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OF MEDICAL RESEARCH: L

Nutrition and Food Science

A Food Desert Simulation

Growth of Lactobacillus Casei

{ Highlights }

Effect of Various Parameters

Monitoring Major Sugars in Greek

Discovering Thoughts, Inventing Future

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Into the (Food) Desert: A Food Desert Simulation

By Mark J. Bonica & Kerryn L. Story

University of New Hampshire, United States

Abstract- Objective: Explore the causal relationship between food deserts and presumed health outcomes of obesity, diabetes, and heart disease.

Design: Simulate food desert conditions by having a subject eat only what he could purchase in convenience stores for 30 days on a financially constrained budget.

Setting: Conducted as a field experiment utilizing local convenience stores. Subjects: One of the coauthors acted as the subject.

Results: The results indicate that typical measures of negative health outcomes associated with food deserts, such as weight gain, elevated cholesterol, and elevated blood sugar, were not supported. However, the intake of many micronutrients was significantly below recommended levels.

Conclusions: Results suggest that further investigation of other diet-induced illnesses should have greater attention in food desert research.

Keywords: food desert, nutrition, food insecurity, food security.

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Into the (Food) Desert: A Food Desert Simulation

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"All models are wrong, some are useful." – George E. Box

I. Introduction

The term "food desert" came into political discourse and later into academic literature initially in Britain during the 1990's when a resident of a public housing project made the claim that his health was adversely affected by his inability to conveniently purchase nutritious food (Cummins & Macintyre, 2002). Michelle Obama, in her role as First Lady and her "Let's Move" campaign, referred to food deserts as "nutritional wastelands that exist across America in both urban and rural communities where parents and children simply do not have access to a supermarket" (the White House, 2010). The term has been invoked to justify policy interventions to subsidize the opening of full-service supermarkets in areas identified as food deserts with tax breaks, grants, and other incentives (Wenger, 2015; Reinvestment Fund, n.d.).

There have been many efforts to define exactly what a food desert is and to define a methodology for identifying geographic areas that qualify as food deserts (Sohi, Bell, Liu, Battersby, & Liese, 2014). The literature generally agrees that a food desert is a geographic area occupied by low income residents who have limited access to retail sellers of fresh fruit and vegetables (Shaw, 2006) (Dutko, Ver Ploeg, & Farrigan, 2012). Most definitions include the claim that a typical resident of a food desert is condemned to purchase her/his food resources from a convenience store or a fast food restaurant (Walker, Keane, & Burke, 2010).

For purposes of this simulation, we will use the United States Department of Agriculture (USDA) definition of a food desert, which follows:

Food deserts are defined as urban neighborhoods and rural towns without ready access to fresh, healthy, and affordable food. Instead of grocery and supermarket stores, these communities may have no food access or are served only by fast food restaurants and convenience stores that offer few healthy, affordable food options. The lack of access contributes to a poor diet and can lead to higher levels of obesity and other diet-related diseases, such as diabetes and heart disease (USDA Agricultural Marketing Service, 2015).

As noted in the USDA definition, food deserts can exist in both rural and urban environments. The operating assumption is that in either case the residents of an area designated as a food desert have difficulty accessing healthy, affordable food options largely as a result of transportation challenges. The USDA uses Census tracts to identify food deserts and relies on two screening criteria: 1) that the community is "low-income" defined as "a) a poverty rate of 20 percent or greater, OR b) a median family income at or below 80 percent of the area median family income"; and 2) that the community is "low access" defined as more than 1/3 of the community lives at least one mile from a super market or large supermarket store in the case of an urban census tract or ten miles from a super market or large supermarket store in the case of a rural census tract (USDA Agricultural Marketing Service, 2015). Using this definition the USDA provides a food desert locator tool ("USDA USDA-ERS Food Desert Locator") which allows researchers and policy makers to locate official food deserts in the United States.

Other researchers have used more sophisticated techniques to attempt to identify food deserts, such as geographic information system (GIS) technology which enables route mapping and more accurate measures of travel requirements between

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residents' homes and supermarkets (Jiao, Moudon, Ulmer, Hurvitz, & Drewnowski, 2012) (Leclair & Aksan, 2014) (Liese, Hibbert, Ma, Bell, & Batterbsy, 2014). Regardless of the methodology for actually identifying the presence of food deserts, the operating assumption is the same: individuals residing in food deserts get the majority of their nutrition from convenience stores and fast food restaurants. The fact that they are limited to seeking nutrition in this environment is suggested to lead to poorer quality diets which in turn contribute to diet-related chronic conditions such as obesity, diabetes, and heart disease (Adams, Ulrich, & Coleman, 2010).

The literature on food deserts and health outcomes has correlational research but little by way of experimental evidence. This led the authors of this study to ask the question, holding all other things constant, what are the effects of being exposed to a food desert? Is geography destiny? Does living in a food desert limit an individual to a poor quality diet? Or is it possible with reasonable care and discipline to consume a nutritious, sustainable diet even under the constraints defined by the food desert literature? To expand our knowledge about these questions, we simulated a journey into the food desert.

II. Methods

The experiment is a single-case, A-B design (Herson, 1984). A single case, A-B design is limited in its generalizability (AHRQ, 2014), but can serve as a qualitative heuristic case to explore a concept that is not well defined (Kleining & Witt, 2000). Given the uncertainty in the literature about the actual effects of attempting to seek nutritious food when one simply does not have access to a supermarket, but the fact that policy has been built around the assumptions of specific chronic illness, this case study attempts to explore the effects of such conditions separate and in isolation from other confounding factors of poverty.

No consent was required as one of the authors (author 1) was the subject. The subject was a male, 68 inches tall, age 44 during the experiment.

a) Simulating Food Desert Conditions

The experimental design was intended to simulate food desert conditions. The design involved the subject eating only food available for purchase at a convenience store for a period of 30 days. The only exception was tap water, which he was allowed to use from any available source. No explicit restrictions were placed on the subject in terms of what he could or could not buy at a convenience store, thus he could have eaten prepared foods such as sandwiches or pizza if it was available. He was allowed to purchase foods which would become ingredients in home prepared meals, such as peanut butter, flour, or tomato sauce as long as he could purchase them in a convenience store.

During the treatment phase, the subject also targeted a daily food resource expenditure of $7.25. This target was selected because it would represent a single hour's wages at the federal minimum wage. The $7.25 target was not hard a restriction. The subject could spend more or less, but the subject targeted average food resource expenditures at this amount. The target functioned as a goal to improve the simulation. Food desert conditions imply not only poor access to full service supermarket stores, but also lower household income levels.

The target food resource expenditure was not necessarily an actual daily expenditure. The subject charged himself only for the value of the food consumed during the course of any given day, not the total amount spent. For example, if the subject purchased a jar of peanut butter, and the cost of the jar was $4.19 but there were 10 servings in the jar, the subject would charge himself $0.42 each time he consumed a serving of peanut butter. Thus, on the day the peanut butter was purchased, if the subject consumed only one serving of peanut butter, the food diary would reflect an expenditure of $0.42, not the whole $4.19.

b) Data Collection

The subject tracked exercise during the treatment phase using a personal activity tracker (a Fitbit Flex™). The subject generally only engaged in walking for intentional exercise in addition to ordinary activities of daily living. Other similar normal activities of daily living such as moving around the kitchen while cooking or walking through a store while shopping were captured by the personal activity tracker, thus the activity tracker is a reasonable estimate of actual exercise.

The subject used a spreadsheet-based food diary to record daily food consumption in terms of cost and caloric value. Calories were derived from a smart phone app (Loseit™). During the 30 day experiment, Lose it™ converted the activity tracker information into calories burned. Following the 30 day period, the subject re-entered the food diary into the US Department of Agriculture's Super Tracker™ web site (https://www.supertracker.usda.gov/) in order to produce a more complete nutritional record of intake over the previous 30 day period. Following the experimental period, the authors reconstructed the cost of foods consumed each day by shopping at a local supermarket store to develop a comparison daily cost if the subject had been able to shop outside in normal conditions, rather than only in convenience stores.

Labs were taken immediately prior to and immediately after the conclusion of the treatment period, providing A-B design. Labs were not redrawn 30 days after the end of the treatment period. The following labs were taken: Serum Cholesterol, LDL Cholesterol, HDL Cholesterol, Triglyceride, Glucose, and Hemoglobin A1C.

c) Defining a "convenience store"

The National Association of Convenience Stores (NACS) divides convenience stores into six format categories based on size and offerings. During the treatment phase the subject shopped at convenience stores that would generally fit the "limited selection convenience store" or "traditional convenience store" formats (NACS, 2015). These store formats are characterized by limited grocery selections and store sizes of less than 2,500 sq ft. The subject occasionally used a smaller convenience store known as "kiosks" or "mini convenience stores" when traveling, but was largely able to stay within the limited or traditional formats. He purposely avoided anything resembling expanded or hyper-convenience store formats (larger store formats bordering on supermarket stores) so as not to have access to more goods than are suggested to be available in a food desert.

During the treatment period, the subject resided in a medium-sized town in southern New Hampshire and commuted to a smaller town for work at a state university. There were several convenience stores in both towns, as well as several standard supermarket stores owned by regional or national chains. The convenience stores were a mix of independent, "mom and pop" stores as well as stores that were part of regional or national chains. The subject used a car for transportation and explored as many convenience stores in the area as possible.

Although many convenience stores now include fast food restaurant counters, the subject did not consume restaurant food during this experiment. Fast food restaurants are included in most food desert definitions, however the cost of such food would have been prohibitive under the $7.25 daily target.

III. Findings

The treatment period began February 2, 2015 and concluded after March 3, 2015. The subject successfully completed the 30 day treatment period, only eating food he could purchase in a convenience store.

a) Cost results

The subject consumed a mean of $8.89 of food per day, with a standard deviation of $3.12. Comparison supermarket store costs were calculated by finding food prices at a local store of a regional supermarket chain in the subject's town.

Table 1: Comparison of convenience store to supermarket store costs

	Convenience Store Costs	Grocery Store Costs
total	$ 262.46	$ 183.67
mean	$ 8.89	$ 6.12
median	$ 7.58	$ 4.29
SD	$ 3.12	$ 3.05
low	$ 5.06	$ 2.72
high	$ 5.57	$ 29.23

Supermarket store food prices were calculated by comparing items that were as close as possible to identical to the ones purchased at convenience stores. This involved finding the same size containers of food, (i.e., looking for the same size box of instant rice) rather than seeking out available economies of scale in a larger box. The comparison supermarket store prices represent the most similar food products to the same degree as possible. A larger difference could have been found by allowing for economies of scale in purchasing, therefore the reported difference underestimates the potential difference had the subject had the ability to shop at a supermarket store.

b) Body Weight change

The subject had a starting weight of 175.8 pounds and a BMI of $26.7 Kg/m^2$, [1] on the morning of February 2nd (day 1 of the 30 day experiment). On the morning of March 4th (the morning following the last day of the treatment, and the day the second set of labs were drawn) the subject weighed 163.5 pounds and had a BMI of $24.9 Kg/m^2$, for a loss of 12.3 pounds. Chart 1 shows the weight change over the 30 day treatment period.

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[1] BMI calculated using National Institute for Health online calculator at http://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm

Chart 1: Weight Change

Subject's daily weight in pounds.

