which is approximately 2.65% of the country's GDP (World Bank, 2012). Beyond the economic losses, micronutrient deficiencies significantly contribute to maternal mortality, with over 1600 maternal deaths annually (World Bank, 2012). Despite the government efforts to eliminate vitamin A deficiency through various interventions for example; nutrition education and supplementation, prevalence of vitamin A deficiency is still high (Tanzania National Nutrition Survey, 2014).

According to Tanzania Demographic Health Survey 2010, 39% of pregnant women and 35% of lactating mothers were vitamin A deficient (NBS, 2011). To deal with these challenges, the Government of Tanzania launched a nationwide food fortification program in 2013 as one of the measures to prevent micronutrients under-nutrition (URT, 2013). Since this fortification program has been launched in a year 2013, evaluation of public intervention using large scale fortification of edible oil has not been conducted in Tanzania. Therefore, as part of the efforts to evaluate the effectiveness of public intervention with fortified oil, this study was conducted to determine factors influencing vitamin A status of lactating mothers in Manyara and Shinyanga regions of Tanzania, which are areas with high prevalence of vitamin A deficiency among women.

Methodology

Description of Study Area

This study was conducted in two regions of Tanzania namely Manyara and Shinyanga. The two regions were selected due to high prevalence of vitamin A deficiency among lactating women (NBS, 2011). These regions were also used as pilot areas to determine effectiveness of public intervention of vitamin A deficiency using fortified sunflower oil.

Study Design, Sampling frame, Sampling Technique and Sample size

A cross sectional study design among lactating women was conducted in June to July 2015. The study population involved all lactating mothers living in the selected districts of Manyara and Shinyanga regions of Tanzania. These regions were purposely selected based on the prevalence of vitamin A deficiency (NBS, 2011) and infrastructure to access remote villages. The sampling frame comprised of lactating mothers of different age groups, i.e., young mothers <20 years, middle age mothers 20-34 years and elder mothers at 35-49 years. Inclusion criteria were lactating mothers who had stayed in the study areas for more than three months at the time of the study and mothers who consented to participate in the study. Exclusion criteria were lactating mothers who had stayed in the study areas for less than three months at the time of the study. Subjects were randomly selected by assigning random numbers and drawing them at random from each qualified age group. Sample size was determined by statistical power analysis using a formula $N=t^2 \times (p \times q)/d^2$, whereby N=was sample size (569), t was the risk of error (1.96), p was expected prevalence (0.5), q was expected non prevalence (0.5) and d was level of precision (0.05) WHO, 1991). A total number of 569 lactating mothers were selected for the study.

Data Collection

A questionnaire adopted from GAIN was used to collect information on factors influencing vitamin A status of lactating mothers. The questionnaire was divided into three sections. Section one established socio-economic and demographic factors e.g. education level, main source of cooking fuel and the main source of energy for light. Section two solicited information about maternal dietary diversity e.g. numbers of meals/food groups consumed for the past 24hrs and section three solicited information regarding knowledge and about vitamin A rich foods and fortified oil. Ten enumerators were trained on how to administer the questionnaire. They were also trained on how to collect blood samples between themselves, which finger to be used for collecting blood sample, how to massage the finger and insert lancet on it, how to use of micropipette to collect blood and dispense on protein saver cards and leave it for a while (five to ten minutes) to allow drying to take place, then packaging into airtight aluminum bags, adding three desiccants in the aluminum bags and seal it. The questionnaire was pretested among ten lactating mothers in Morogoro urban and rural districts of Tanzania. The villages in which the questionnaires were pre-tested were Bigwa, Kichangani and

Mazimbu in Morogoro town and Mgeta and Mlali in Morogoro rural districts. Necessary adjustments were made on the questionnaire. The questionnaire was administered by home visits in which ten enumerators conducted face to face interviews with the lactating mothers in the early hours of the day.

Samples Collected

Household cooking oils: A sample of cooking oil (capacity 5 ml) was also collected from the household after the interview. The oil samples were used to determine concentration of vitamin A in the oil that was consumed by the household. Vitamin A concentration in the oil was determined by HPLC. The cut-off point used to identify vitamin A concentration in the oil was 20-40 mg/kg (EAC, 2013).

***Blood samples*:** Blood samples were drawn from the lactating mothers using safety lancets by finger prick method. About 50µL of blood was taken by a micropipette and dispensed into a protein saver paper (903 Protein Saver Card, Whatman International, UK) and allowed to dry in air for 5-10 minutes then packaged in airtight aluminum bags in which three pieces of desiccant were added to keep the blood sample dry. Retinol in the DBS was determined by enzyme-linked immunosorbent assay (ELISA) method. The cut-off point used to identify vitamin A deficiency for the lactating mothers was plasma retinol concentration of <1.05 µmol/L or 26.04 µg/ml of retinol binding protein (RBP) (WHO, 2008).

Maternal Dietary Intake

Maternal dietary intake was assessed by using a 24 hour recall method. Maternal Dietary diversity score (MDDS) was calculated based on the consumption of the following nine food groups: (i) starchy staples (ii) legumes, nuts and seeds (iii) milk and milk products

(iv) meat and fish (v) dark green leafy vegetables (vi) other vitamin A rich fruits and vegetables (vii) eggs (viii) other fruits and vegetables (ix) organ meat. Consumption of <3 food groups was classified as low dietary diversity, 3 food groups as moderate-low dietary diversity, 4 food groups as medium dietary diversity and ≥5 food groups as high dietary diversity (FAO and FHI, 2016).

Mothers Knowledge about Vitamin A

Mother's knowledge about vitamin A was assessed using a five point Likert Scale. There were twelve statements asked about vitamin A. Mothers were required to indicate whether they strongly agree, agree, undecided, disagree or strongly disagree for each statement as indicated in Table 5. Strongly agree carried the weight of 5, agree 4, undecided 3, disagree 2 and strongly disagree carried the weight of one.

By using a five- point rating responses (5 strongly agree, 4 agree, 3 undecided, 2 disagree or 1 strongly disagree), means and standard deviation values were determined (Table 9). For the purposes of data to be interpreted, the means of each item that were ranging from 2 and above were characterized as knowledgeable while for the values which were below the mean of 2 were considered low knowledge.

Data Analysis: Data from the questionnaire were coded, cleaned and analyzed using SPSS computer program version 20. Descriptive analysis was used to calculate frequencies and percentages in categorical variables such as age, sex and level of education. The relations between variables were determined through binary logistic regression analysis in which retinol status of lactating mothers was taken as a dependent variable that was affected by independent variables.

Ethical Consideration: Ethical clearance was obtained from the Ethics Committee of the National Institute for Medical Research (NIMR) and the ethics committees for research on human subjects in Canada. Subjects were required to fill in a consent form to affirm their willingness to participate in the study. Confidentiality of the data collected was ensured and no body outside the research team was allowed to access the information containing personal identifiers. All subjects were identified by numbers instead of their real names.

Results

Socio-economic and demographic characteristics of the lactating mothers

Table 1 presents socio-economic and demographic characteristics of the lactating mothers. Majority of lactating mothers 74.3% (95% CI: 70.14-78.45), had attained primary school education level. Only few had informal/secondary education level 25.7% (95% CI: 18.61-32.79). Most of the lactating mothers 99.1% (95% CI: 98.32-99.88) were married. About 76.2% (95% CI: 72.05-80.35) of the lactating mothers came from families with more than 4 people in the household. Most of the lactating mothers 89.5% (95% CI: 86.84-92.16) were housewives while majority of households 98% (95% CI: 96.84-99.16) were headed by males.

Vitamin A deficiency by regions

Vitamin A deficiency in maternal by regions is shown in figure 1. Prevalence of vitamin A deficiency among the maternal was high in both regions regardless of their geographical location although it was worse off in Manyara 83.3% (95% CI: 81.50-85.48) than Shinyanga (79.6% CI: 78.77-80.10).

Vitamin A deficiency by age groups and maternal education level

Vitamin A deficiency by age groups and maternal education level of the respondents are shown in Table 2. Majority of lactating mothers 53.4% (95% CI: 47.79-59.01) who were vitamin A deficient were in middle age group (20-34 years). All groups had high deficiency of vitamin A, although the younger mothers group (15-19 years) was worse off. Prevalence of vitamin A deficiency among lactating mothers was high regardless of their education levels. The most affected group with vitamin A deficiency was primary school education mothers 64.3% (95% CI: 59.39-69.21).

Vitamin A deficiency by district of residence

Table 3 shows vitamin deficiency by district of residence. Prevalence rate of vitamin A deficiency in rural districts was higher than that of urban districts. Prevalence of vitamin A deficiency in rural districts was 63.6% (95% CI: 58.64-68.56) while that in urban districts was 16.7% (95% CI: 9.2-24.2).