Chart 2: Calories consumed and exercise
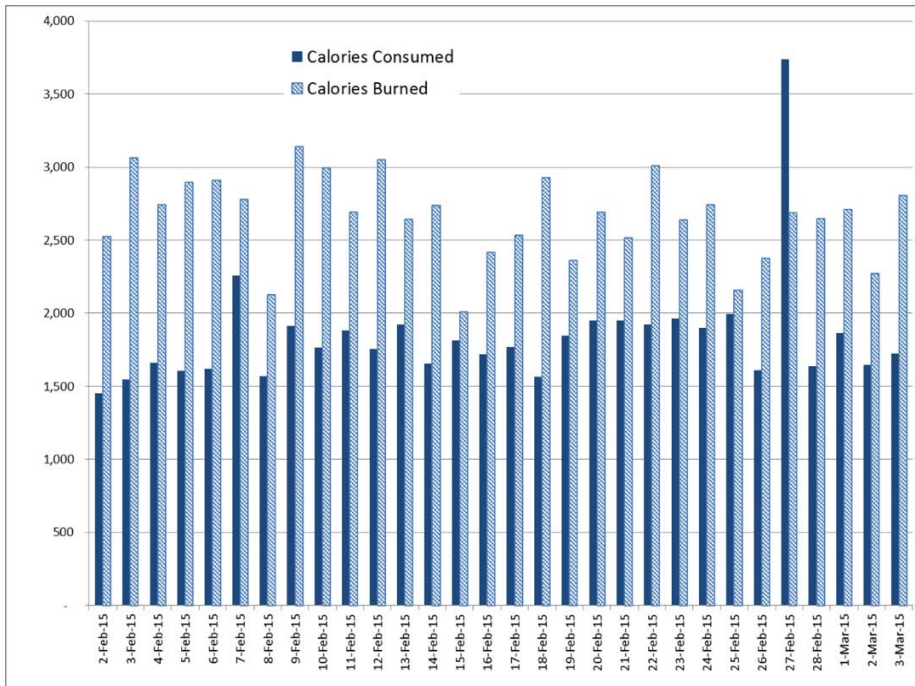
Table 2: Calories consumed

	Calories Consumed	Calories Burned
mean	1,852	2,660
median	1,763	2,693
SD	187	289
low	1,453	2,007
high	3,739	3,139

4

According to National Institutes of Health BMI standards (National Institutes of Health, 2015), the subject should maintain a weight between 125 (19Kg/m^2 BMI) and 164 pounds (25Kg/m^2 BMI) to be in the normal range. Using the Harris Benedict and Mifflin-St Jeor equations (Amirkalali, Heshmat, & Larijani, 2008) and a mid-point of 144 Paunds, the Harris-Benedict predicted resting energy expenditure (PREE) for the subject was 1,531 calories and 1,518 calories respectively. Caloric intake was well above the PREE throughout the experiment with a mean daily intake of 1,852 kcal. For comparison, a survey of residents from two communities residing in food deserts self-reported a daily caloric intake of 1,727 and 1,861 kcal (Dubowitz, et al., 2015). The subject was employed in a relatively sedentary job throughout the experiment, however, he engaged in moderate exercise on most days (walking), with an average of 11,890 steps per day, with a daily range from 2,137 to 20,275. The activity tracker application calculates total expenditure to include resting energy expenditure. Calories consumed (as estimated by LoseIt ® app) and calories burned (as estimated by the FitBit ® app) are depicted in chart 2 and a summary is given in table 2. Assuming the accuracy of these applications, the subject incurred a daily caloric debt of 808 calories.

Lab results

Lab results are summarized in Table 3.

Table 3: Lab Results

Test Name	Pre	Post	Reference Range	Units
Cholesterol, Serum	168	169	(</=-200)	mg/dL
HDL	62	62	(40-60)	mg/dL
LDL	97	90	(</=-130)	mg/dL
Triglyceride	45	84	(</=-150)	mg/dL
A1C	5.4	5.6	4.8-5.6	%
Fasting Plasma Glucose	102	89	(70-110)	mg/dL

"Pre" represent values at the beginning of the experiment.
"Post" represent values from the day following the experiment.
"Reference range" is the standard range for normal results.

The LDL cholesterol and fasting plasma glucose decreased between pre and post experiment readings. Fasting plasma glucose readings should be interpreted with caution, however, as readings may vary from day to day, and can be affected by some medications, posture when sample was drawn, sample handling, prolonged fasting, exercise, and acute stress (Sacks, 2011).Thus, a single elevated fasting plasma glucose reading warrants further testing. During the experiment, the subject's A1C and triglyceride levels rose. Since A1C is a reflection of long term glycemic exposure, it is a more accurate measurement of glycemic control over time. A1C levels are used in the diagnosis of diabetes. In the case of the subject, although A1C levels rose during the experiment, the level remained within the reference range. The increase could be a reflection of the intake of simple sugars throughout the course of the experiment.

c) *Nutritional Analysis*

All nutritional analyses were extracted from reports generated by USDA's Super Tracker database based on the subject's entry of foods consumed during the experiment. The subject entered all foods with serving sizes consumed into the database on a daily basis during the experiment. All food intake was self-reported and based on the subject's measurement of serving sizes. The subject maintained food logs and entered foods consumed into Super Tracker for a period of 30 days. Nutrient analyses were generated through the database.

With regard to macronutrient intake, the subject exceeded recommended grams of carbohydrate during most of the 30 days tracked. This is likely because many foods consumed were processed and therefore higher in carbohydrate content. Protein target was met on all but five of the days tracked. The third macronutrient, fat, must be categorized as monounsaturated, polyunsaturated, or saturated fat in order to provide meaningful nutritional interpretation of daily intake. During eight of the 30 days, or 26% of the time, the subject's intake of saturated fat sources exceeded the 2010 Dietary Guidelines for Americans' recommendations for daily intake (<10% of total fat) (health.gov, 2015). There are no daily targets or limits set by the Dietary Guidelines for Americans for monounsaturated or polyunsaturated fat intake, however, the goal is to eat foods rich in these fats while staying within the total fat allowance for the day. Sixteen

percent of calories consumed by the subject were of monounsaturated fat sources, and 9% were of polyunsaturated fat sources.

Chart 3: Macronutrients
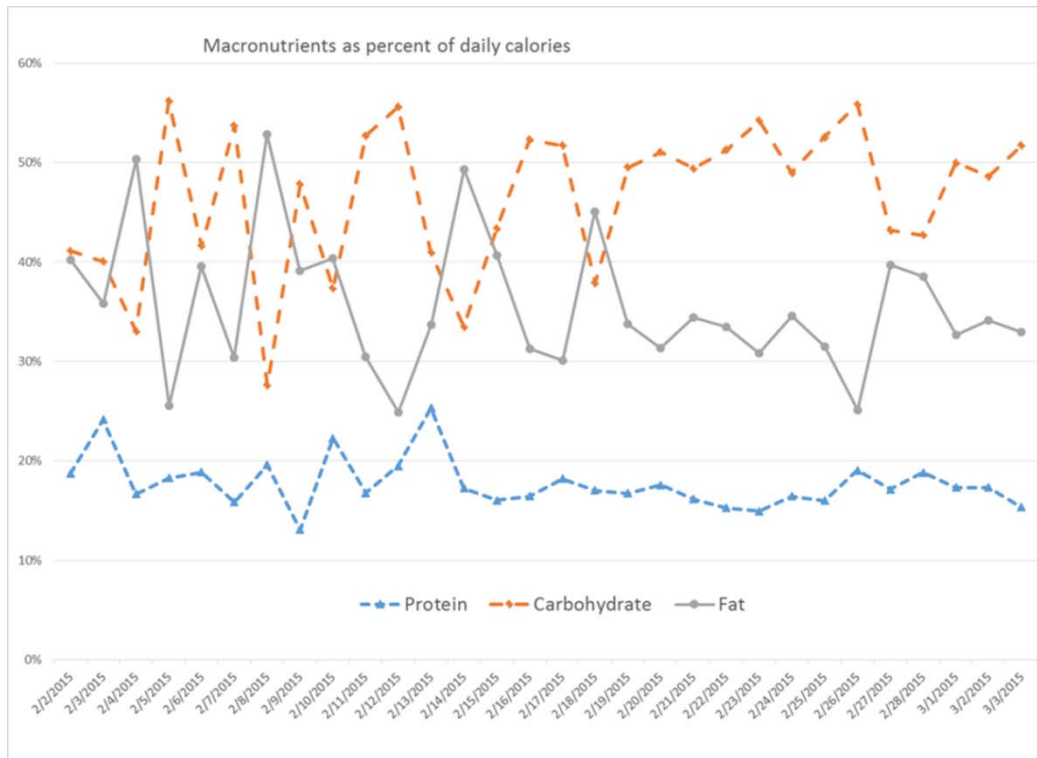
Table 4: Micronutrient Shortfalls

Micronutrient	Percent of Recommended Daily Intake
Calcium	73
Potassium	59
Megnesium	85
Zinc	81
Vitamin A	93
Vitamin C	54
Vitamin D	13
Vitamin E	73
Vitamin K	62
Choline	64
Fiber	63

With regard to micronutrient intake, averaged out over the 30 day period, the subject fell short in meeting the recommended daily intakes for some vitamins and minerals:

Fiber intake was also inadequate to meet the recommended intake on all but one day during the 30 day trial. Fiber averaged 24 grams per day, meeting only 63% of daily needs (38 gram target). The diet exceeded the recommended daily intake for sodium and iron.

Over a period of 30 days, the lack of or overconsumption of micro or macronutrients would not be overly concerning. In the case of this experiment, for this subject, there was an end date to the experiment, a time when foods no longer had to be purchased only at a convenience store. However, such is not the case for many individuals who live within a food desert and must survive for years on foods lacking nutrient density. Vitamin and mineral deficiencies or excesses, over time, manifest in clinical symptoms.

Another interesting finding in the nutrient analysis was that the subject's intake of sugars, particularly added sugars, trended upwards from the first day of the diet.

Chart 4: Dietary Sugar
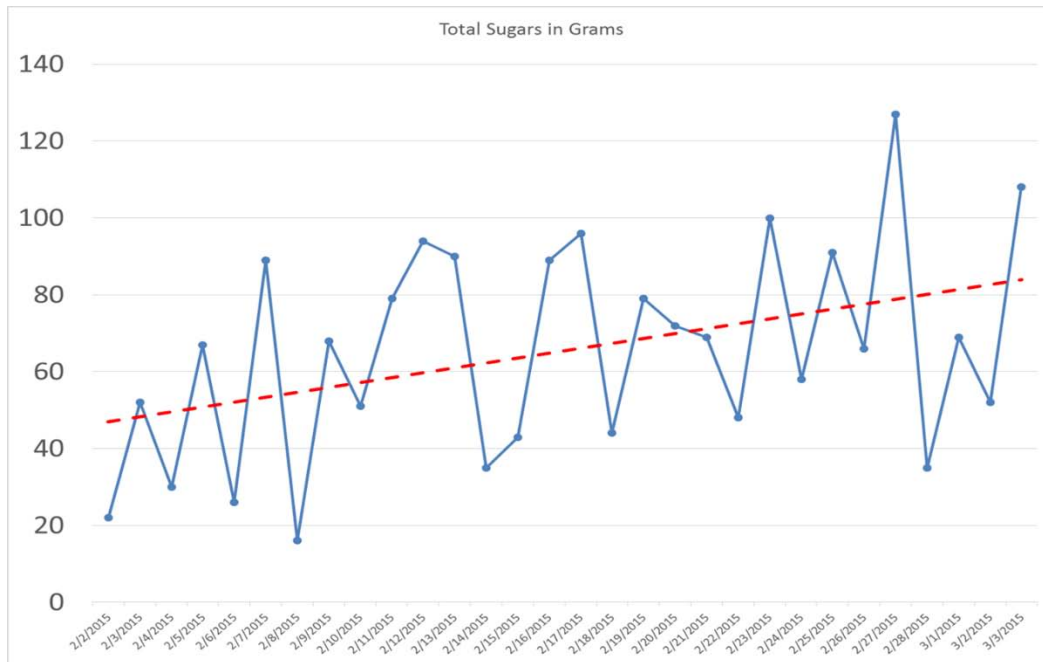
The chart tracks the daily total grams of sugar consumed in the diet. The trend line shows an upward drift in the number of grams consumed each day.

Sugars provide no additional nutrient value but do supply additional calories and account for the sweetened taste of some foods, particularly baked goods. Added sugars include sources such as white sugar, high fructose corn syrup, maple syrup, honey, molasses, or brown sugar, to name a few. The upwards trend could have represented the subject's preference for sweeter foods the longer he consumed the diet, or it could be because foods available at the convenience stores in the food desert contained added sugars to improve their flavor.

IV. DISCUSSION

The literature on food deserts includes a variety of methods for identifying food deserts in practice, but nearly all of the literature on the subject of identifying food deserts includes the assumption that what researchers are looking for is an environment where financially constrained (poor) residents are limited to consuming a diet that comes from convenience stores and fast food restaurants, as identified by the USDA. It is important to remember that the definition is two part: access limitations and financial limitations.

The experimental results do not support the broader claim that the access portion of food deserts cause obesity, high cholesterol, or diabetes. Even within the very restrictive definition used in this experiment, there were still choices that could be made that allowed

the subject to maintain a healthy weight, and showed no major changes in indicators for heart disease. The slight elevation of A1C reflects an increase in the consumption of carbohydrates, as does the increase in triglycerides. Both of these measures were within normal ranges as of the end of the experiment, but it would be interesting to see how long term exposure to this diet would affect those measures. A longer term exposure could answer whether A1C and triglyceride levels would continue to rise or level out.

Based on these results, it does not appear eating in a food desert is unambiguously harmful with regard to the primary conditions typically associated with food deserts (obesity, heart disease, and diabetes). Much can be done to mitigate the influence of the environment by adhering to some simple rules. The subject employed relatively simple rules of thumb throughout the experiment, such as avoiding chips, candy, and sweetened beverages when possible. He sought to have at least one vegetable and one fruit each day. After an initial purchase of a kielbasa sausage, for example, he tried to avoid meats of higher fat content (hot dogs, sausages) that were available in most stores where meat was sold. None of the stores the subject shopped in sold fresh meats; only packaged and canned meats were available. These simple rules combined with careful tracking of total calories consumed and a conscientious effort to exercise, even if exercise was limited to walking, ensured that the subject did not gain weight and actually progressed towards a healthier weight. The subject engaged in this experiment with intentions of losing weight to reach a healthier goal

weight. He averaged a caloric deficit of approximately 808 calories per day, after calculating calories burned from exercise. If the experiment had continued beyond the 30 days and once the subject reached his goal weight, he would need to add additional calories to his diet to prevent continued weight loss, assuming exercise levels remained the same. Increasing calories to reach a maintenance weight would have resulted in higher daily expenditures and would potentially alter the nutritional composition of the diet. Relatively inexpensive increases could be made to the diet by increasing calories from fats (butter, peanut butter) or carbohydrates (pasta and bread), but both of those may have led to increased negative health outcomes.

As noted, the subject did, over time, increase dietary sugar intake, despite careful tracking of food intake. For example, he started making peanut butter and jelly sandwiches instead of peanut butter and banana sandwiches on day sixteen and beyond. He also purchased and consumed several packages of honey roasted peanuts toward the end of the 30 day period. These rules do not require extensive knowledge of nutrition and functioned reasonably well for the subject. The unintentional drift toward added sugar demonstrates how easy it is to allow a diet to deteriorate, even when one is keeping careful track of foods consumed. Nevertheless, given the other options that were available, these were relatively small slips.

In addition to subtle drifts towards a less nutritious diet, daily stresses could lead to failures of discipline that violated both nutrition and cost goals. The limitations imposed by the food desert required continuous planning and preparation to both achieve the twin goals of reasonable nutrition at a low cost. Day 12 is evidence of the fact that a failure to plan, combined with the normal stresses of life, can cause costs to escalate rapidly. The subject failed to plan properly for an out of town trip, and as a result he spent $19.88, nearly three times his daily target. The failure to plan included two factors: first, he did not pack sufficient food for the trip, requiring him to purchase food on the go; and second, he did not know his route well enough and became lost (twice) when looking for fuel. The psychology literature on self-control shows that self-control is weaker when we are tired, hungry, or mentally exhausted (Kahneman, 2011). Sustaining both nutrition and cost is a balancing act that wealthier individuals do not have to consider, as food costs are a small fraction of the average household's budget[2]. This simulation forced the subject to have to think about two things at the same time, as well as deal with the ordinary stresses of daily life. Having to think about two things rather than simply focus on one or the other is inherently more stressful and therefore more likely to fail when other

distractions are experienced by the subject. In this case, the subject experienced a high degree of frustration and violated his standing rule of not purchasing chips or sugary drinks and did both.

Successfully navigating the challenges of the food desert required the subject to exert continuous effort. Despite success in preventing weight gain and increases in measures such as cholesterol, the subject was not able to create a diet that met all of the recommended micronutrient levels. The lack of a variety of micronutrients could lead to long term deficiencies and therefore lead to other types of diet-related illness. A longer term study would need to be conducted to make stronger claims on either the macro or micronutrient influence. The food desert literature has largely focused on issues such as obesity, diabetes, and heart disease, but has not discussed the issue of micronutrients. This would be something to consider for longer-term studies.

A major issue the subject dealt with was the problem of scale. Food desert conditions do not allow for economies of scale. Even when healthier options are available, they are often only available in more expensive, smaller size packages. For example, when the subject first purchased flour, he initially only found a two pound bag for $2.99 ($1.50/pound). By comparison, a five pound bag of flour can be purchased in a nearby supermarket store for $1.75 ($0.35/pound). The subject was pleased to eventually find frozen peas for $1.79 (9 oz bag, $0.20/oz). Canned peas were available in convenience stores ranging for as little as $0.10 per ounce (though the price of a can of peas varied between stores), therefore frozen peas were less affordable than canned. A two pound bag of frozen peas could have been purchased at a nearby supermarket store for $1.98 (32 oz, $0.06/oz), which would have made the frozen peas less expensive than the canned peas from the convenience store, but still slightly more expensive than canned peas from the supermarket store at $0.04 per ounce. Convenience stores typically did not offer multiple sizes of the same product – such as a nine ounce and two pound bag – with a commensurately lower cost per unit, thus a shopper in a food desert cannot scale up and generate savings by buying in bulk. Had the subject included his family in this experiment, his cost to provide for the family would have simply been a multiple of what he spent on himself.

Frozen vegetables were not available in most of the convenience stores the subject visited, but frozen vegetables, as with peas, offer nutritional values very close to their fresh counterparts at a fraction of the cost. Frozen peas (because this was the one vegetable the subject purchased frozen during the experiment) deliver 90% of the potassium on a per gram basis that fresh peas do, while canned (drained and rinsed) peas deliver only 44% (USDA, 2015). Potassium was one of the

[2] The average US family spent 9.8% of their disposable income on food in 2013 (USDA Economic Research Service).

micronutrients the subject failed to consume sufficient volumes of during the experiment. Frozen peas also minimize added sodium, with only 25% more sodium per gram than fresh, while canned carried more than 56 times more sodium per gram. A fresh green pepper costed $1.29 in a convenience store, $0.90 in the local supermarket store (at the time of the experiment), but a fourteen ounce bag of frozen sliced peppers only costed $1.94. If we assume a medium green pepper weighs 4.4 ounces, a fourteen ounce bag would contain 3.2 peppers, for a cost of $0.61/pepper. Supermarket stores commonly stock a variety of frozen vegetables, some in large quantities giving significant scale. A five pound bag of mixed vegetables (peas, carrots, corn, green beans) was available in the local supermarket store for $5.98 ($0.07/ounce). Had the subject been able to purchase a large bag of frozen mixed vegetables at such a price he could have added more vegetables to his diet without significant cost. Frozen vegetables also have the advantage of being long lasting for both the grocer and consumer. Much of the food desert literature has focused on a lack of fresh vegetables, but it would seem that from both the consumer's and retailer's perspectives, frozen would be more economical.

One could argue that people who are impoverished enough to live in an official food desert might not have access to a freezer large enough to maintain a five pound bag of mixed vegetables in order to capture the benefits of scale. Dried legumes and grains also have lengthy shelf lives. Dried legumes sell for a fraction of the cost of canned legumes in a standard supermarket store, and tend to be healthier because salt has not been added, which is ordinarily done during the canning process. The subject was able to locate a few cans of legumes, but in none of the convenience stores he visited was he able to locate and purchase dried legumes such as dried pinto beans (peanuts, also a legume, were available everywhere). The subject was also only able to find dehydrated, precooked white rice (minute rice) in the convenience stores he visited. Rice and dried beans are traditional staples that could provide a nutritional base to a low cost diet. The precooked rice and canned beans were not low cost in the stores included in the simulation and were less healthy than the traditional alternatives.

Fresh foods were predictably expensive when available, though the comparative costs were not as high as some of the non-fresh items, such as flour. As mentioned, a green pepper costed $1.29 (at one of the few convenience stores that actually carried green peppers) and $0.90 at a nearby supermarket store, for a cost ratio of 1.43. Likewise, flour had a cost ratio of 4.29. Instant oatmeal had a cost ratio of almost four times, and jelly was more than five times as expensive. Thus, some of the most expensive items relative to their counterparts in supermarket stores were actually not the fresh items.

Ironically, it was possible to buy a variety of gourmet ice creams, craft beer, and even lobster (if one happens to live in coastal New Hampshire), but fresh fruit and vegetables were extremely limited or non-existent. As the food desert literature has documented, there are abundant cheap, empty calories in every store included in the simulation.

The problems the subject faced, particularly when he had days of failure, were a result of the interaction between poverty and access, rather than simply access. It seems also likely that the subject had deeply ingrained preferences that he fell back on either immediately during times of stress, and gradually and subtly over time. The overwhelming presence of unhealthy food and drink in the stores the subject frequented presented a continuous temptation to revert to unhealthy preferences.

a) *Limitations of the study*

The first limitation of the study was the fact that where the subject was living and shopping was not an actual food desert. In most cases a full supermarket store was only a few minutes down the road, therefore it would not have been rational for the convenience store managers to have stocked bulky, low margin items such as dried beans and rice. The lack of nutritious foods in the convenience stores frequented by the subject could simply represent a specialization by the stores in foods that people (especially college students) would want for a late night snack or a quick bite to eat. People residing in the subject's locale had ready access to several full supermarket stores, and were not reliant on the convenience stores he shopped in for all of their shopping. In a real food desert, convenience stores may have stocked more items that are more useful in maintaining a normal diet because there would be more potential demand from reliant customers. If the assumption that convenience stores in the subject's area actually intentionally stocked fewer nutritious options is true, it would have made the effort more difficult than if the subject had actually shopped in a real food desert. Thus the negative results could be overstated.

A second important limitation of the study is the fact that poverty is a multi-dimensional challenge for those who live with it. A food desert as defined by the USDA includes the resident being poor; there are wealthy people who live in urban and rural environments who are farther than one or ten miles (respectively) from a supermarket, but the presumption is the non-poor can afford transportation, and therefore the simple geographic distance does not constitute food desert conditions since their wealth allows them to overcome the geographic limitations. A study by Dubowitz, et al., found that 86% of the residents who lived in a food desert community shopped at full-service supermarkets

or supercenters (2015), which implies access is more complex than simply not having a supermarket nearby.

Being poor does not just present the challenge of constructing a nutritious diet with few financial resources and poor access to nutritious food, it also potentially includes uncertainty about physical safety, shelter, child care, health care, and so forth. The authors acknowledge that this experiment isolated the effects of food desert conditions from all of the rest of the challenges of actually being poor. However, many policies being proposed based on food desert research, such as trying to bring farmers' markets into areas identified as food deserts (USDA Food and Nutrition Service, 2015), are similarly one-dimensional and do not address the full impact of poverty. The fact that the subject did not face all of the dimensions of poverty simultaneously does allow the effects of food desert conditions to be isolated, which is an advantage over conducting the same analysis with all of the factors of poverty operating on the subject simultaneously. In this sense, the fact that the subject only faced the nutrition challenges of poverty allows the study to more precisely identify the effects of food desert conditions.

A third limitation is that the study is a single case (N of 1). The same diet and exercise combination may have had different impacts on other subjects in terms of laboratory outcomes and weight gain/loss. Furthermore, some of the lab tests are very sensitive to the timing of consumption (glucose, triglycerides). Thus, foods consumed closer to the end of the experiment might have dominated the outcome of the labs. Had the same food been eaten, but in different order, such that for example days 1-3 had been days 28-30, different lab results might have been found.

Other limitations include some of the rules by which the simulation was set up. Commenters have offered that only charging what is used on a given day is unrealistic, and that a fixed dollar amount should have been used for the entire experiment, such as $7.25 x 30 = $217.50. There are many ways that the rules of the simulation could be adjusted. We recommend other interested parties run their own simulations with rule variations to see what the respective effects are.

V. Impact and Conclusions

All simulations are imperfect; we hope this one was useful. This experiment simulated a food desert with regard to nutrition while holding all other factors constant, allowing us to see the effects of living in a food desert an otherwise healthy person's health, at least in the short term. Some of the common assumptions about the harms of food deserts, such as weight gain, high cholesterol, and dangerous sugar consumption were controlled by carefully monitoring caloric intake, applying a few simple rules while shopping, and engaging in regular moderate exercise. Laboratory evidence showed some modest improvements and some modest decreases in health status. However, a detailed analysis of the nutritional content of the diet indicates that some micronutrients were underrepresented in the diet. This could lead to other long term health issues and chronic illnesses, but these are not the illnesses typically associated with food deserts. This experiment suggests that further research might be warranted to look at the effects of micronutrient deprivation in food desert conditions.

The experiment showed that despite planning and reasonable effort, the subject was unable to develop significant economies of scale, supporting the operating assumptions that eating relatively healthily in a food desert is significantly more expensive than eating where larger scale Supermarket retailers are accessible. The relatively high value of frozen and dried foods (higher nutritional value and relatively lower cost) discussed in this paper suggest that further consideration be given to emphasizing policies that encourage the greater availability of frozen and dried foods over fresh. Policies that might result in greater availability of these foods in areas designated as food deserts may be worth further exploration.

This simulation helped to show that food deserts, as defined by the USDA, would contribute to less nutritious diets, however, not in the way typically suggested. Problems of obesity, heart disease, and diabetes require an interaction between the diet available in the food desert, and the broader challenges of poverty that make it difficult to sustain the healthy behaviors that mitigated the effects of the food desert during this simulation. While a relatively balanced macronutrient diet was possible, this simulation showed that there would be a significant problem achieving adequate micronutrients in the diet.