Vitamin A deficiency by type of cooking oil and maternal dietary diversity

Vitamin A deficiency among the lactating mothers by type of cooking oil used in households and maternal dietary diversity are presented in table 4. According to the East African Standard EAS 269: 2017, the recommended levels of fortification of vitamin A in edible oil at factory level is 30-40 mg/kg, while the concentration of vitamin A at retail and household level (end users) should be 20-40 mg/kg. The most popular oil in Manyara was sunflower oil which has some natural but low content of retinol while in Shinyanga was palm oil which is fortified. Prevalence of vitamin A deficiency among mothers indicated that, 45.3% (95% CI: 39.23-51.37) of the households had oil with retinol concentration of <20 mg/kg. Others with vitamin A deficiency had edible oil with retinol concentration in the range of 20-40 mg/kg and above 40 mg/kg of oil. Prevalence of vitamin A deficiency among lactating mothers was high in all groups, although deficiency was higher for mothers with the lowest dietary scores 40.8% (95% CI: 34.48-47.12). The mostly consumed food group 97% (95% CI: 96.99-97.01) was staples followed by other fruits and vegetables and dark green vegetables (Fig. 1).

Practices toward consumption vitamin A rich foods and Knowledge about vitamin A

Figure 2 describes food groups consumed by lactating mothers in the previous 24hours. The findings in Table 5 indicate mothers score on the items of the Likert type scale used to assess knowledge about vitamin A. For all twelve phrased statements, the maternal scores on their knowledge had means of less than 2 (Table 6). This implies that, maternal knowledge about vitamin A among the lactating mothers in this study was low. Sources of the knowledge about vitamin A are shown in Table 7.

Binary Logistic Regression for the determinants of the maternal retinol status

Binary logistic regression was run to compute the strength of association between maternal retinol status and age, maternal education, household cooking oil, geographical location, occupation, income earnings, source of light, main source of cooking fuel, type of roof, type of floor and vitamin A rich foods and maternal dietary diversity is presented in Table 7. There was a significant difference at $p<0.05$ between the maternal retinol status and geographical location.

Discussion

It was revealed in this study that, vitamin A deficiency was high in Manyara (83.3%) than Shinyanga (79.6%) (Fig.1). These prevalence rates were higher than the Nation average whereby the proportional of vitamin A deficiency among the lactating mothers in

Manyara was 47.6% and Shinyanga was 41.5% (NBS, 2011). Vitamin A status of lactating mothers with informal education was better as compared to their peers with primary school and secondary school education (Table 2). These findings are contrary to those of Rahman and Sapkota (2014) who reported that, vitamin A status of lactating mothers with primary and secondary education was better than those with informal education. The benefits of maternal education to nutrition and child health care have been demonstrated (Vikram *et al.,* 2012; Sabates, 2013; Wang *et al.,* 2013; Rawlings, 2014). According to Abuya *et al.* (2012) maternal education contributes to a decline in morbidity and mortality. Maternal education improves child nutrition through adopting better child-care practices such as good selection of healthy foods and health care services (Makoka, 2013), since mothers are good custodians of family health (Burchi, 2012; Bain *et al.,* 2013).

It was also noted in this study that, vitamin A deficiency rate was higher among lactating mothers in rural than urban areas (Table 3). These results are in agreement with those of Wallace *et al.* (2014) who reported that, prevalence of VAD among lactating mothers was higher in rural areas in comparison to the urban locations. High prevalence of VAD in rural areas has also been documented by Mbah *et al.* (2013) and Abebe *et al.* (2014). This disparity could be due to the fact that, mothers in rural areas are less educated (Agarwal *et al.,* 2014; Wallace *et al.,* 2014) and therefore, they are less likely to participate in health programs or utilize available health services (Makoka, 2013). Also this disparity could be due to low income hindering dietary diversification and consumption of fortified foods (Haileslassie *et al.,* 2016).

Most of the households in this study were using edible oil with retinol concentrations below the recommended levels (<20 mg/kg) while some other oil samples had retinol concentrations above the recommendation (>40 mg/kg) (Table 4). This is because fortification of edible oil in low income countries including Tanzania is poorly managed (Akhtar *et al.,* 2013). This has been observed in this study whereby much of the oil which was sold in retail outlets was not fortified and even the little that was fortified contained either too little vitamin A concentration or excess amount of it. These findings are in line with those of Sandjaja *et al.* (2015) and Rohner *et al.* (2016). Poor fortification of oils with vitamin A might be due to poor quality control and monitoring at factory level (Soekirman *et al.,* 2012; Andarwulan *et al.,* 2014).

Vitamin A deficiency by maternal dietary diversity showed that mothers with low dietary diversity (scores less than 3) were worse off as compared to their peers with medium (4 scores) and high (5 scores and above) dietary diversity (Table 4). Several studies have reported high prevalence of vitamin A deficiency among lactating mothers in low income countries with the lowest dietary diversity score (Haileslassie *et al.,* 2016; Chagomoka *et al.,* 2016; Na *et al.,* 2016). This was attributed to low education and poverty which influence food choices and access. Maternal Dietary Diversity Score is important to both, mothers and their nursing children (Amugsi et *al.,* 2015). According to Weldehaweria *et al.* (2016), dietary diversity during lactation is essential because of increased nutritional needs especially minerals and vitamins. When a lactating mother gets sufficient vitamin A through the diet, the breastfed child also benefits since the concentration of vitamin A in the mother’s milk also increases with the mother’s diet (WHO, 2011). When lactating mothers do not get enough energy and micronutrients from their diets, they are at risk of getting micronutrient deficiencies (Henjum *et al.,* 2015). Some micronutrient deficiency negatively affects the quality of the breast milk as the mother’s milk would also be deficient in those micronutrients. This in turn adversely affects the health of the breastfed baby (Weldehaweria *et al.,* 2016).

Food consumption by the mothers in this study was mostly based on the staples. Consumption of vitamin A rich foods such as animal foods e.g. meat, fish, eggs and dairy products especially in rural areas was very limited, despite the fact that, many households in the districts of Manyara and Shinyanga regions were animal keepers. Also, consumption of fruits and/or vegetables that were yellow/orange in color such as papaya, carrots and orange fleshed sweet potatoes was very low (Fig. 2). Findings of this study are in agreement with those reported by Haileslassie *et al.* (2016) who stated that, consumption of vitamin A rich foods including animal products, fruits and/or vegetables yellow/orange in color in rural communities was generally low but high consumption of staple foods. Low consumption of vitamin A rich foods among women in low income countries was mostly associated with factors such as low economic status (Amugsi *et al.,* 2015) and non-availability of vitamin A rich foods especially fortified foods (Haileslassie *et al.,* 2016). Intakes of micronutrients less than the recommended levels increase the risks of micronutrient deficiencies and reduce lactation performance (Henjum *et al.,* 2015.) Adequate consumption of vitamin A rich foods among lactating mothers is beneficial for the health of both mothers and their nursing children (WHO, 2011).

Majority of the lactating mothers in this study lacked knowledge about vitamin A including benefits of vitamin A, sources of vitamin A and ways to avoid vitamin A rich foods (Table 6). Sources of information about vitamin A for the mothers were mainly from health centers and community health workers (Table 7). These results concurred with those reported by Aktar *et al.* (2013), who found that mothers in Pakistan lacked knowledge about the health implications of Vitamin A deficiency and only few women in India had knowledge about vitamin A rich foods. Knowledge about the beneficial effects of consuming vitamin A rich foods, such as green leafy vegetables, poultry, meat, cereal, and dairy products, may help to reduce the risk of VAD among lactating mothers (Aktar *et al.,* 2013). According to Chagomoka *et al.* (2016), maternal knowledge about vitamin A is important to reduce vitamin A deficiency by achieving dietary diversification through consumption of locally produced vitamin A rich foods.

In this study there was also a strong relationship between the maternal vitamin A status and their geographical location (Table 8). The association observed suggested that, rural residence was an important factor that influenced the retinol status of the lactating mothers. The findings are in line with those of Samba *et al.* (2013) and Hanson *et al.* (2016), who in their studies reported that, retinol status among the lactating mothers was significantly influenced by rural residence. Rural residence is an important factor in determination of maternal retinol status because mothers in rural settings have low income and purchasing power in comparison to those from urban (Samba *et al.*, 2013). Thus, they are less likely to diversify diets and consume vitamin A rich foods including fortified foods (Sealey-Potts and Potts, 2014).

Conclusions and Recommendations

Generally vitamin A deficiency among lactating mothers was high. The major factor that influenced vitamin A deficiency in this socio group was residence. Other factors which had insignificant influence included fortification of cooking oil and consumption of animal-based foods such as eggs fish/meat and dairy foods, plant based foods such as cereal, roots and tubers, yellow or orange fruits, dark green vegetables, other fruits and vegetables, maternal knowledge about vitamin A rich foods and maternal dietary diversity. Starchy foods were commonly consumed by the respondents while animal-based foods were rarely consumed. It is recommended based on this study that, to address the problem of vitamin A deficiency, nutrition and healthy planners should put more efforts in food fortification especially of edible oil and promote consumption of diverse diets at the household level. Also lactating mothers and the community as a whole should be educated on the importance of consuming fortified oil and selection of foods rich in vitamin A. Furthermore, the community should be educated on how to identify the fortified oil in the market by recognizing the fortification logo or reading the label before buying the oil or any other fortified food product.

Acknowledgements

The authors are grateful to the Government of Canada who through the Global Affairs Canada (GAC) and IDRC-CIFSRF Grant, Phase 2 provided the financial support to undertake this study.