On a personal level, the experiment is a taste of someone else's life. Eating this way, even for a short period of time shifts the way one looks at food and nutrition. As noted in the limitations, there were many other variations on this experiment that could be performed. We would encourage others to try them both to test the value of the simulation and to experience what it is like to live the theory we are discussing.

VI. Acknowledgements

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Monitoring Major Sugars in Greek Commercial Fir Honey and their Role in Geographical Differentiation, using Chemometrics

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Abstract- The aim of the present study was a) to provide information on fructose, glucose, sucrose and maltose content, along with sum of fructose and glucose content, fructose/glucose ratio, and sum of the four sugars, of a lees common type of honey produced in Greece namely fir, and b) investigate the possibility of geographical differentiation using above parameters in combination with chemometrics. For this purpose, 30 commercial fir honey samples were collected during the harvesting period 2011 from 4 different regions in Greece. The analysis of saccharides was performed by high pressure liquid chromatography coupled to a refractive index detector.

Results showed that sugar content of fir honey was affected by geographical origin (p<0.05). Application of linear discriminant analysis (LDA) to sugar parameters resulted to the correct geographical differentiation of commercial fir honeys recording 80% correct classification rate using the original, and 76.7% the cross validation method, respectively.

Keywords: commercial fir honey; sugars; high pressure liquid chromatography; refractive index; differentiation.

GJMR-L Classification: NLMC Code: QU 145.5

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Results showed that sugar content of fir honey was affected by geographical origin ($p<0.05$). Application of linear discriminant analysis (LDA) to sugar parameters resulted to the correct geographical differentiation of commercial fir honeys recording 80% correct classification rate using the original, and 76.7% the cross validation method, respectively.

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I. Introduction

Sugars (saccharides) are the main components of honey. They are produced by honeybees from nectar sucrose, which is transformed through the action of several enzymes, mainly a-and b-glycosidase, a-and b-amylase and b-fructosidase (Huidobro et al.,1995; De la Fuente et al., 2011).

Fructose and glucose (monosaccharides) are the major constituents of honey, being the dominant components in almost all types, except for some honeys of dandelion (*Taraxacum officinale*), blue curl (*Trichostema lanceolatum*), and rape (*Brassica napus*) origin, where glucose is present in higher amounts (Cavia, et al., 2002). The content of fructose and glucose, as well as their ratio, has been considered as useful indicator for the classification of unifloral honeys (Oddo et al., 1995; Terrab et al., 2001; Oddo & Piro, 2004; De La Fuente et al., 2007; Manikis et al., 2011). Besides these two main constituents, there are also oligosaccharides (disaccharides, trisaccharides, and tetrasaccharides). These compounds are formed, mainly by the action of honey enzymes.

Ruiz-Matute et al. (2010), reported 25 trisaccharides and 10 tetrasaccharides, for Spanish and New Zealand honeys. The trisaccharides planteose and α-3-glucosylisomaltose were reported in honey for the 1st time by these authors.

Thus, new developments in analytical techniques enhance the possibilities of searching for more precise and representative geographical and botanical origin markers (De La Fuente et al., 2006).

Dvash et al. (2002) used NIR spectroscopy for the analysis of avocado (*Persea Americana* Mill.) honey and found that carbohydrate alcohol perseitol (d-glycerod-galacto-heptitol) in spite of its low content (average value 0.48g/100g) could be used as a marker of avocado honey. The same compound was reported in avocado honey by de La Fuente et al. (2006), at a higher amount of 0.75g/100g.

Honey carbohydrate composition has been commonly determined by high performance liquid chromatography (HPLC) or by gas chromatography (GC). Since a high number of carbohydrate isomers are present in honey, resulting in very complex chromatograms with a high degree of overlapping, several methods have been proposed for their quantification (De La Fuente et al., 2006).

HPLC allows the determination of high molecular weight oligosaccharides (Swallow & Low, 1990; Weston & Brocklebank, 1999; Morales et al., 2006), while GC provides better resolution for many important minor sugars as disaccharides and trisaccharides (Low & Sporns, 1988; Gómez-Bárez et al., 2000; Cotte et al., 2004; Sanz et al., 2004).

Carbohydrate derivatization is required for gas chromatography (GC) analysis, and when trimethylsilyl oximes are used, they produce two peaks for reducing sugars and only one for non reducing sugars (Gómez-Bárez et al., 2000; De La Fuente et al., 2011).

Greece holds a leading position internationally in hives and honey production, regarding its population and area. While in all European countries the quantity of colonies decreased or remained constant, over the last twenty years in Greece have increased, by approximately two colonies per km. What is interesting, is that there are a quite few studies in Greece dealing with the characterization of fir honey based on sugar profile (Manikis et al., 2011; Spilioti et al., 2014).

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Thus, the aim of the present study was to characterize and investigate the possibility of differentiating fir honey according to geographical origin based on its major sugars determined with HPLC, and by using chemometrics.

II. Materials and Methods

a) Honey samples

Thirty fir honey samples were collected from professional beekeepers during the harvesting period 2011 from 4 different geographical regions: Messinia (8 samples), Lakonia (10 samples), Arkadia (8 samples), Karditsa (4 samples). Samples were stored in glass containers, shipped to the laboratory and maintained at $4\pm1\ ^{\circ}$C until analysis.

b) Standards and chemicals

Fructose, glucose, sucrose and maltose, were obtained from Merck (Darmstadt, Germany). All chemicals used in the present study were of analytical grade and deionized water was used to prepare all solutions. Acetonitrile (HPLC grade), methanol (HPLC grade), ammonium hydroxide and ethylenediamine were also obtained from Merck (Darmstadt, Germany).

c) Preparation of standards

The preparation of the standard solutions of saccharides was carried out based on the method described by Bogdanov and Baumann (1988).

d) Preparation of honey samples

5g of honey was weighed into a beaker and dissolved in 40 mL deionized water. Then, 25 mL of methanol was pipetted into a 100 mL volumetric flask and the honey solution was quantitatively transferred into the flask. It was filled to the mark, with deionized water. Finally, the obtained solution was filtered through a 0.45μm membrane filter prior to HPLC analysis (IHC, 1997). Each sample was run in duplicate (n=2).

e) Quantification analysis

Saccharides were quantified by comparison their chromatographic peak areas with the calibration curves of the standards. The calibration curves were made in triplicate (n=3) for each individual standard at five different concentrations (100-20000 mg/L). The determination coefficients for the calibration curves were: R^2=0.993 for fructose, R^2=0.996 for glucose, R^2=0.995 for sucrose, and R^2=0.996 for maltose respectively. Limit of detection (LOD) and limit of quantification (LOQ) were: LOD= 0.11 and LOQ = 0.37 mg/Kg for fructose, 0.21 and 0.71 mg/Kg for glucose, 0.06 and 0.19 mg/Kg for sucrose, 0.05 and 0.18 mg/Kg for maltose, respectively. Figure 1 shows a representative chromatogram of a mixture of the four standard sugars.

f) HPLC Analysis

i. Apparatus

HPLC analysis was performed with a SHIMADJU LC solution (Kyoto, Japan), consisting of a quaternary pump (LC-20AD), a thermostated column oven (CTO-10A), a 20 μL loop injector and a SHIMADJU chemstation for data analysis. Detection was carried out using a SHIMADJU refractive index (RID-10A).

ii. HPLC conditions

A separation column (Zorbax Rx-SIL, 250 mm x 4.6 mm i.d., 5 μm, Hewlett-Packard, USA) was used. The column temperature was held at 25°C. The mobile phase for isocratic elution was a mixture of water/acetonitrile (1:2.6 v/v) containing 0.03% (v/v) ethylenediamine as a modifier and ammonium hydroxide (0.05%, v/v), which was used to adjust the pH to 9-10. The flow rate was 1.0 mL/min. Before analysis, a mixture of water/acetonitrile (1:2.6, v/v) containing 0.3% (v/v) ethylenediamine was run through the column forming a dynamic coating layer on the silica surface (Wei & Ding, 2000).

g) Statistical analysis

Data processing was performed using the SPSS 20.0 statistics software (SPSS Inc., 2012). Comparison of the means was achieved using multivariate analysis of variance (MANOVA), while correct classification ability according to the production area of fir honey was performed using LDA to sugar data collected at the confidence level $p<0.05$ (Karabagias et al., 2014).
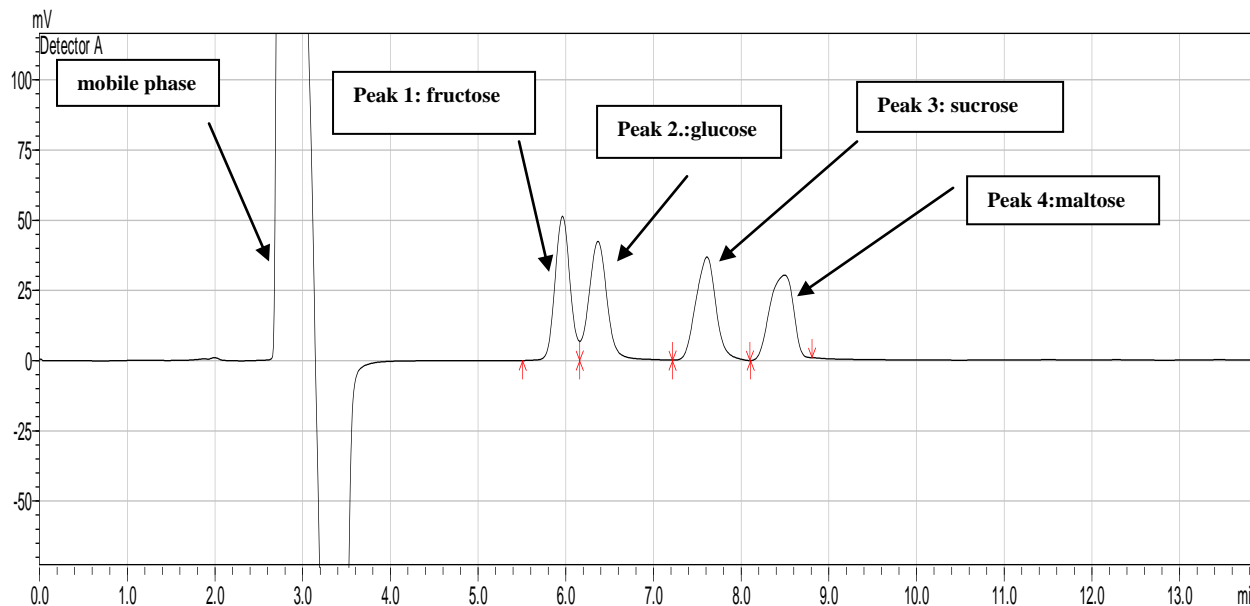
Figure 1: A typical HPLC-RI chromatogram of a standard mixture (100 mg/L) of sugars obtained with the applied method.

III. Results and Discussion

a) *Sugar content and sugar parameters of commercial fir honey according to geographical origin*

Fructose (g/100g), ranged between 21.87 (sample no.5 from Arkadia) and 42.48 (sample no 1. from Messinia). Glucose (g/100g), ranged between 6.56 (sample no.2 from Arkadia) and 39.21 (sample no.1 from Messinia). Maltose (g/100g), ranged between 0.21 (sample no.4 from Lakonia) and 5.69 (sample no.10 from Lakonia), while it was not detected in two samples. Finally, sucrose (g/100g) ranged between 0.27 (sample no.1 from Arkadia) and 7.81 (sample no. 9 from Lakonia).

According to directive 127/2004 of the Greek Ministry of Agricultural Development and Food (''Classification of monofloral honeys''), the sum of fructose and glucose (F+G) must be≥45g/100g. In most of the fir honey samples analyzed, (F+G) was higher than 45g/100g. All the Arcadia samples (Menalon fir honey) gave (F+G) ≤45g/100g. This is in great agreement with Manikis et al. (2011) who reported (F+G) ranging between 32.60-38.20 g/100g for Menalon fir honey. In the same directive the sucrose content must be ≤ 5g/100g. Only five samples from Lakonia (nos. 5-9) exhibited this upper limit. No limits have been set for the maltose content in fir honey by the Greek Ministry of Agricultural Development and Food or the European Council Directive relating to honey (110/EC, 2001).

Oddo et al., (1995) in 52 honeydew honeys (*Abies spp.*) analyzed reported fructose, glucose, sucrose and maltose values (g/100g) ranging between: 24.50 and 35.80, 18.0 and 28.60, 0.4 and 1.8 and 0.4 and 1.60, respectively.

Mateo and Bosch–Reig (1997), in an effort to characterize honeydew Spanish honeys reported values

(g/100g) ranging from 32.60-35.90 for fructose, 22.70-28.50 for glucose, 0.02-0.75 for sucrose and 3.43-6.22 for maltose, respectively. These values are in very good agreement with present results regarding fir honeys collected from the regions of Messinia, Karditsa, and Lakonia.

Maltose was the major disaccharide present in 80 genuine Brazilian honey samples (mostly *Eucalyptus* spp., extra-floral, and multifloral honeys) with a mean value of 3.05g/100 g (Da Costa Leite et al., 2000). In this case, maltose was considered as marker for the geographical classification of honey. These reported values for maltose are in very good agreement with present results regarding fir honeys collected from Lakonia and Arkadia regions (Table 1).

Cotte et al. (2004) determined the predominant disaccharides in several types of honeys from France: maltose and turanose in acacia; maltulose and turanose in chestnut and linden; turanose and trehalose in fir; and sucrose, maltose in lavender honey. In the same study, these authors characterized 37 fir honey samples reporting mean values of fructose 31.49 (g/100g), glucose 24.17 (g/100g), sucrose 0.04 (g/100g) and maltose 0.17 (g/100g), respectively. The reported values for fructose and glucose are in very good agreement with present results regarding fir honeys from Messinia, Karditsa, and Lakonia regions. The lower glucose content reported in the present study for fir honeys from Arkadia, may be attributed to these samples were collected from mountain Menalon. It is widely known that this region gives the only PDO honey in Greece, and it is characterized by its low glucose content as compared to other types of honey (Manikis et al., 2011).

Finally, de la Fuente et al. (2011) in a study dealing with the carbohydrate composition of Spanish unifloral honeys (*Eucalyptus, Echium, Rosemary, Heather, Citrus, Rosaceae, Miscellaneous*) reported

that fructose and glucose were the main sugars in all samples analyzed with a mean value of 37.14 (g/100g) and 30.02 (g/100g), respectively. Such values are higher than those obtained in the present study (Table 1).

Table 1: Sugar content (g/100g) of commercial fir honeys according to geographical origin

Region	Fructose	Glucose	Maltose	Sucrose	F/G	F+G	F+G+M+S
Messinia	42.48	39.21	nd	0.28	1.08	81.70	81.97
Messinia	38.01	38.00	nd	2.68	1.00	76.01	78.69
Messinia	25.02	11.91	0.33	1.04	2.10	36.94	38.30
Messinia	34.18	36.93	1.37	1.84	0.93	71.11	74.32
Messinia	25.43	24.25	0.61	0.83	1.05	49.68	51.13
Messinia	37.24	24.25	0.58	0.97	1.54	61.49	63.03
Messinia	35.15	23.64	0.41	2.10	1.49	58.79	61.30
Messinia	38.23	24.40	0.45	1.97	1.57	62.63	65.06
Mean	34.47	27.82	0.47	1.46	1.34	62.29	64.23
±SD	6.21	9.44	0.43	0.80	0.40	14.43	14.54
Karditsa	30.38	28.60	0.21	1.16	1.06	58.98	60.35
Karditsa	27.62	27.05	0.73	1.37	1.02	54.67	56.77
Karditsa	34.55	25.91	0.68	1.19	1.33	60.46	62.33
Karditsa	31.39	25.40	0.99	1.07	1.24	56.79	58.86
Mean	30.99	26.74	0.65	1.20	1.16	57.73	59.58
±SD	2.86	1.42	0.33	0.13	0.15	2.53	2.35
Lakonia	28.45	27.89	0.82	2.53	1.02	56.34	59.69
Lakonia	26.75	26.38	0.62	2.23	1.01	53.13	55.98
Lakonia	26.30	24.36	0.31	1.55	1.08	50.66	52.52
Lakonia	26.87	25.40	0.21	1.76	1.06	52.27	54.23
Lakonia	28.77	20.88	2.23	5.19	1.38	49.64	57.06
Lakonia	32.53	25.85	5.38	6.99	1.26	58.38	70.76
Lakonia	32.64	25.98	5.27	7.53	1.26	58.62	71.42
Lakonia	32.99	27.39	5.30	7.68	1.20	60.38	73.36
Lakonia	29.77	19.87	5.65	7.81	1.50	49.64	63.11
Lakonia	30.96	22.55	5.69	4.92	1.37	53.51	64.13
Mean	29.60	24.65	3.15	4.82	1.21	54.26	62.23
±SD	2.57	2.71	2.50	2.61	0.17	3.93	7.57
Arkadia	25.51	8.46	4.35	0.27	3.01	33.97	38.59
Arkadia	26.04	6.56	3.61	0.45	3.97	32.60	36.66
Arkadia	22.21	7.76	3.66	0.78	2.86	29.96	34.40
Arkadia	22.89	7.90	3.39	0.82	2.90	30.79	35.01
Arkadia	21.87	7.55	5.00	0.83	2.90	29.42	35.25
Arkadia	23.94	9.56	3.72	0.82	2.51	33.49	38.03
Arkadia	24.86	9.50	3.72	0.82	2.62	34.36	38.90
Arkadia	27.04	13.24	3.72	0.82	2.04	40.28	44.82
Mean	24.30	8.82	3.90	0.70	2.85	33.11	37.71
±SD	1.88	2.05	0.52	0.22	0.55	3.44	3.35

F/G: fructose/glucose ratio, F+G: sum of fructose and glucose (g/100g), F+G+M+S: sum of fructose, glucose, maltose, and sucrose (g/100g).

The results are the mean of two replicates (n=2). MANOVA in comparison of means ($p<0.05$), nd: not detected.

b) Classification of commercial fir honeys according to geographical origin based on sugar data

MANOVA analysis was applied to the sugar data of the thirty commercial fir honey samples in order to point out which sugar parameters are significant for the differentiation of honeys from the four different geographical origins. Dependent variables included the 7 sugar data while geographical origin was taken as the independent variable. Pillai's trace= 1.806 (F=4.752, df=21, *p-value=0.000<0.05*) and Wilk's Lambda= 0.018 (F=8.445, df=21, *p-value=0.000<0.05*) index values showed the existence of a significant multivariable effect of geographical origin on the identity of fir honey sugar data. Four sugar parameters (Table 2) were found to be significant ($p<0.05$) for the differentiation of fir honeys. Thus, these 4 sugar parameters were subjected to LDA.

Results showed that two statistically significant discriminant functions were formed: Wilk's Lambda= 0.028, X^2=89.523, df=12, *p-value=0.000<0.05* for the first function, and Wilk's Lambda= 0.322, X^2=28.334, df=6, *p-value=0.000<0.05* for the second. These significant values of Wilk's Lambda index shows that the discriminant functions created were basic for the differentiation of the investigated regions.

The first discriminant function accounted for 84.7% of total variance, the second accounted for 14.4%. Both accounted for 99.1% of total variance, an excellent rate.

In Figure 2 it is shown that fir honeys from Arkadia are fully separated. Fir honeys from Karditsa and Messinia are close, the latter seems to be not well separated. Honeys from Lakonia are also separated, as compared to honeys from Arkadia.

The overall correct classification rate was 80% using the original and 76.7% the cross validation method, a quite satisfactory value especially for the second method. Correct classification (100%) was obtained for honey samples from Arkadia, followed by those of Karditsa (correct classification 75%), Lakonia (correct classification 70%) and Messinia (correct classification 62.5%) (Table 3).

Cotte et al. (2004), using a much larger number of honey samples (280) produced in the wider area of France, and belonging to 7 botanical origins (acacia, chestnut, rape, lavender, fir, linden, sunflower) reported that the 17 carbohydrates determined along with fructose/glucose ratio, resulted to a classification rate of 72.1% according to honey type, after the application of principal component analysis.

Nozal et al. (2005) characterized 77 honeys belonging to several botanical origins (ling, spike lavender, French lavender, thyme, forest, and multifloral) from a single (identical) geographical area, the Province of Soria (Spain), using 14 carbohydrates in combination with chemometrics. These authors, managed to classify above types of honey, reporting an overall classification rate of 90%.

Finally, de la Fuente et al. (2011) in a study dealing with the characterization of 59 Spanish floral honeys (citrus, rosemary, heather, rosaceae, eucalyptus, and echium) in terms of carbohydrate composition, reported that the carbohydrates determined did not allow an unambiguous classification of honeys according to their type, after application of chemometric analyses (correct classification rate < 70%).

Table 2: Contribution of each discriminant function to fir honey geographical differentiation chemometric approach and standardized canonical discriminant function coefficients

Sugar data (g/100g)	Discriminant function 1 84.7%	Discriminant function 2 14.4%	F	*p*
Sucrose	-1.496	0.694	12.343	*<0.001*
Maltose	1.370	0.318	9.472	*<0.001*
F/G	0.589	-0.157	37.949	*<0.001*
Fructose	-0.067	-0.809	9.665	*<0.001*

F: Fisher's linear discriminant functions, *p*: probability, F/G: fructose/glucose ratio

Canonical Discriminant Functions
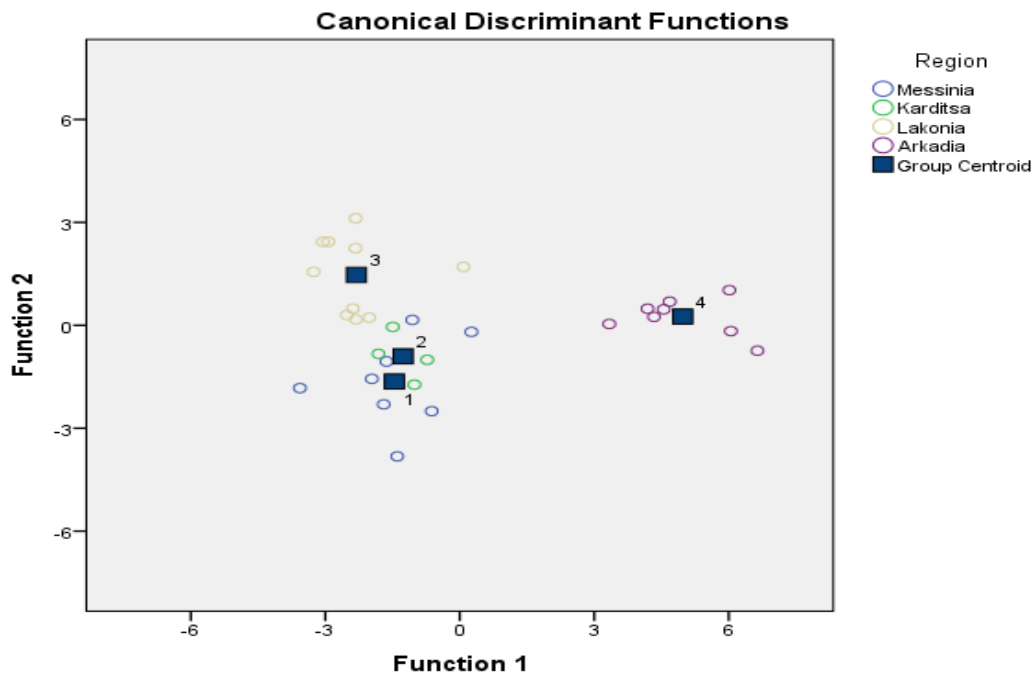
Figure 2: Geographical differentiation of commercial fir honey based on sugar data

1: Messinia, 2: Karditsa, 3: Lakonia, 4: Arkadia

Table 3: Differentiation ability of the proposed chemometric model using sugar data (g/100g) of commercial fir honey

Classification Results[a,b]

		Region	Predicted Group Membership*				Honey
			Messinia	Karditsa	Lakonia	Arkadia	samples
Original[a]	Count	Messinia	5	3	0	0	8
		Karditsa	1	3	0	0	4
		Lakonia	0	2	8	0	10
		Arkadia	0	0	0	8	8
	%	Messinia	62.5	37.5	0	.0	100.0
		Karditsa	25.0	75.0	0	.0	100.0
		Lakonia	0	20.0	80.0	.0	100.0
		Arkadia	0	0	0	100.0	100.0
Cross-validated[b]	Count	Messinia	5	3	0	0	8
		Karditsa	1	3	0	0	4
		Lakonia	0	3	7	0	10
		Arkadia	0	0	0	8	8
	%	Messinia	62.5	37.5	.0	.0	100.0
		Karditsa	25.0	75.0	.0	.0	100.0
		Lakonia	.0	30.0	70.0	.0	100.0
		Arkadia	0	0	.0	100.0	100.0

a. 80.0% of original grouped cases correctly classified.

b. 76.7% of cross-validated grouped cases correctly classified.

*Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions Variables ordered by absolute size of correlation within function.

IV. Conclusion

In the present study results showed that sugar content of commercial fir honey is affected by geographical origin ($p<0.05$). This is the first attempt to differentiate fir honeys produced in different regions in Greece, using selected sugar parameters, this constituting the novelty of the present work. The classification rate obtained is within the range reported previously in the literature (Cotte et al., 2004; Nozal et al., 2005; De La Fuente et al., 2011). It is worth mentioning that the sugar content of the honeydew secretions is greatly variable and depends strongly on the insect and plant species, as well as on the climate in a specific area (Salvucci and Crafts-Brander, 2000), affecting thus fir honey sugar content.