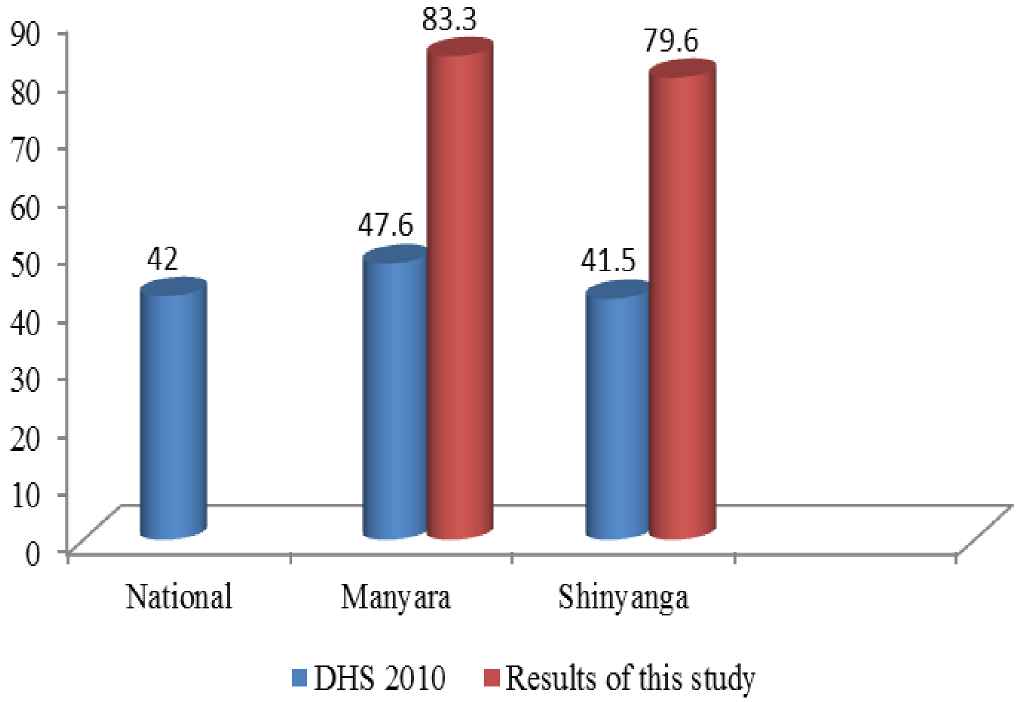

Figure 1: Prevalence of vitamin A by region

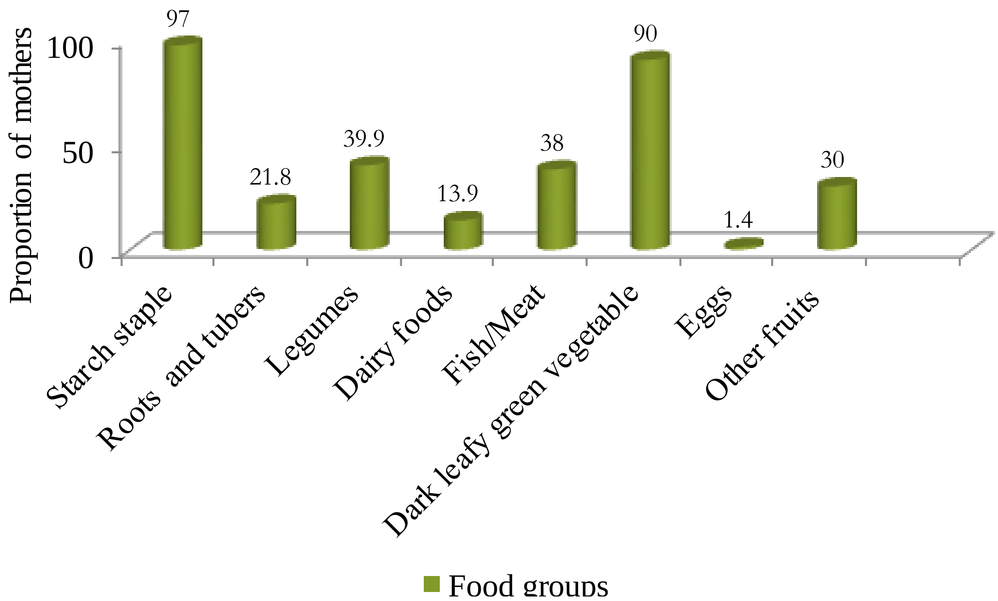

Figure 2: Consumption of food groups by maternal during the previous 24 hours

Table 1: Socio-economic and demographic characteristics of the lactating mothers (n=569)

Parameter	n	%	95% CI
Age			
15- 19	25	4.4	3.64-12.440
20 – 34	383	67.3	62.60-72.00
35 – 49	161	28.3	28.00-28.65
Education level			
Primary education	423	74.3	70.14-78.45
Informal education	83	14.6	7.00-22.20
Secondary education	63	13.4	5.00-21.81
Marital status			
Married	564	99.1	98.32-99.88
Single	5	0.9	16.08-34.08
Household family size			
≤4	165	23.8	17.30-30.30
>4	404	76.2	72.05-80.35
Occupation			
Housewife	509	89.5	86.84-92.16
Petty business	25	4.4	3.64-12.44
Employed for wage	15	2.6	5.45-10.65
Self employed	20	3.5	4.55-11.55
Household head			
Male	558	98.0	96.84-99.16
Female	11	2.0	6.27-10.27

Table 2: Vitamin A deficiency by age groups and maternal education level (n=569)

Parameter	**With vitamin A deficiency (% , 95% CI)**	**Without vitamin A deficiency (% , 95% CI)**	**DBS not analyzed (% , 95% CI)**	**Total (% , 95% CI)**
Age groups				
15-19	3.9 (4.12-11.99)	0.5 (7.48-8.48)	0.0 (0)	4.4 (3.64-12.44)
20-34	53.4(47.79-59.01)	9.8 (2.01-17.59)	4.0 (4.01-12.01)	67.3 (62.5-71.9)
35-49	23 (15.79-30.21)	3.2 (4.93-11.33)	2.2 (6.1-10.5)	28.3 (21.43-35.17
Education level				
Primary	64.3 (59.39-69.21)	10.0 (2.21-17.79)	0.0 (0)	74.3 (70.14-78.46)
Informal	6.5 (1.81-13.01)	1.9 (6.17-9.97)	6.2 (1.79-14.19)	14.6 (7.00-22.20)
Secondary	9.1 (1.43-16.77)	1.6 (6.60-9.80)	0.0 (0)	13.4 (5.0-21.81)

Table 3: Vitamin A deficiency by district of residence (n=569)

Districts	With vitamin A deficiency (% , 95 CI)	Without vitamin A deficiency (% , 95 CI)	BDS not Analyzed (% , 95 CI)	Total (% , 95 CI)
Urban				
Babati urban	9.3 (1.48-17.12)	0.5 (7.48-8.48)	0.9 (7.38-9.18)	10.7 (2.94-18.46)
Shinyanga urban	7.4 (0.52-15.32)	1.1 (7.25-9.45)	0.2 (1.56-8.96)	8.6 (0.75-16.45)
Total urban	**16.7 (9.2-24.2)**	**1.6 (6.60-9.80)**	**1.1 (7.25-9.45)**	**19.3 (11.92-26.68)**
Rural				
Babati rural	11.8 (4.08-19.52)	1.8 (6.44-10.04)	0.7 (7.47-8.87)	14.1 (6.6-21.80)
Hanang	10.1 (2.21-15.11)	1.4 (6.74-9.54)	2.1 (6.01-10.21)	13.5 (5.87-21.13)
Mbulu	20.7 (13.39-28.01)	4.9 (3.1-12.90)	1.1 (7.25-9.45)	26.7 (19.67-33.73)
Kishapu	7.2 (0.71-15.11)	1.4 (6.74-9.54)	0.2 (8.56-8.96)	8.9 (1.01-16.79)
Shinyanga Rural	7.2 (0.71-15.11)	11.4 (6.74-9.54)	0.2 (8.56-8.96)	8.9 (1.01-16.79)
Kahama	6.6 (1.63-14.83)	2.1 (6.01-10.21)	0.4 (8.35-9.15)	8.6 (0.75-16.45)
Total rural	**63.6 (58.64-68.56)**	**12.1 (4.40-19.80)**	**4.7 (3.14-12.54)**	**80.7 (77.09-84.31)**

Table 4: Vitamin A deficiency by type of cooking oil used at the household and Mothers Dietary Diversity Score (MDDS) (n=569)

Parameter	**Cut-off point**	**With vitamin A deficiency (% , 95 CI)**	**Without vitamin A deficiency (% , 95 CI)**	**DBS not analyzed (% , 95 CI)**	**Total (% , 95 CI)**
Household oil (mg/kg)					
	< 20	45.3 (39.23-51.37)	6.9 (1.05-14.85)	7.0 (1.88-3.28)	59.1(53.84-64.36)
	20-40	20.2 (12.86-27.54)	4.4 (3.64-12.44)	1.6 (6.60-9.80)	26.2(19.14-33.26)
	>40	11.2 (3.42-18.99)	1.4 (6.74-9.54)	1.6 (6.60-9.80)	14.7(6.94-22.46)
Maternal Dietary Diversity Score					
	Low (<3)	40.8 (34.48-47.12)	7.0 (0.9-14.91)	2.6 (5.45-10.65)	50.4 (44.62-56.18)
	Moderate low (=3)	29.3 (22.40-36.20)	5.4 (2.56-13.56)	1.4 (6.74-9.54)	36.2 (29.64-42.76)
	Medium (=4)	8.4 (0.55-16.26)	1.0 (7.0-9.0)	1.8 (6.44-10.04)	11.2 (3.47-18.93)
	High (≥5)	1.8 (6.44-10.04)	0.0 (0)	0.4 (8.35-9.15)	2.2 (6.10-10.50)

Table 5: Mothers score on the items of the Likert type scale used to assess knowledge about Vitamin A

Statement about vitamin A	**Strongly agree**		**Agree**		**Undecided**		**Disagree**		**Strongly Disagree**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Vitamin A Helps with child growth (benefit of VA)	22	3.87	10	1.8	143	25.1	45	7.9	349	61.3
Vitamin A Strengthens immunity to illness (benefit of VA)	33	5.8	26	4.6	143	25.1	29	5.1	338	59.4
Vitamin A improves vision (benefit of VA)	15	2.6	14	2.5	143	25.1	157	27.6	230	40.4
Vitamin A improves health/reduces illness (benefit of VA)	18	3.2	33	5.8	143	25.1	85	14.9	290	51.0
Vitamin A avoids night blindness (benefit of VA)	2	0.4	2	0.4	143	25.1	120	21.1	302	53.1
Vitamin A reduces mortality (benefit of VA)	0	0	3	0.5	143	25.1	123	21.6	300	52.7
Foods rich in Vitamin A (animal sources)	5	0.9	14	2.5	143	25.1	10	1.8	397	70
Foods rich in Vitamin A (plant sources)	10	1.8	12	2.1	143	25.1	33	5.8	369	64.9
Consumption of balanced diet (way to avoid VAD)	22	3.9	11	1.9	143	25.1	94	16.5	266	46.7
Consumption of foods rich in vitamin A (way to avoid VAD)	11	1.9	24	4.2	143	25.1	30	5.3	350	61.5
Consumption of vitamin A Supplement (way to avoid VAD)	10	1.8	6	1.1	143	25.1	91	16.0	318	55.9
Consumption of foods fortified with vitamin A (way to avoid VAD)	3	0.5	4	0.4	143	25.1	91	16.0	328	57.6

VAD= Vitamin A deficiency, VA= Vitamin A

Table 6: Respondents score on their knowledge using mean and standard deviation about vitamin A (n=569)

Statement	Mean	SD
Vitamin A Helps with child growth (benefit of VA)	1.79	0.47
Vitamin A Strengthens immunity to illness (benefit of VA)	1.92	0.56
Vitamin A improves vision (benefit of VA)	1.93	0.63
Vitamin A improves health/reduces illness (benefit of VA)	1.95	0.68
Vitamin A avoids night blindness (benefit of VA)	1.74	0.36
Vitamin A reduces mortality (benefit of VA)	1.73	0.44
Foods rich in Vitamin A (animal sources)	1.63	0.64
Foods rich in Vitamin A (plant sources)	1.69	0.55
Consumption of balanced diet (way to avoid VAD)	1.90	0.64
Consumption of foods rich in vitamin A (way to avoid VAD)	1.74	0.42
Consumption of vitamin A Supplement (way to avoid VAD)	1.76	0.28
Consumption of foods fortified with vitamin A (way to avoid VAD)	1.70	0.34

VAD= Vitamin A Deficiency, VA= Vitamin A

Table 7: Sources of mothers knowledge about vitamin A (n=569)

Source	n	%
Health Center	393	69.2
Community health worker	18	3.2
Neighbors	3	0.5
Radio	20	3.5
Television	2	0.4
Neutral	143	25.1
Total	**569**	**100.00**

Table 8: Binary Logistic Regression for the determinants of the maternal vitamin A status

Parameter	B	S.E.	Wald	df	p-value	Exp (B)
Geographical area	0.802	0.296	7.397	1	0.016**	2.229
Maternal age	0.413	0.526	0.615	1	0.433	1.511
Education	-0.198	0.251	0.623	1	0.430	0.820
Family size	0.265	0.916	0.084	1	0.772	1.304
Occupation	0.994	0.829	1.438	1	0.230	2.701
Income	-0.998	0.995	1.005	1	0.316	0.369
MDDS	-0.070	0.591	0.014	1	0.905	0.932
Hh Cooking oil	0.218	0.623	0.123	1	0.726	1.244
Source of lighting	-0.275	0.397	0.480	1	0.488	0.759
Cooking fuel	0.660	0.625	1.113	1	0.291	1.934
Floor	-0.523	0.813	0.414	1	0.520	0.592
Roof	-0.737	0.595	1.536	1	0.215	0.478
Constant	**-2.588**	**1.159**	**4.986**	**1**	**0.026**	**0.075**

Dependent Variable: Vitamin A deficiency
Independent variables: Geographical area, maternal age, education, family size, occupation, family income, maternal dietary diversity scores (MDDS), Hh cooking oil, source of lighting, cooking fuel, floor and roof

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CHAPTER THREE

3.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions

It was concluded from the study that, consumption of sunflower oil fortified with vitamin A, significantly improved the serum retinol concentrations of both lactating mothers and children. Administration of mega doses of vitamin A supplement contributed to the improvement in the serum retinol concentration of children under the age of five years. Likewise, dietary diversity improved vitamin A status of both lactating mothers and children. Edible oil fortified with vitamin A, mega doses of vitamin A supplements and dietary diversification significantly improved the linear growth (height for age) of the children. Vitamin A was stable in fortified sunflower oil during six months of storage and during retail by scooping. Retention of vitamin A in the oil samples was high with minimal degradation of the vitamin with increase in the storage time. The quality attributes of the fortified sunflower oil sold at retail by scooping and those of the stored oil remained within the acceptable levels. Some sunflower oil samples collected from the SMEs had lower concentration of vitamin A than the recommended fortification levels. Majority of the oils collected from the households and retail shops however had normal vitamin A concentrations.

Only few oil samples collected at the market (retail shops) and households had vitamin A concentration below the recommended levels. Results from this study further revealed that, all the sunflower oil samples collected from the factory, retail shops and households met the limits for the recommended quality attributes. Majority of the children had low dietary diversity and poor anthropometric status, in which most of them had low weight for age, height for age and weight for height. The most common food group consumed by almost all children was starchy staples. A strong association exists between low dietary diversity and underweight and stunting. Rural residence and family size were the other factors that were strongly associated with low dietary diversity. Prevalence of vitamin A deficiency among children was significantly higher than the national average. VAD was significantly higher among non-breastfed than among breastfed children and also higher among males than among females because males have high physiologic needs compared to females thus expending their vitamin A stores rapidly. Most of the children with vitamin A deficiency also suffered from one or more forms of under-nutrition (underweight, stunting and/or wasting). Vitamin A status was strongly associated with age of the child and stunting condition. Age and height-for-age status of the child were therefore good anthropometric attributes for predicting vitamin A status of children aged 6-36 months. Vitamin A deficiency among lactating mothers was high. The major factor that influenced vitamin A deficiency in this socio group was residence.

3.2 Recommendations

Therefore, to address this problem of vitamin A deficiency,

i. Nutrition and health planners should promote fortification of edible oil with vitamin A, consumption of vitamin A rich foods such as green leafy and yellow vegetables and consumption of diverse diets at a household level

ii. Oil processors should abide to the fortification specifications stipulated in the regulations to avoid fortifying the oils with vitamin A below the recommended levels. Also, oil processors should maintain good manufacturing and hygiene practices to ensure high quality oil. Retailers should observe good handling practices of fortified sunflower oil so as to maintain the quality of the oil and ensure high retention of vitamin A in the oil. Retailers must also keep the oil under good storage conditions by avoiding direct light, exposure to air, dust and metals contamination which accelerate oxidation thus reducing the quality of the oil.

iii. Producers should adhere to the National fortification guideline. Also oil producers should adhere to Good Manufacturing Practices including Hazard Analysis Critical Control Point program to ensure high quality of the oil.

iv. There is a need for interventions to combat under-nutrition particularly by promoting consumption of diverse of food groups that are locally available and affordable.

v. These findings call for coordinated and sustainable intervention programs to reduce under-nutrition and micronutrient deficiencies such as vitamin A deficiency to optimize growth among children age 6-36 months.

vi. Lactating mothers and the community as a whole should be educated on the importance of consuming fortified oil and selection of foods rich in vitamin A. Furthermore, the community should to be educated on how to identify the fortified oil in the market by recognizing the fortification logo or reading the label before buying the oil or any other fortified food product.