Thus, the classification rate presented in the present study will be further evaluated by collecting honeydew secretions from the same regions. In that sense, a more sophisticated differentiation model will be constructed for fir ''honeydew'' honey.

V. Acknowledgements

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Quality Characteristics of Breads Fortified with Sesame Seed

By Fabian Uchenna Ugwuona & Nwamaka A. Obeta

Abstract- This study evaluated the quality of bread fortified with sesame seed. Breads with added full fat and defatted sesame seed meals were baked and analysed for nutrient compositions, physical and sensory properties and storage stability. Full fat and defatted sesame seed meal respectively had 31.28% and 46.00% carbohydrate, 23.07% and 29.9% protein, 31.05% and 11.89% fat, and 13.20% and 12.14% crude fibre. Fortification with sesame seed improved the nutrient composition, storage stability, physical and sensory properties of bread. Fortification with 20% full fat or defatted sesame seed flours respectively improved protein from 11.80% to 13.93% or 14.89%, crude fibre from 1.63% to 3.44% or 5.25%, fat from 0.15% to 6.80% or 3.85%, but decreased carbohydrate from 55.44% to 50.37% and 48.68% respectively. Sensory scores (colour, texture, flavour, and overall acceptability), loaf and specific volume and shelf life of bread increased with increasing sesame seed addition.

Keywords: bread, sesame, fortification, quality, shelf-life.

GJMR-L Classification: NLMC Code: WA 695

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Fabian Uchenna Ugwuona[α] & Nwamaka A. Obeta[σ]

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I. Introduction

Bread is wheat based baked product widely accepted and consumed throughout the world (O'Brian *et al*, 2003). In Nigeria, wheat is produced in limited quantity while a greater proportion of wheat flour is imported to meet local flour needs for bakery products. Bread is made about 60% wheat as the base material (Akubor, 2003). The impact of various ingredients, other than wheat on sensory and nutritional quality of bread have been extensively studied (Heinio *et al*., 2003; Barcenas and Rossel., 2005; Plessas *et al*., 2005) Efforts have been made to use composite flours in which flours of high protein grains grown locally replace a portion of wheat flour, thereby decreasing the high cost of imported wheat and at the same time producing protein-enriched bread (Almazan, 1987). Non-wheat flours, particularly of legumes / oilseeds and other high protein seed flours, up to 20%, have been shown to improve baking properties, nutritional and sensory quality of bread (Kallasapathy *et al*., 1985; Misra *et al*., 1991; Doxastakis *et al*., 2002). While nutritionist are more interested in food composition and health related factors, sensory properties are still the most important criteria that consumers stick to when choosing bakery goods (Giami *et al*, 2004).

In this regard, the researchers strives to produce high quality bread of long shelf life from blends of full fat or defatted sesame seed and wheat flours that would meet the recommended nutritional need and sensory requirement of popular consumers. Sesame seeds contain high amount of tocopherol and lignan compounds which give the oil resistance to oxidation (Mohamed and Awati, 1998; Suja *et al*., 2004b). Sesame is cultivated at commercial quantity in many parts of North Central states of Nigeria and is highly valued as soup and snack ingredient among these people. It is collected from these states and exported out of Nigeria to industrialized nations of the Western world (Bedigan, 2003)

II. Materials and Methods

a) Materials

White sesame seed (*Sesamum indicum*) variety was purchased from Akwanga market while wheat flour, sugar, margarine, baking powder, yeast, common salt, vanilla flavour, eggs and Calcium propionate were purchased from commercial stockers in Lafia main market, all in Nasarawa state, Nigeria. All laboratory reagents used were of analytical grade.

b) Processing of full fat and defatted sesame seed flours

Dried Sesame seeds were dehulled by pounding lightly in a mortar, and then winnowing away the husks. Part (300g) of the nibs (dehulled seeds) was milled to a fine paste using a laboratory mill (Numex Pep Grinding Mill, India) and then oven-dried (55^{OC}) for 24h. This was re-milled to sesame seed powder and sieved through $160 \mu m$ pore sieve. The remaining 500g of the nibs was ground to a coarse paste. The paste was fat-extracted batch-wise (100g) with n-hexane (1:5, w/v) as proposed by Boadright and Hetiarachchy (1995) and then oven-dried (60^{OC}) for 24h. This was then milled into powder and sieved through $160 \mu m$ pore sieve. Both the full fat and defatted flours were used to fortify bread.

c) Flour blend preparation

Commercial wheat flour was blended with 0%, 10% or 20% of either whole or partially defatted sesame seed flour and the blends are shown in Table 1

d) Preparation of bread

Breads were prepared using the flour blends in Table 1 and other ingredients. The dough was prepared by blending flour (500g) with other ingredients, yeast (15g), sugar (37.5g), salt (12g), calcium propionate (1.5g), nutmeg (2.5g), citric acid (0.25g), fat (25g), egg

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(83ml), water (200 ml to 268 ml), in a Kenwood mixer (Model A 907D) using the method of Chauhan *et al*. (1992). The dough from each was kneaded repeatedly by pressing, folding, turning and stretching it out to develop. The dough were fermented for about 80 minutes in plastic bowls covered with muslin cloth at room temperature (26 ± 2^{OC}); and then later scaled to 150 g pieces. These were proofed for 90 minutes at room temperature and then baked at 200^{OC} for 30 minutes. Breads were cooled for 2h and assessed the following day for chemical composition, physical features, and then for sensory properties by 15-member panellists using a 7-point hedonic scale rank order test.

III. Chemical Analysis

The method of the Association of Official Analytical Chemists (AOAC, 2000) was used proximate analysis. . Moisture content of wheat flour, whole and defatted sesame meal and bread samples was determined by drying subsamples (3g) of each at 121^{OC} for 4h in hot air-oven (Astell-Hearson, Great Bratain) at 121^{OC} for 4h, and the loss in weight recorded as the moisture content. The micro-Kjeldahl method was used for nitrogen determination and the crude protein contents expressed as N x 6.25. Crude fat was estimated by exhaustive extraction of the samples (5g) using petroleum ether (boiling point $40\text{-}60^{OC}$) in a Soxhlet apparatus. The fat-free samples after ether extraction were digested alternatively with 1.25% H_2SO_4 and 1.25% NaOH under specified conditions. The loss in weight on ignition of the residues to white ashes at 525^{OC} in a muffle furnace were reported as crude fibre contents while the net weight was recorded as ash (a measure of mineral content) content. The carbohydrate content (excluding fibre) was obtained by subtracting the sum of crude protein, crude fat, crude fibre and ash from the analyse sample of each sample.

Mineral elements were determined in wet-digests of the samples (Walsh, 1971). Calcium, iron and zinc were determined using an atomic absorption spectrophotometer while the phosphomolybdate method of Yen and Pollard (1955) was used to estimate phosphorus content.

a) Physical analysis of bread

Physical properties were evaluated by measuring loaf volume and specific volume. Loaf volume was measured by seed displacement method (Onwuka, 2005) using dehulled sesame seed in place of rape seed. A box of fixed dimensions (3.4x 2.1 x 4.2 cm), with internal volume 30cm³ was put into a tray, half filled with dehulled sesame seed, shaken vigorously for four (4) times, and then filled till slightly overfilled so that the overspill fell into the tray. The box was shaken again twice, and then a straight edge was used to press across the top of the box once to give a levelled surface. The seeds were decanted from the box into a receptacle

and weighed. This procedure was repeated three times and the mean value for seed weight was noted (B g). A weighed loaf was placed in the box and levelled off as before. The overspill was weighed and from the weight obtained the weight of seeds around the loaf and the volume of seeds displaced by the loaf were calculated using the following formulas:

Seed displaced by loaf (L) = B g + overspill weight- 20.82g

$$\text{Volume of loaf (V)} = \frac{\text{L x 23.59cm}^3}{\text{B}}$$

b) Sensory analysis

A blind method of analysis was used where bread samples were coded with randomly selected two (2) digits and one (1) letter (Mellgaard *et al*, 1999). The samples were evaluated by twelve-(12)-member trained panellists. The panellist were instructed to evaluate the organoleptic quality (i.e. the colour, texture, flavour and overall acceptability) using a seven-point hedonic scale where7 (seven) represents liked extremely and 1 (one) represents disliked extremely.

Consumer testing was conducted at the home economics sensory analysis laboratory. The products were served to each panellist in similar sample retaining plate. The panellist were instructed to rinse their mouth with clean water which was provided to each of them before and after testing a product to avoid carry over effect.

c) Storage Studies

The storage study of five bread samples with 100% wheat flour, 10 and 20% substituted whole or defatted sesame seed flour were evaluated by 15-member trained panellists who scored for softness, springiness, moisture and flavour on the 2nd, 4th and 6th day of storage at ambient condition (26 ± 2^{oC}) after baking, using a seven-point hedonic scale.

d) Statistical analysis

Analysis of Variance (ANOVA) was used to test differences of nutritional value and sensory evaluation. Least Significant difference (LSD) test was used to test for significant differences between the samples at ($P< 0.05$).

IV. Results and Discussion

a) Nutrient composition of whole and defatted sesame seed flours

Table 2 shows the nutrient composition of full fat (FF) and defatted sesame seed flours (DF). The results show significant variations in moisture (6.6 and 8.0%), crude fibre (23.1 and 27.9%), lipid (31.1 and 11.9%), ash (3.4 and 5.4%) and. digestible carbohydrate (31.28 and 48.01%) contents in full fat and defatted sesame seed flour. The minerals calcium, phosphorus, zinc and iron were comparatively higher in the defatted

seed flour than in the full fat seed flour. The content of most minerals (calcium, phosphorus, zinc and iron) in both full fat and defatted seed flours were high enough as good food sources of these minerals. Thus, both the full fat and defatted sesame seed flours were rich in most of the needed nutrients, particularly protein, crude fibre and ash, but the defatted seed flour at most instances had higher contents of each of these nutrients. However, the full fat seed flour was comparatively higher in fat (31.1%) content than the defatted seed flour (11.9), precisely due to the incomplete fat extraction. The nutrient, particularly crude protein and fibre, contents of both full fat and defatted sesame seed fours were very high to warrant their consideration for use to complement cereal-based products to meet the recommended daily nutrient intake of human (National Research Council, 1989). Nutrient composition of bread samples prepared with different levels of whole and defatted sesame seed flour.

Table 3 shows the nutrient composition of bread samples fortified with different levels (0%, 10% and 20%) of full fat or defatted sesame seed flours. The moisture content decreased from 27.95% for the control (0% fortification) to 23.60% for the 20% full-fat sesame seed flour-fortified bread sample. Increasing sesame seed flour (full fat and defatted) decreased residual water content of bread samples proportionally. Substituting whole and defatted sesame seed flours for wheat flour increased protein, fibre, ash and mineral content but decreased carbohydrate content of the bread samples. The residual moisture content of the bread samples decreased with increased levels of sesame seed flour (defatted and whole) substituted 10% defatted sesame seed flour for wheat flour increased the protein (11.80%), fat (0.15%), fibre (1.63%), ash (0.90%), calcium (40.00mg/100g) and phosphorus (15.00mg/100g) content to 14.59%, 2.90%, 5.11%, 1.85%, 1.40%, 43.00mg/100g and 19.00mg/100g content respectively in the bread samples. Also substituted 20% defatted sesame seed flour for wheat flour increased the protein content from 11.80% to 14.93%, fat from 0.15% to 3.85%, fibre content from 1.63% to 5.25%, ash content from 0.90% to 1.85% in the bread samples. The digestible carbohydrate contents (55%) in the 100% wheat bread sample decreased to 51.80% in 20% substituted whole sesame seed bread to 48.10% in 20% substituted defatted sesame seed bread samples. Carbohydrate which is abundant in wheat flour was reduced with the low carbohydrate of defatted and whole sesame seed flours. This is a good approach to increase the nutritional quality of bread from such blends over those of 100% wheat flour (Misra *et al*., 1991).

b) *Sensory and physical quality of bread samples*

Table 4 shows the sensory and physical quality of bread samples prepared with different levels (0%,

10%, 20%) of whole and defatted sesame seed flour. Panellists scored the colour of the Bread samples with 0% sesame seed flour had higher (6.07) higher and bread samples with 20% whole sesame seed flour (5.07) lower. The colour, texture, flavour, mouth feel, overall acceptability of all the bread sample did not differ significantly ($p > 0.05$). Bread samples with 10% whole sesame seed flour had higher score in terms of texture (5.93), flavour, (5.80), mouth feel (5.80) and overall acceptability (5.80). The defatted and whole (10 and 20%) sesame seed flour bread showed equal acceptability. The bread sample with 100% wheat flour had the least mean loaf volume (8.07) and mean specific volume (2.07) while bread samples with 20% whole sesame seed flour had the highest mean for loaf volume (11.01) and mean specific volume (2.72). Storage (6 days) stability of bread samples fortified with different levels of whole and defatted sesame seed flour. Table 5 shows the storage stability of bread samples fortified with different levels of defatted and whole sesame seed flour stored at ambient temperature ($29 \pm 2^{O}C$) for 6 days. The O-fat rancidity, moisture, flavour, musty, softness and springiness of the bread samples on the 4th (6.43, 1.79, 6.72, 6.85, 2.41, 5.28) and 6th day (6.01, 1.77, 5.89, 6.52, 2.41, 1.87) significantly ($P < 0.05$) differed from the bread samples of the 2nd day (6.15, 3.03, 6.29, 6.40, 1.97, 4.08) respectively. The bread samples on the 2nd day (4.08) were springy and became less springy on the 4th day (5.28) and 6th day (5.08). The bread sample substituted with whole (U4) and defatted (D5) sesame seed flour did not go rancid and were more stable than the control (A1) during the storage period.