APPENDICES

Appendix 1: Baseline Store Questionnaire for Promoting Use of Locally Produced Fortified Sunflower Oil by E-Vouchers

Store ID: …………………………Date:……………...………………………
Interviewer's ID: …………………….Shop no………………………
Name of the shopkeeper ………………Mobile……………………………………

N°	QUESTIONS	ANSWERS	SKIPS
S1	Are you the owner of this store?	YES / NO	
S2	If No, what is your relationship to the store owner?	Relative/employee/other (specify)	
S3	What is the approx. time from your shop to the nearest store where oil is also sold?	a) Minutes ………………… b) Seconds………………….	
S4	Within a street/village, how many other stores sell cooking oil?	Write in numbers ………………	
S5	Size of store: interviewer to estimate approximate area devoted to displaying merchandise for sale (suggest appropriate units)	a) Length- in footsteps………….. b) Width-in footsteps…………….	
S6	Approximately how many liters of cooking oil do you sell?	a) Per day …………… b) Per week …….....	
S7	Do you carry the following kinds of cooking oil? Cottonseed oil Yes/No a) Cotton seed oil b) Unfortified oil c) Fortified oil d) Palm oil e) Sunflower oil f) Others (specify)	a) YES/NO b) YES/NO c) YES/NO d) YES/NO e) YES/NO f) YES/NO	
S8	Of all the types of oil you carry, which do you sell the most?	Type of oil……………………………………..	
S9	What share of all oil sold, does this account for? (Ask if is < ¼, ¼, ½, ¾, etc of all types of oil)	In fraction………………………………………	
S10	Which is the next most popular type of oil you sell?	Type of oil………………………………………	
S11	Of this second type of oil, what share of all oil sold does this account for? (Ask if is < ¼, ¼, ½, ¾, etc. of all types of oil)	In fraction……………………………………….	
S12	Which is the next most popular type of oil you sell?	Type of oil………………………………………	

S13	After this second type of oil, what share of all oil sold does this account for? (Ask if is < ¼, ¼, ½, ¾, etc. of all types of oil)	In fraction……………………………………….	
S14	What share of all oil sold is accounted for by sunflower oil?	a) ………..liter of oil per week(all types) b) ………...Liter of sunflower oil	
S15	Of the sunflower oil you sold in the last week, what proportion was fortified?	Liter	
S16	When customers buy sunflower oil, what size package is most common (in terms of number of customers)?	a) 1 litre……. b) Half litre…… c) Scoops d) Cup…..other (specify)…………………..…..	
S17	Where did you get your oil supply?	Wholesaler…………..… Factory ……………..…. Other (specify)…………	
S18	a) If you buy oil in containers of 20 L how long (days) do they last b) If you buy oil in 2 containers of 10 L how long (days) do they last c) c) If you buy oil in 4 containers of 5 L how long (days) do they last d) If you buy oil in 20 containers of 1L how long (days) do they last	a) …………………… b) …………………… c) …………………… d) ……………………	
S19	How do you store your packaged oil (non - opened? a) In dark/ low light? b) In normal light and temperature? c) In l dry place? d) Protect dust and other contaminants from getting to the container?	a) YES/NO b) YES/NO c) YES/NO d) YES/NO	
S20	After opening the 20 L containers, what special care do you provide to the oil? a) Control the light from reaching the oil b) Control atmospheric moisture from getting into the oil c) Protect air from getting into the oil container d) Protect the oil from getting warm e) Protect dust and other contaminants from getting into the container	a) YES/NO b) YES/NO c) YES/NO d) YES/NO e) YES/NO	
S21	a) Have you ever had any lesson about entrepreneurship?	YES/NO	
	b) Who provided it?	…………………………………………………	

	Price		
S22	1Lter	a) 1Liter of fortified sunflower oil ………. b) 1Liter of fortified sunflower oil ……….(the same brand) Tsh c) 1Liter of fortified sunflower oil ……….(other brand) Tsh	
S23	20Liter	a) 20Liter of fortified sunflower oil ………. (the same brand) Tsh b) 20Liter of fortified sunflower oil ………. (the same brand) Tsh 20Liter of fortified sunflower oil ……… (other brand) Tsh	
	Sunflower Oil		
S24	Quantity of sunflower oil sold last week?	a) Container of 20liter fortified ……………Tsh b) Container of 20Liter unfortified (the same brand)……Tsh c) Container of 20Liter unfortified (other brand) Tsh	
S25	Sample of edible oil sold	Interviewer should ask on all types of oil sold at the shop	

Appendix 2: Endline (follow up) store Questionnaire for Promotion of Locally Produced Fortified Sunflower Oil by E-Vouchers

Store ID: …………………………Date:……………...………………………
Interviewer's ID: ………………………….Shop no.…………………………………...
Name of the shopkeeper …………………..…… Mobile………………………………

N°	QUESTIONS	ANSWERS	SKIPS
S1	Are you the owner of this store?	YES / NO	
S2	If No, what is your relationship to the store owner?	Relative/employee/other (specify)	
S3	What is the approx time from your shop to the nearest store where oil is also sold?	a) Minutes …………………… b) Seconds……………………	
S4	Within a street/village, how many other stores sell cooking oil?	Write in numbers ………………	
S5	Size of store: interviewer to estimate approximate area devoted to displaying merchandise for sale (suggest appropriate units)	a) Length- in footsteps…………. b) Width-in footsteps…………….	
S6	Approximately how many liters of cooking oil do you sell?	a) Per day …………… b) Per week …….........	

S7	Do you carry the following kinds of cooking oil? Cottonseed oil Yes/No g) Cotton seed oil h) Unfortified oil i) Fortified oil j) Palm oil k) Sunflower oil l) Others (specify)	a) YES/NO b) YES/NO c) YES/NO d) YES/NO e) YES/NO f) YES/NO	
S8	Of all the types of oil you carry, which do you sell the most?	Type of oil…………………………..………	
S9	What share of all oil sold, does this account for? (Ask if is < ¼, ¼, ½, ¾, etc of all types of oil)	In fraction……………………………………..	
S10	Which is the next most popular type of oil you sell?	Type of oil……………………………………..	
S11	Of this second type of oil, what share of all oil sold does this account for? (Ask if is < ¼, ¼, ½, ¾, etc. of all types of oil)	In fraction…………………………………….	
S12	Which is the next most popular type of oil you sell?	Type of oil……………………………………..	
S13	After this second type of oil, what share of all oil sold does this account for? (Ask if is < ¼, ¼, ½, ¾, etc. of all types of oil)	In fraction……………………………….…….	
S14	What share of all oil sold is accounted for by sunflower oil?	a) liter of oil per week(all types) b) Liter of sunflower oil	
S15	Of the sunflower oil you sold in the last week, what proportion was fortified?	Liter	
S16	When customers buy sunflower oil, what size package is most common (in terms of number of customers)?	a) 1 litre……. b) Half litre…… c) Scoops d) Cup…..other (specify)………………..	
S17	Where did you get your oil supply?	Wholesaler…………… Factory ……………..…. Other (specify)………….	
S18	e) If you buy oil in containers of 20 L how long (days) do they last f) If you buy oil in 2 containers of 10 L how long (days) do they last g) c) If you buy oil in 4 containers of 5 L how long (days) do they last h) If you buy oil in 20 containers of 1L how long (days) do they last	e) …………………… f) …………………… g) …………………… h) ……………………	

S19	How do you store your packaged oil (non - opened? e) In dark/ low light? f) In normal light and temperature? g) In l dry place? h) Protect dust and other contaminants from getting to the container?	e) YES/NO f) YES/NO g) YES/NO h) YES/NO	
S20	After opening the 20 L containers, what special care do you provide to the oil? f) Control the light from reaching the oil g) Control atmospheric moisture from getting into the oil h) Protect air from getting into the oil container i) Protect the oil from getting warm j) Protect dust and other contaminants from getting into the container	f) YES/NO g) YES/NO h) YES/NO i) YES/NO j) YES/NO	
S21	c) Have you ever had any lesson about entrepreneurship?	YES/NO	
	d) Who provided it?	…………………………………………	
	Price		
S22	1Lter	a) 1Liter of fortified sunflower oil ………. b) 1Liter of fortified sunflower oil ………. (the same brand) Tsh c) 1Liter of fortified sunflower oil other brand) Tsh	
S23	20Liter	a) 20Liter of fortified sunflower oil ……… .(the same brand) Tsh b) 20Liter of fortified sunflower oil ………. (the same brand) Tsh 20Liter of fortified sunflower oil ………. (other brand) Tsh	
	Sunflower Oil		
S24	Quantity of sunflower oil sold last week?	a) Container of 20liter fortified ………. Tsh b) Container of 20Liter unfortified (the same brand) Tsh c) Container of 20Liter unfortified (other brand) Tsh	
25	Sample of edible oil sold	Interviewer should ask on all types of oil sold at the shop	

S26. In the last week, did any customers use e-vouchers to buy sunflower oil? Yes/No
S27. In the last week, what share of sunflower oil was sold using e-vouchers?
…………………………………………………………………………

S28. Why do you think some customers like to use e-vouchers?
……………………………………………………………………
S29. Why do you think some customers do not like to use e-vouchers?
……………………………………………………………………

VITAMIN A KNOWLEDGE, ATTITUDE AND PRACTICES			
N°	QUESTIONS	ANSWERS	SKIPS
va01	Have you heard information about vitamin A?	Yes / No .	If No, skip to end
va02	If yes, what are the main benefits? *(can select more than one answer if appropriate: do not prompt)*	Helps with child growth......................1 Strengthens immunity to illness…......2 Improves vision ……………….……….3 Improves health/reduces illness……..4 Avoids night blindness …………...…5 Reduces mortality………………...…6 Other ……………………………..….7 (specify)	
va03	If yes, where did you hear about vitamin A?	Health center…..................................1 Community health worker..................2 Neighbours..3 Radio ....4 Television..5 Other …………………...……………6 (specify)..	
va04	Could you list up to three foods rich in Vitamin A?	1……………………………… 2………………………………… 3…………………………………	
va05	What methods can one use to avoid a diet poor in vitamin A?	Balanced diet......................................1 Foods rich in vitamin A......................2 Vitamin A supplement........................3 Foods fortified with vitamin A............4 Don’t know …...………………….…5 Other ………………………………...6 (specify)	

Appendix 3: Household Baseline Questionnaire

HOUSEHOLD CHARACTERISTICS			
N°	QUESTIONS	ANSWERS	SKIPS
hc01	What is the main source of drinking water for members of your household?	Piped water Piped into dwelling………………………….11 Piped to year/lot……………………….….12 Public tap/Standpipe………………………..13 Neighbor's tap…………………………….....14 Tube well or borehole………………………21 Dug well Protected well……………………………..…31 Unprotected well……………………….….…32 Water from spring Protected spring……………………………41 Unprotected spring…………………………42 Rainwater……………………….……….51 Tanker truck…………………………..….61 Cart with small tank…………………….....71 Surface water (river/dam/lake/pond/ Stream/canal/irrigation channel……….....81 Bottled water……………………………..91 Other ……………………………………….96 (specify) .	 →hc03 →hc02 →hc02 →hc02 →hc02 →hc02 →hc04 →hc02 →hc02 →hc02 →hc02 →hc02
hc1A	Who is providing water at your main source?	Authority……………………………………1 NGO/CBO…………………………………..2 Private operator……………………..……3 Don't know…..…………………………..….4	
hc1B	*Check hc01: if circled 11 or 12, go to hc03; if circled 13, go to hc04*		
hc02	Where is that water source located?	In own dwelling……………………….…..1 In own yard/plot…………………………..2 Elsewhere…………………………………….3	→hc04 →hc04
hc03	How long does it take to go there, get water, and come back? .	Minutes……………………… ☐☐☐ .Don't know………………..998	
hc04	What kind of toilet facility do members of your →household usually use?	Flush or pour flush toilet Flush to septic system………………………11 Flush to septic tank……………………….....12 Flush to pit latrine…………………………..13 Flush to somewhere else…..………………..14 Flush, don't know where……………………15 Pit latrine Ventilated, improved (VIP)………………...21 Pit latrine with slab (washable……………..22 Pit latrine with slab (not washable)………...23 Pit latrine without slab/ open pit……........24 Composting toilet/Ecosan…………………..31 Bucket……………………………………….41 No facility/bushfield………………..……….51 Other_____________________________ 96 (specify)	hc07
hc05	Do you share this facility with other households?	Yes…………………………………………....1 No…………………………………………….2	 →hc07
hc06	How many households share this toilet facility?	No. of households if less than 10 ☐☐ 10 more more households.……..95 Don't know…………………….98	
hc07	Does your household have		

	Electricity that is connected? A battery or generator for power? A paraffin lamp in working condition? A radio in working condition? A television in working condition? A mobile telephone in working condition? A non-mobile telephone in working condition? An iron? (Charcoal or electric)? A refrigerator in working condition?	..YES NO Electricity…………………………….1 2 Battery/generator…………………….1 2 Paraffin lamp……………………...…1 2 Radio………………………………….1 2 Television……………………………..1 2 Mobile telephone……………………..1 2 Non-mobile telephone……………….. 1 2 Iron…………………………………….1 2 Refrigerator…………….....................1 2	
hc08	What type of fuel does your household use mainly for cooking?	Electricity…………………………………...01 Bottled gas………………………………...02 Biogas………………………………………03 Kerosene……………………………….…04 Charcoal……………………………………05 Firewood……………………………………06 Straw/shrubs/grass…………………….….07 Agricultural crop……………….……...….08 Animal dung………………………………..09 No food cooked in household……………...95 Other ______________________________96 (specify)	
hc09	What is the main source of energy for lighting in the household?	Electricity…………………………………..01 Solar………………………………………..02 Gas……………………………………….....03 Paraffin-Hurricane lamp…………………..04 Paraffin-Pressure lamp…………………..…05 Paraffin-Wick lamp……………………..….06 Firewood…………………………………...07 Candles…………………….……………....08 Other______________________________09 (specify)	
hc10	*Main material of the floor* *RECORD OBSERVATION*	Natural floor Earth/sand……………………………….11 Dung………………………….…………12 Rudimentary floor Wood planks/Timber…………………....21 Palm/Bamboo……………………………...22 Finished floor Parquet or polished wood………………….31 Vinyl or asphalt strips……………….....…32 Ceramic tyle, terrazzo……………………...33 Concrete/Cement………..…………………34 Carpet……………………………………….35 Other____________________96 (specify)	
hc11	*Main material of the roof* *RECORD OBSERVATION*	Natural roofing Grass/Thatch/Mud…………………………11 Finished roofing Iron sheets……………...............................21 Tiles……………………………………..….22 Concrete/Cement…………………………..23 Asbestos sheets……………………………..24 Other____________________ 96 (specify)	
hc12	*Main material of the exterior walls* *RECORD OBSERVATION*	Natural walls Palm/Trunks/Bamboo………………..….11 Dirt/Mud…………………………………12	

		Rudimentary walls Bamboo poles with mud………………...21 Stone with mud…………………………..22 Sun-dried bricks/mud brick…………….....23 Baked bricks……………………….…...24 Cement blocks……………………..…...25 Wood planks…………………………..…26 Other_______________________ 96 (specify)	
hc13	How many rooms in this household are used for sleeping? *INCLUDE ROOMS OUTSIDE MAIN DWELLING*	Rooms……………………. [ ][ ]	
hc14	How many sleeping spaces such as mats, rugs, mattresses or beds are used in this household?	Sleeping spaces…………………….. [ ][ ]	
hc15	Does any member of the household own A watch? A bicycle? A motorcycle or motor scooter? An animal-drawn cart? A car or truck? A boat with a motor?	Yes No Watch…………… 1 2 Bicycle…………… 1 2 Motorcycle/scooter… 1 2 Animal-drawn cart… 1 2 Car/truck…………….1 2 Boat with motor…… 1 2	
hc16	Does any member of the household own any agricultural land?	Yes……………………………………….…..1 No……………………………………….……2	→hc20
hc17	How many acres of land for farming or grazing do members of this household own? *IF NONE, RECORD 0000.0* *IF 9500 ACRES OR MORE OR TOO LARGE TO ESTIMATE, RECORD 9500.00* *IF DOESN"T KNOW, RECORD 9999.8*	Acres for farming…… [ ][ ][ ][ ].[ ] Acres for grazing……. [ ][ ][ ][ ].[ ]	
hc18	Does the household use land for farming or grazing that it doesn't own?	Yes, rented……………………………………1 Yes, sharecropped……………………………2 Yes, private land provided free………………3 Yes, open access/communal……………….....4 No…………………………………………......5	→hc20
hc19	How many acres of land are used? *IF NONE, RECORD 0000.0* *IF 9500 ACRES OR MORE OR TOO LARGE TO ESTIMATE, RECORD 9500.00* *IF DOESN"T KNOW, RECORD 9999.8*	Acres for farming…… [ ][ ][ ][ ][ ] Acres for grazing……. [ ][ ][ ][ ][ ]	
hc20	Does the household own any livestock, herds, other farm animals, or poultry?	Yes……………………………1 No……………………………..2	→hc22
hc21	How many of the following animals does this household own? *IF 95 OR MORE, ENTER '95'*	Cattle……………………… [ ][ ] Cows/Bulls……………….. [ ][ ]	

	IF UNKNOWN, ENTER '98' Cattle? Milk cows or bulls? Horses or donkeys? Goats? Sheep? Pigs? Chickens or other poultry?	Horses or donkeys……….. Goats…………………….. Sheep…………………….. Pigs……………………….. Chickens/poultry…………..	
hc22	Does any member of this household have a bank account?	Yes……………………….……1 No…………………………..…2	
hc23	How far is it to the nearest marketplace? *IF LESS THAN 1 KM, ENTER '00'* *IF MORE THAN 95 KM, ENTER '95'*	KILOMETERS………	

HOUSEHOLD ROSTER

	A. Name	B. Sex	C. Age *(in years OR months)*		For persons aged > 5 years	
			Years	Months	D. Currently attending school or college?	E. 7 or more years of education?
Caregiver		M / F			Yes / No	How many years of education? *Don't know enter 98*
Child		M / F				
3		M / F			Yes / No	Yes / No
4		M / F			Yes / No	Yes / No
5		M / F			Yes / No	Yes / No
6		M / F			Yes / No	Yes / No
7		M / F			Yes / No	Yes / No
8		M / F			Yes / No	Yes / No
9		M / F			Yes / No	Yes / No

10		M / F	☐☐	☐☐	Yes / No	Yes / No
11		M / F	☐☐	☐☐	Yes / No	Yes / No
12		M / F	☐☐	☐☐	Yes / No	Yes / No
13		M / F	☐☐	☐☐	Yes / No	Yes / No
14		M / F	☐☐	☐☐	Yes / No	Yes / No
15		M / F	☐☐	☐☐	Yes / No	Yes / No
16		M / F	☐☐	☐☐	Yes / No	Yes / No

Check the roster regarding completion!