V. Conclusion

It is apparent that substituting defatted and whole sesame seed flour for wheat flour in bread improved the nutrient composition of the product without adversely reducing its sensory quality. Sesame seed is locally available and easily processed into flour for use. It could form a good substitute for dietetic bread with good keeping quality. The colour, texture, flavour and mouth feel were acceptable by the consumers.

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Table 1: Flour blends

Flour blend Ratio	Defatted sesame flour(g)	whole sesame flour(g)	Wheat Flour(g)
W: FF_0 (0:100)	0	0	500
W: FF_1 (10:90)	0	50	450
W: DF_5 (10:90)	50	0	450
W: FF_2 (20:80)	0	100	400
W: DF_6 (20:80)	100	0	40

W=Wheat flour, FF= Full fat Sesame seed flour, DF=Defatted sesame seed flour

Table 2: Nutrient compositions of wheat flour, whole and defatted sesame seed flours

	FF	DF
Moisture (%)	6.60±1.10	8.00±0.90
Crude protein (%)	23.07±1.00	27.90±1.00
Crude fibre (%)	12.14±0.06	12.20±0.02
Lipid (%)	31.05±1.70	11.89±1.09
Ash (%)	3.40±0.01	5.45±0.01
Digestible carbohydrate (%)	31.28±1.00	46.02±0.90
Calcium (mg/100g)	70.00±1.90	73.00±3.20
Phosphorus (mg/100g)	31.00±1.80	41.00±2.30
Iron (mg/100g)	0.19±0.00	0.29±0.01
Zinc (mg/100g)	5.90±1.00	
	6.0±1.50	

ND= Not determined, UF= whole sesame seed flour, DF= Defatted sesame seed flour

Table 3: Nutrient composition of bread samples fortified with different levels of defatted and whole sesame seed flour

D/UDSF	Control	Whole	Defatted	Whole	Defatted
% Flour substituted	**0 (A1)**	**10 (A2)**	**10 (A3)**	**20 (A4)**	**20 (A5)**
Moisture	27.95±2.30	24.85±3.00	26.65±2.60	23.60±1.80	23.75±1.90
rude protein	11.80±0.10	12.48±0.90	14.59±1.00	13.93±0.90	14.93±0.90
Crude fibre	1.63±0.00	3.71±0.00	5.11±0.00	3.44±0.00	5.25±0.00
Lipid	0.15±0.00	4.65±0.00	2.90±0.01	6.80±0.02	3.85±0.00
Ash	0.90±0.00	0.95±0.00	1.40±0.00	1.30±0.01	1.85±0.02
Digestible carbohydrate	55.44±1.80	55.28±1.80	51.80±1.50	50.37±1.90	48.68±2.00
Calcium (mg/100g)	40.00±1.20	43.00±1.60	43.00±1.10	50.00±1.06	50.00±2.01
Phosphorus (mg/100g)	15.00±0.70	20.00±0.40	19.00±0.80	24.00±0.90	23.60±1.00
Iron (mg/100g)	0.24±0.40	0.40±1.00	0.28±0.95	0.30±1.00	0.26±0.70
Zinc (mg/100g)	3.92±1.00	4.90±1.20	4.70±1.00	4.42±1.00	4.21±1.20

Values are means ± standard deviation of two determinations.

A1, A2, A3, A4 and A5 are bread samples with 0%,10% whole, 10% defatted, 20% whole and 20% defatted sesame seed flour respectively.

Table 4: Sensory and physical quality of bread samples fortified with different levels of whole or defatted sesame seed flour

	A1	A2	A3	A4	A5	Grand mean	LSD	Significance
% Sesame seed flour		10%	10%	20%	20			
Colour	6.07±1.49	5.80±1.32	5.47±1.59	5.07±1.79	5.40±1.88	5.56±1.62	1.12	0.51[NS]
Texture	5.27±1.71	5.93±1.34	5.13±1.59	5.00±1.89	4.87±1.64	5.24±1.64	0.99	0.44[NS]
Flavour	5.60±1.64	5.80±1.15	4.67±1.76	4.67±1.84	4.20±2.18	4.99±1.81	0.96	0.07[NS]
Mouth feel	4.87±2.20	5.80±1.32	4.33±1.95	4.47±2.13	4.53±2.26	4.80±2.02	1.12	0.27[NS]
O/A	5.33±1.76	5.80±1.47	5.33±1.59	5.00±181	4.73±1.98	5.24±1.72	0.95	0.53[NS]
Loaf volume	8.04	9.97	9.20	11.01	10.65	9.77	1.42	
Specific volume	2.02	2.38	2.42	2.72	2.58	2.43	0.34	

Values are means ± standard deviation of two determinations.

A1, A2, A3, A4 and A5 are bread samples with 0%,10% whole, 10% defatted, 20% whole and 20% defatted sesame seed flour respectively.

Table 6: Sensory and physical quality of bread samples fortified with different levels of undefatted or defatted sesame seed flour

% of Sesame Seed Flour	0	10	10	20	20	Grand mean	LSD	Significance
D/UDSF		UD	D	UD	D			
Colour	6.07±1.49	5.80±1.32	5.47±1.59	5.07±1.79	5.40±1.88	5.56±1.62	1.12	0.51[NS]
Texture	5.27±1.71	5.93±1.34	5.13±1.59	5.00±1.89	4.87±1.64	5.24±1.64	0.99	0.44[NS]
Flavour	5.60±1.64	5.80±1.15	4.67±1.76	4.67±1.84	4.20±2.18	4.99±1.81	0.96	0.07[NS]
Mouth feel	4.87±2.20	5.80±1.32	4.33±1.95	4.47±2.13	4.53±2.26	4.80±2.02	1.12	0.27[NS]
O/A	5.33±1.76	5.80±1.47	5.33±1.59	5.00±181	4.73±1.98	5.24±1.72	0.95	0.53[NS]
Loaf volume	8.04	9.97	9.20	11.01	10.65	9.77	1.42	
Specific volume	2.02	2.38	2.42	2.72	2.58	2.43	0.34	

Values are means ± standard deviation of three determinations, NS= Not significant. (P> 0.05).O/A= Overall acceptability, D/U DSF= Defatted or undefatted sesame seed flour, D- defatted, UD= Undefatted

Table 5: Storage (6 days) stability of bread samples fortified with different levels of undefatted and defatted sesame seed flour

	Days		Sample Codes					
		A1	A2	A3	A4	A5	LSD	Significance
% of undefatted or Defatted Seed flour		0	10	10	20	20		
O-fat rancidity (Days)	2	5.87±1.36	6.00±1.13	6.27±0.59	6.33±0.72	6.27±0.88	0.54	0.64[NS]
	4	7.00±0.00	7.00±0.00	7.00±0.00	5.40±1.55	5.73±1.03	0.56	0.00***
	6	7.00±0.00	7.00±0.00	6.93±0.26	4.53±1.89	4.60±1.29	0.68	0.00***
Moisture (Days)	2	2.80±0.94	3.20±0.78	3.00±0.54	3.07±0.70	3.07±0.70	0.38	0.68[NS]
	4	1.47±0.91	1.40±0.83	2.80±0.78	1.73±1.22	1.53±1.06	0.57	0.00***
	6	1.40±0.63	1.33±0.49	2.80±0.68	1.67±0.98	1.67±1.05	0.46	0.00***
Flavour (Days)	2	6.13±0.74	6.07±0.79	6.47±0.52	6.40±0.51	6.40±0.51	0.30[NS]	
	4	6.80±0.41	6.87±0.35	6.87±0.35	6.80±0.41	6.27±0.46	0.19	0.00***
	6	6.07±0.26	5.93±0.46	6.00±0.38	5.93±0.46	5.53±0.83	0.34	0.00***
Musty (Days)	2	6.47±0.64	5.93±0.46	6.33±0.72	6.33±0.49	6.47±0.64	0.37	0.00***
	4	6.93±0.26	6.40±0.74	6.93±0.26	6.93±0.26	6.53±0.52	0.17	0.96[NS]
	6	6.87±0.35	6.93±0.26	6.67±0.49	6.60±0.63	5.67±1.11	0.38	0.00***
Softness (Days)	2	2.00±1.00	6.80±0.41	1.93±0.79	1.87±0.92	1.80±0.94	0.33	0.00***
	4	1.13±0.35	2.27±0.70	3.40±0.83	3.53±0.99	3.53 ±0.99	0.49	0.64[NS]*
	6	1.07±0.26	1.13±0.35	2.67±0.82	4.53±1.19	2.67±0.82	0.51	0.00**
Springiness (Days)	2	4.07±1.10	1.13±0.35	3.80±1.08	4.27±0.88	4.40±0.83	0.59	0.00***
	4	6.87±0.35	3.87±0.99	4.53±1.06	6.80±0.41	2.67±1.39	0.55	0.41 [NS]
	6	6.73±0.46	7.45±0.26	3.47±0.92	6.73±0.46	2.53±0.92	0.49	0.00***

Values are means ± standard deviation of two determinations.

A1, A2, A3, A4 and A5 are bread samples with 0%,10% whole, 10% defatted, 20% whole and 20% defatted sesame seed flour respectively.

Assessment of the Nutritional Status and Associated Factors of Orphans and Vulnerable Preschool Children on Care and Support from Nongovernmental Organizations in Hawassa Town

By Bisrat Getaneh, Dr. Usha Kulkarni & Mr. Yemane G/Mariam

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Abstract- Background: United Nation Children"s Fund estimates that as of 2010 there were 153 million orphaned children and adolescents living in the world. Thirty six percent of the world"s orphans lives in Sub Saharan region. As of 2012, Ethiopia is estimated to have 1,988,731 Orphans of whom 530,630 are orphans due to HIV /AIDS and is one of the largest populations of Orphan and Vulnerable Children in Africa. However there is a little information about the effect of orphan hood and child vulnerabilities on child nutritional status in the study setting.

Objective: To assess the nutritional status and associated factors among orphans and vulnerable preschool children on care and support from a nongovernmental organization.

Keywords: nutritional status, orphans and vulnerable preschool children, care and support, hawassa.

GJMR-L Classification: NLMC Code: WS 115

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Strictly as per the compliance and regulations of:

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Bisrat Getaneh [α], Dr. Usha Kulkarni [σ] & Mr. Yemane G/Mariam [ρ]

Abstract- *Background:* United Nation Children"s Fund estimates that as of 2010 there were 153 million orphaned children and adolescents living in the world. Thirty six percent of the world"s orphans lives in Sub Saharan region. As of 2012, Ethiopia is estimated to have 1,988,731 Orphans of whom 530,630 are orphans due to HIV /AIDS and is one of the largest populations of Orphan and Vulnerable Children in Africa. However there is a little information about the effect of orphan hood and child vulnerabilities on child nutritional status in the study setting.

Objective: To assess the nutritional status and associated factors among orphans and vulnerable preschool children on care and support from a nongovernmental organization.

Methods: Community based Cross-sectional study was conducted on 364 orphan and vulnerable preschool children who were on care and support from Non Governmental Organization in Hawassa town, Southern Ethiopia. The data collection instruments were a structured pretested questionnaire, 24 dietary recall and anthropometric measurements. Data were entered using EPi-info software and exported to SPSS for analysis. The prevalence of malnutrition among Orphans and Vulnerable preschool Children was assessed by calculating the percentages using ENA for SMART 2007 software and analysis was made using WHO Standard cut off point below- 2 S.D using z-scores. Logistic Binary and multivariate analysis were carried out to see the effect of each independent variable on nutritional status explained as stunting ,wasting and underweight. Logistic regression was used to control any confounders at p value 0.05 with 95% CI.