HOUSEHOLD HUNGER SCALE (FOOD SECURITY)			
N°	QUESTIONS	ANSWERS	SKIPS
hh1	How many times in the last month was there ever no food to eat of any kind in your house because of lack of resources to get food?	Number of times ☐☐ *If 'none', record 00.*	
hh2	How many times in the last month did you go to sleep at night hungry because there was not enough food? *If 'none', record 00.*	Number of times ☐☐ *If 'none', record 00.*	
hh3	How many times in the last month did you go a whole day and night without eating anything at all because there was not enough food? *If 'none', record 00.*	Number of times ☐☐ *If 'none', record 00.*	

INFANT AND YOUNG CHILD FEEDING (IYCF)			
N°	QUESTIONS	ANSWERS	SKIPS
f1	Is …………………….. (Name) currently breastfed?	Yes / No	
f2	Does ……………………………… (Name) take any food or drink other than breast milk?	Yes / No	
f3	How many times was …………………………………………… (Name) fed porridge/pap or stiff porridge/ rice/ potatoes/ other adult foods as a meal or a snack since this time yesterday?	Number of times ☐☐	
MOTHER AND CHILD DIETARY DIVERSITY			

Since this time yesterday, what food did you and …………………………… (Name) eat?
Probe for items not mentioned: Did you and ………………………. (name) have any of the following things to eat or drink? Meat, fruits, vegetables, juices, milk

Circle Yes if eaten since this time yesterday, and No if not eaten.

N°	ITEMS	A. Caregiver	B. Child
dd01	Plain water?	Yes / No	Yes / No
dd02	Tinned, powdered, infant formula such as S26, Cerelac, Nido, Nan, Lactogen, Ninolac, Lailac, or milk (excluding breast milk)	Yes / No	Yes / No
dd03	Sweetened or flavoured water, minerals, malt drinks, tea, coffee, liquor, beer, soda	Yes / No	Yes / No
dd04	Any food made from grain such as maize, millet, wheat, millets, sorghum, rice	Yes / No	Yes / No
dd05	Any food made from fruits or vegetables that have yellow or orange flesh (carrots, mangoes, papaya, squash, melon)	Yes / No	Yes / No
dd06	Any dark green leafy vegetables (spinach, cassava leaves, amaranth, potato leaves, moringa leaves, okra leaves, cowpea leaves)	Yes / No	Yes / No
dd07	Any food made from roots or tubers (potatoes, sweet potatoes, yams, cassava)	Yes / No	Yes / No
dd08	Any food made from beans, peas, nuts, or seeds (palm seed, peanuts, soybeans, sesame, sunflower)	Yes / No	Yes / No
dd09	Any other fruits or vegetables (coconut, eggplant, tomatoes, peppers, avocado, lemon, banana, plantain, orange, mandarin, apple, cucumber, okra, onion) and fruit juices (orange, passion, roselle, ginger, baobab)	Yes / No	Yes / No
dd10	Liver, kidney, heart, or other organ meats	Yes / No	Yes / No
dd11	Any meat such as beef, pork, lamb, mutton, goat, chicken, duck, guinea fowl	Yes / No	Yes / No
dd12	Fresh or dried fish, shellfish, or seafood, snails, crabs (makerel, sea bream, capitaine, carp, shrimp fish, shrimps)	Yes / No	Yes / No
dd13	Cheese, yoghurt, or other milk products	Yes / No	Yes / No
dd14	Eggs (chicken and quail)	Yes / No	Yes / No
dd15	Sugary foods such as sugar cane, sweets, candies, chocolate, cakes, and biscuits	Yes / No	Yes / No
dd16	Any food made with oil, fat, or butter	Yes / No	Yes / No
dd17	Red palm oil	Yes / No	Yes / No

MOTHER AND CHILD HEALTH AND NUTRITION DATA			
N°	QUESTIONS	ANSWERS	REMARKS
MOTHER			
preg	Are you currently pregnant?	Yes / No	

lact	Are you currently breastfeeding a child?	Yes / No	
vasm	Have you taken a vitamin A capsule in the past 6 months?	Yes / No / Don't know	
CHILD			
vas	Has………………………………… (Name) taken a vitamin A capsule in the past 6 months? *Show vitamin A capsule.*	Yes / No / Don't know	
hght	*Take the height of the child* *If 'refused' write 666.6*	cm ☐☐☐.☐	
wght	*Take the weight of the child* *If 'refused' write 66.666*	Kg ☐☐.☐☐☐	
bcg	*Check for BCG scar on left arm*	Yes / No	
oed	*Check for oedema*	Yes / No	*If oedema present → Refer!*
bloo	*Take finger prick blood sample for child*	*Sticker here with sample number or write 'refused'*	

OIL FORTIFICATION COVERAGE (1)			
N°	QUESTIONS	ANSWERS	SKIPS
of1	What is the <u>main</u> edible <u>oil</u> consumed by your household (*clarify with: the oil that you use on most days in most meals in the home*)? *Circle only <u>one</u> answer.*	Red Palm oil ………..…....................1 Soybean oil...2 Groundnut oil.....................................3 Sunflower oil.....................................4 Olive oil..5 Cotton seed oil ……………………...6 Vegetable blend oil............................7 Don't know/Don't remember……….8 Other: __________________9	
of2	Can you tell me where you usually get this ……………………………….……… oil? *Circle only <u>one</u> answer.*	Purchased from supermarket……….1 Purchased from shops........................2 Purchased from open markets............3 Purchased from market / street stand..4 Purchased from moving street vendor ....5 Made it at home..................................6 Received from food aid.......................7 Don't know/Don'tremember………..8 Other: __________________9	If 'Made it at home' (20), skip to next page
of3	This ………………………..…… oil that you consume, when you get it, is it usually packaged or open? *Open means packaged in another bottle than the original one, in a sachet or from a big container.*	Packaged...1 Open...2 Don't know / Don't remember...........8 Other: __________________9	
of4	Can you tell me the brand of this oil you use?	VOIL.....................................1	

	Circle only <u>one</u> answer.	SUNOLA................................2 WESSON................................3 KORIE....................................4 MPISHI...................................5 SUNDROP..............................6 SAFI.......................................7 MARHABA............................8 SUNFLOWER OIL...............9 OKI......................................10 VIKING...............................11 OKAY..................................12 AFYA ……..……….……..13 FLORAL………………….14 ALFA……………………..15 SUNFRY………………….16 ASMA…………………….17 MSWANU………………..18 OLIEN……………………19 MAISHA…………………20 GOLDEN…………………21 JOLIE…………………….22 SUNLITE………………...23 Don't know/Don'tremember..88 Other: _________________99	

OIL FORTIFICATION COVERAGE (2)

N°	QUESTIONS	ANSWERS	SKIPS
of5	The <u>last time</u> your household received/purchased ………………………………… (name of oil), how much did you get? *Write in the number <u>and</u> circle the unit.* *If 'don't know', record 8888.*	A. Quantity ☐☐☐☐ B. Kg...1 g...2 L...3 mL..4 CFA..5	
of6	How long does this amount usually last in your household? *Write in the number <u>and</u> circle the unit.* *If 'don't know', record 88.*	A. Duration ☐☐ B. Day(s)..1 Month(s)..2	

VITAMIN A KNOWLEDGE, ATTITUDE AND PRACTICES

N°	QUESTIONS	ANSWERS	SKIPS
va01	Have you heard information about vitamin A?	Yes / No	If No, skip to end
va02	.If yes, what are the main benefits?	Helps with child growth..........................1	

	(can select more than one answer if appropriate: do not prompt)	Strengthens immunity to illness..............2 Improves vision……………………….....3 Improves health/reduces illness……......4 Avoids night blindness…………………5 Reduces mortality………………………6 Other_______________7 (specify)	
va03	If yes, where did you hear about vitamin A?	Health center……..................................1 Community health worker......................2 Neighbours...3 Radio..4 Television...5 Other_________________________ 6 (specify)	
va04	Could you list up to three foods rich in Vitamin A?	________________ ________________ ________________	
va05	What methods can one use to avoid a diet poor in vitamin A?	Balanced diet….......................................1 Foods rich in vitamin A..........................2 Vitamin A supplement............................3 Foods fortified with vitamin A………...4 Don't know…………………………….5 Other__________________________6 (specify)	

*** CHECK THE QUESTIONNAIRE & THANK THE MOTHER!***

Appendix 4: Follow Up Household Questionnaire

dateint	Date of interview	DD / MM / YY ☐☐ / ☐☐ / 1 6			
teamid	Team identifier	☐☐		Interviewer identifier	☐☐
wardid	District				☐☐
village	Ward				
psu	Village/Street				
psuname	Hamlet leader				
hh	Household head				
Cons	Consent obtained?	Yes, written/ Yes, oral / No			If yes, begin If no, end
Cell	Cellphone number available?	Yes / No			

HOUSEHOLD ROSTER					
	A. Name	B. Sex	C. Age *(in years OR months)*		Is this individual still a member of the household?
			Years	Months	
Caregiver		M / F	☐☐		Yes / No
Child		M / F		☐☐	Yes / No

Check the roster regarding completion!

HOUSEHOLD HUNGER SCALE (FOOD SECURITY)			
N°	QUESTIONS	ANSWERS	SKIPS
hh1	How many times in the last month was there ever no food to eat of any kind in your house because of lack of resources to get food?	Number of times ☐☐ *If 'none', record 00.*	
hh2	How many times in the last month did you go to sleep at night hungry because there was not enough food? *If 'none', record 00.*	Number of times ☐☐ *If 'none', record 00.*	
hh3	How many times in the last month did you go a whole day and night without eating anything at all because there was not enough food?	Number of times ☐☐ *If 'none', record 00.*	

	If 'none', record 00.		

INFANT AND YOUNG CHILD FEEDING (IYCF)

N°	QUESTIONS	ANSWERS	SKIPS
f1	Is …………………….. (Name) currently breastfed?	Yes / No	
f2	Does ……………………………… (Name) take any food or drink other than breast milk?	Yes / No	
f3	How many times was …………………………………………… (Name) fed porridge/pap or stiff porridge/ rice/ potatoes/ other adult foods as a meal or a snack since this time yesterday?	Number of times ☐☐	

MOTHER AND CHILD DIETARY DIVERSITY

<u>Since this time yesterday</u>, what food did you and …………………………… (Name) eat?

Probe for items not mentioned: Did you and ………………………. (name) have any of the following things to eat or drink? Meat, fruits, vegetables, juices, milk

Circle Yes if eaten since this time yesterday, and No if not eaten.

N°	ITEMS	A. Caregiver	B. Child
dd01	Plain water?	Yes / No	Yes / No
dd02	Tinned, powdered, infant formula such as S26, Cerelac, Nido, Nan, Lactogen, Ninolac, Lailac, or milk (excluding breast milk)	Yes / No	Yes / No
dd03	Sweetened or flavoured water, minerals, malt drinks, tea, coffee, liquor, beer, soda	Yes / No	Yes / No
dd04	Any food made from grain such as maize, millet, wheat, fonio, sorghum, rice	Yes / No	Yes / No
dd05	Any food made from fruits or vegetables that have yellow or orange flesh (carrots, mangoes, papaya, squash, melon)	Yes / No	Yes / No
dd06	Any dark green leafy vegetables (spinach, cassava leaves, amaranth, potato leaves, moringa leaves, okra leaves)	Yes / No	Yes / No
dd07	Any food made from roots or tubers (potatoes, sweet potatoes, yams, cassava, tiger nuts)	Yes / No	Yes / No
dd08	Any food made from beans, peas, nuts, or seeds (palm seed, peanuts, soybeans, sesame)	Yes / No	Yes / No
dd09	Any other fruits or vegetables (coconut, eggplant, tomatoes, peppers, zucchini, avocado, lemon, banana, plantain, orange, mandarin, apple, cucumber, okra, onion) and fruit juices (orange, passion, roselle, ginger, baobab)	Yes / No	Yes / No
dd10	Liver, kidney, heart, or other organ meats	Yes / No	Yes / No
dd11	Any meat such as beef, pork, lamb, mutton, goat, chicken, duck, guinea fowl	Yes / No	Yes / No
dd12	Fresh or dried fish, shellfish, or seafood, snails, crabs (makerel, sea	Yes / No	Yes / No

	bream, capitaine, carp, shrimp fish, shrimps)		
dd13	Cheese, yoghurt, or other milk products	Yes / No	Yes / No
dd14	Eggs (chicken and quail)	Yes / No	Yes / No
dd15	Sugary foods such as sugar cane, sweets, candies, chocolate, cakes, and biscuits	Yes / No	Yes / No
dd16	Any food made with oil, fat, or butter	Yes / No	Yes / No
dd17	Red palm oil	Yes / No	Yes / No

MOTHER AND CHILD HEALTH AND NUTRITION DATA			
N°	QUESTIONS	ANSWERS	REMARKS
MOTHER			
preg	Are you currently pregnant?	Yes / No	
lact	Are you currently breastfeeding a child?	Yes / No	
vasm	Have you taken a vitamin A capsule in the past 6 months?	Yes / No / Don't know	
CHILD			
vas	Has………………………………… (Name) taken a vitamin A capsule in the past 6 months? *Show vitamin A capsule.*	Yes / No / Don't know	
hght	*Take the height of the child* *If 'refused' write 666.6*	cm ☐☐☐.☐	
wght	*Take the weight of the child* *If 'refused' write 66.666*	Kg ☐☐.☐☐☐	
bcg	*Check for BCG scar on left arm*	Yes / No	
oed	*Check for oedema*	Yes / No	*If oedema present → Refer!*
bloo	*Take finger prick blood sample for child*	*Sticker here with sample number or write 'refused'*	

OIL FORTIFICATION COVERAGE (1)			
N°	QUESTIONS	ANSWERS	SKIPS
of1	What is the main edible oil consumed by your household (*clarify with: the oil that you use on most days in most meals in the home*)? *Circle only one answer.*	Red Palm oil ……………………………….....1 Soybean oil...2 Groundnut oil..3 Sunflower oil...4 Olive oil..5	

		Cotton seed oil ……..………………………...6 Vegetable blend oil...7 Don’t know / Don’t remember.........................8 Other: ______________________9	
of2	Can you tell me where you usually get this ……………………………….……… oil? *Circle only <u>one</u> answer.*	Purchased from supermarket............................1 Purchased from shop / superette......................2 Purchased from boutique / kiosk......................3 Purchased from market/street stand…………4 Purchased from moving street vendor ……....5 Made it at home...6 Received from food aid...................................7 Don’t know/Don’t remember...........................8 Other: _____________________..................._9	If ‘Made it at home’ (20), skip to next page
of3	This ……………………….…… oil that you consume, when you get it, is it usually packaged or open? *Open means packaged in another bottle than the original one, in a sachet or from a big container.*	Packaged...1 Open...2 Don’t know / Don’t remember..........................8 Other ______________________9	
of4	Can you tell me the brand of this oil? *Circle only <u>one</u> answer.*	VOIL...1 SUNOLA...2 WESSON...3 KORIE...4 MPISHI...5 SUNDROP...6 SAFI...7 MARHABA...8 SUNFLOWER OIL...9 OKI..10 VIKING...11 OKAY...12 AFYA ………………………………………13 FLORAL……………………………………14 ALFA……………….………………………15 SUNFRY……………………………………..16 ASMA………………………………………17 MSWANU………………………………......18 OLIEN…………………………………..…..19 MAISHA……………………………………..20 GOLDEN……………………………………21 JOLIE………………………………………..22 SUNLITE……………………………………23 Don’t know/Don’t remember……………….88 Other: ………………………………………99	

OIL FORTIFICATION COVERAGE (2)			
N°	QUESTIONS	ANSWERS	SKIPS
of5	The <u>last time</u> your household received/purchased ………………………………… (Name of oil), how much did you get? *Write in the number <u>and</u> circle the unit.* *If ‘don’t know’, record 8888.*	A. Quantity ☐☐☐☐ B. Kg..1 g...2 L...3 mL..4 CFA...5	

of6	How long does this amount usually last in your household? *Write in the number* *<u>and</u>* *circle the unit.* *If 'don't know', record 88.*	A. Duration ☐☐ B. Day(s)..1 Month(s)..2	
	In areas where e-voucher is available, continue with questions below. In control area, skip to va01		
of7	Have you used the e-voucher to buy oil in the last month?	Yes / No	→of8b
of8a	If yes, did you like using the e-voucher?	Liked it……………………………………….1 Did not like it………………………………...2 No opinion………………………………..…..3 Other _____________________________ 4 (specify)	
of8b	If no, why not?	Didn't have access to cell phone…………..….1 Desired quantity too small……………..……...2 Store did not accept e-voucher……………….3 Did not like to use e-voucher………………...4 Don't know…………………………………...…5 Other______________________________ 6 (specify)	

VITAMIN A KNOWLEDGE, ATTITUDE AND PRACTICES			
N°	QUESTIONS	ANSWERS	SKIPS
va01	Have you heard information about vitamin A?	Yes / No	If No, skip to end
va02	.If yes, what are the main benefits? *(can select more than one answer if appropriate: do not prompt)*	Helps with child growth.................................1 Strengthens immunity to illness…………….2 Improves vision……………………………..3 Improves health/reduces illness……….…...4 Avoids night blindness………………….….5 Reduces mortality……………………….….6 Other____________________________ 7 (specify)	
va03	If yes, where did you hear about vitamin A?	Health center……..1 Community health worker.............................2 Neighbours...3 Radio..4 Television..5 Other_____________________6 (specify)	
va04	Could you list up to three foods rich in Vitamin A?	____________________________ ____________________________ ____________________________	
va05	What methods can one use to avoid a diet poor in vitamin A?	Balanced diet…...1 Foods rich in vitamin A................................2 Vitamin A supplement..................................3 Foods fortified with vitamin A……………......4 Don't know…………………………………5	

		Other____________________6 (specify)	

***** CHECK THE QUESTIONNAIRE & THANK THE MOTHER! *****