Results: This study revealed that, 35.1%, 8.9 % and 7.5 % of orphans and vulnerable children were stunted, underweight and wasted, respectively. The main associated factors of stunting were found to be children who have parents (AOR 3.717; 95% CI 1.405, 9.804), single care takers (AOR.259; 95% CI .751, .089) and Educational status secondary and above (AOR 2.777; 95% CI 1.272, 6.063), first complementary food were milk (AOR 2.463; 95% CI 1.328, 4.568).Cough prior to 2 weeks of this survey (AOR 2.272; 95% CI 1.997, 5.181), HH food security (AOR 2.667; 95% CI 1.072,6.667)and food and nutritional support from NGO"s. (AOR.6.251; 95% CI. 1.427, 9.778)) Were variable associated with wasting. Underweight was associated with family size < 5 (AOR 2.778; 95% CI 1.148, 6.721) and duration of breast feeding (AOR 3.257; 95% CI 1.344, 7.891).

Conclusion and recommendation: from the findings of this study, can be concludes that malnutrition is still an important problem among Orphans and vulnerable preschool Children on care and support from NGO"s and more attention needed to be given by stakeholders.

Keywords: nutritional status, orphans and vulnerable preschool children, care and support, hawassa.

I. Introduction

a) Background

An estimated 24 million children across the world live without their parents, and numbers of children live outside parental care are. The effects of the loss of parental care on children can be devastating. Children without parental care find themselves at greater risk of discrimination, inadequate care, abuse and exploitation. Inadequate care can impair children"s education, emotional and physical development and health. It is widely recognized that the most preferable form of such alternative care is usually care within a family setting such as kinship care or foster care, and that large scale institutional care should be avoided where possible (1).

Poverty and deprivation have a major impact on children"s ability to stay with their parents, and may also affect the ability of extended or other families to offer homes for children. Poverty also interacts with other determinants of children"s care choices, such as HIV, migration and abuse or neglect in the home (2).

Historically, the fostering of children by extended family members, including aunts, uncles, grandparents, and other relatives, is common throughout sub-Saharan Africa. Extended family members have fostered children for a variety of reasons including the deaths of mothers in childbirth (3). The tradition of fostering by extended family continues today and is a vital coping mechanism in nations with high HIV prevalence and growing orphan populations. Throughout sub-Saharan Africa, an estimated 90% of

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orphaned children in households live with extended family members (4). The advantages of extended-family fostering are that it is culturally acceptable and assumed to be sustainable throughout a child"s development, partially because communities will band together to support these households. In most cases, children can find stability, love, and emotional support in relatives" homes (5).

United Nation Children"s Fund estimates that as of 2010 there were 153 million orphaned children and adolescents living in the world. While13% of the world"s children under the age of 18 years live in Sub- Saharan Africa, 36% of the world"s orphans lives in Sub Saharan region (6). Approximately 27% of these orphans were orphaned due to HIV/AIDS (7).

With a total population projection of over 86 million, Ethiopia is the second most populous country in Africa, More than half (55.5%) of the population is constituted by children below the age of 18 years (8). Though the national prevalence of HIV in Ethiopia, estimated to be 1.3%, is considerably lower than rates in other sub-Saharan African countries, As of 2012, Ethiopia is estimated to have 1,988,731 Orphans of whom 530,630 are orphans due to HIV /AIDS, one of the largest populations of Orphan and vulnerable children (OVC) in Africa (9).

A number of factors have been suggested to affect both the level of food security at household level and the children"s nutritional status, some of which are independently associated with households in which orphans live. These can broadly be classified into child characteristics (e.g., age and gender), household characteristics (e.g., household income, and number of children in the household), parental characteristics (e.g., occupation, education level and age of the household head) and community factors (e.g., water supply and sanitation) (7).

b) Statement of the problem

Despite being highlighted as one of the priority development issues under the Millennium Development Goals framework, malnutrition remains an important public health concern and one of the main causes of early child morbidity and mortality in developing countries (10). Many OVC suffer from cycles of poverty as a result of the illness and death of their parents and they are especially vulnerable and are at an increased risk of malnutrition and ill-health (11).

Communities and families in sub-Saharan Africa have been faced with a growing challenge of providing care for orphans and vulnerable children. Over 90% of all orphans not living with a surviving parent are cared for by extended families (12). Malnutrition is among the most series health problem facing in Ethiopia .The prevalence of child malnutrition that is stunting, wasting and under weight is 44.1%, 7.6% and 28.3% respectively for SNNPR. However, there is little information about the

effect of orphan hood and child vulnerability on child nutritional status in study setting and only Few evidence examining the nutritional status of Orphans and vulnerable children (OVC) who are on care and support from NGO while children 6 to 59 month is one of the critical window of opportunity for Intervention to address under-nutrition through the Lifecycle Approach as Stated in the National Nutrition Program and moreover OVC are potentially at greater risk of poor health and nutrition because they are more likely to be extremely poor, may receive less care and may themselves be HIV-infected via parent-to-child transmission.

Therefore, this study will address the information gap by estimating the magnitude and identifying the potential factors affecting the nutritional status of OVC who are on care and support from NGO.

c) Significance of the study

Information regarding the nutritional status of orphan and vulnerable children is limited in the study setting and most studies mainly focus on the general population than these segments of population. Currently a number of Non Governmental Organization in Ethiopia are providing different types of care and support to OVC based on the Guideline developed by the Ministries Of Women"s Affaires on care and support for orphan and vulnerable children, However; little is known about their nutritional status and therefore determining the magnitude and associated factors affecting the nutritional status of the Orphans and Vulnerable Children will be helpful to provide information for policy maker, NGO"s and other stakeholders and the information can be used as a baseline for further research.

II. Literature Review

a) Prevalence of malnutrition in Orphans and Vulnerable Children

Malnutrition is a leading cause of morbidity and mortality among children in the developing world, contributing to more than half of all child deaths (13). Worldwide, nearly one in four children under five ages are stunted, an estimated 101 million children of under five age are underweight and 52 million children are moderately or severely wasted (14).

In Sub Saharan Africa, Malnutrition is a leading cause of morbidity and mortality. More than one third of countries in sub Saharan Africa with high prevalence rates 40% of children are stunted, 25% of children are underweight and wasted (14).

A study conducted on influence of socio-economic factors on nutritional status of children in a rural community of osun state, Nigeria revealed that the prevalence rates of underweight, wasting and stunting were 23.1%, 9 % and 26.7% respectively (15).

Nationally, The National Demographic Health Survey conducted by Central Statistic agency (CSA) in Ethiopia in 2011 showed that the prevalence of stunting,

wasting, and underweight was 44%,10% and 29% and varies from region to region (8).

A cross sectional study conducted in North West Ethiopia shows prevalence of malnutrition in the community with 28.5% of the children underweight, 24 % stunted and 17.7% wasted (16). A community based cross-sectional survey conducted in West Gojam zone revealed that 49.2 % children were found to be under-weight, 43.2 % of the children under age five were suffering from chronic malnutrition and 14.8 % acutely malnourished (17). The cross sectional survey conducted in rural communities of Tigray region also revealed that, the levels of stunting, under weight and wasting were 42.7%, 38.3% and 13.4%, respectively (18). A cross sectional study conducted in Aynalem village in Tigray region, the overall prevalence of stunting, underweight and wasting were 45.7%,43.1% and 7.1% ,respectively (19). According to research conducted in Gimbi district, Oromia region indicated that, 32.4 % stunted, 23.5 % underweight and 15.9% of the children were wasted. Prevalence of severe stunting, severe underweight and severe wasting respectively were 15.7%, 8.0 % and 5.7% (20). A community-based cross-sectional study conducted in rural kebeles of Haramaya district revealed, the prevalence of stunting, underweight, and wasting were 42.2%, 36.6%, and 14.1%, respectively (21).

The prevalence of stunting, wasting, and underweight reported for SNNPR is 44.1%, 7.6% and 28.3% respectively (8).

b) Factors affecting the nutritional status of an OVC

i. *Inadequate Dietary intake*

Globally, 39 per cent of infants less than 6 months old were exclusively breastfed and only 60 per cent of children aged 6-8 months receive solid, semi-solid or soft foods, highlighting deficiencies in the timely introduction of complementary foods in 2011 (14). The study conducted in Nairobi, Kenya prevailed that orphans living in households in informal settlements in Nairobi are indeed more vulnerable with regards to food security than non-orphaned children, most particularly paternal orphans. In preliminary studies completed by UNICEF in Malawi and Jamaica, the percentage of *non-*orphans who were food insecure was 15%, compared to 39% among paternal and double orphans in the same region (22).

ii. *Disease and Malnutrition*

It is clear that Infection and nutritional status of children are interrelated where malnutrition can accelerate disease progression, and Infection worsens malnutrition by weakening the immune system and hindering nutrient intake, absorption, and storage. Globally, between 1995 and 2005, one in three preschool-age children were deficient in vitamin A due to inadequate dietary intake. A study conducted by FAO shows that one in four had experienced symptoms of

illness including fever, cough, and/or diarrhea in the previous two weeks; and 55% had been ill during the previous 6 months. Four percent were reported to have tested HIV positive, and HIV infection was suspected in another 2.0 % (23).

In sub-Saharan Africa, AIDS is the leading cause of death among those aged 15-59 years old and 80.0% of them who have lost a parent by AIDS in the developing world are living in this region. Even once the HIV infection rates stabilize or begin to decline, the number of orphans will continue to grow or remain high for many years due to the time lag between HIV infection and death. Orphaned children are at an increased risk for malnutrition and illness in addition to a lack of access to health care (24).

A study conducted in Angolela tera Woreda north Ethiopia revealed that One-third of the participants were found to have a protozoan infection, while 7.1% were found to have a helminthic infection. Approximately 11% of the students were stunted, 19.6% were wasted, and 20.8% were underweight. (25).

iii. *Child care and OVC*

Care affects nutritional status in three ways: through feeding practices such as breast-feeding and the preparation of nutritious foods for weaned infants and others in the household; through health and hygiene practices both within the family and within the community; and through support to the mother, both by the family and by the community, so that she has sufficient time to care for the child. (1)

A study conducted about attributes of care giving Reveled that, Caregivers, whose mean age at enrolment was 42.9 (S.D 13.3) years, were predominantly female (87%), and 25% per cent were married. Fifty-five percent were biological parents of the child participants. Forty-five percent of caregivers were known to be illiterate, and the mean number of years of education was 4.9 (S.D 3.7). Forty-five percent of caregivers reported their own health to be fair, poor, or very poor; 24% reported symptoms in the previous two weeks; and 56% reported illness in the previous 6 months (26).

iv. *Socio demographic variables*

A study conducted about poor health in less wealthy country showed the median age at enrolment was 10 (standard deviation, S.D 2.1) years. Fifty-seven percent of the OAC were paternal orphans; 16% were maternal orphans, and 17% were double orphans (27).

Vulnerable children who live in household sizes of 4-6 members and vulnerable children who live with non-relatives had greater odds of inadequate immunization (OR = 1.51, 95% CI: 1.13-2.01, OR = 9.02, 95% CI: 4.62-17.62). Paradoxically, vulnerable children living with non-relative caregivers were at lower risk for inadequate food (OR = 0.19, 95% CI 0.07-0.33). Single orphans with an HIV positive parent were less

likely to be fully immunized than single orphans with an HIV negative parent (28).

v. *Environmental and hygiene and OVC*

Unsafe water, poor sanitation and unhygienic conditions claim many lives each year. An estimated 1.2 million children die before the age of 5 years from diarrhea. Poor urban areas where insufficient water supply and sanitation coverage combine with overcrowded conditions tend to maximize the possibility of fecal contamination (29). Globally, urban dwellers enjoy better access to improved drinking water sources (96 per cent) than do people living in rural areas (78 per cent). Even so, improved drinking water coverage is barely keeping pace with urban population growth; access to an improved water source does not always guarantee adequate provision. Without sufficient access to safe drinking water and an adequate water supply for basic hygiene, children‟s health suffers (29).

vi. *Impact of malnutrition in OVC*

Malnutrition prevents children from reaching their full physical and mental potential. Health and physical consciences of prolonged states of malnourishment among children are: delay in their physical growth and motor development; lower intelligent quotient (IQ), greater behavioral problems and deficient social skills; susceptibility to contracting disease. Under nutrition and micronutrient deficiencies contribute substantially to the global burden of disease. Under nutrition reduces immunological capacity to defend against diseases, and diseases deplete and deprive the body of essential nutrients. Under nutrition and infectious diseases further exacerbate poverty through lost wages, increased health care costs, and most insidiously impaired intellectual development that can significantly reduce earning potential (30).
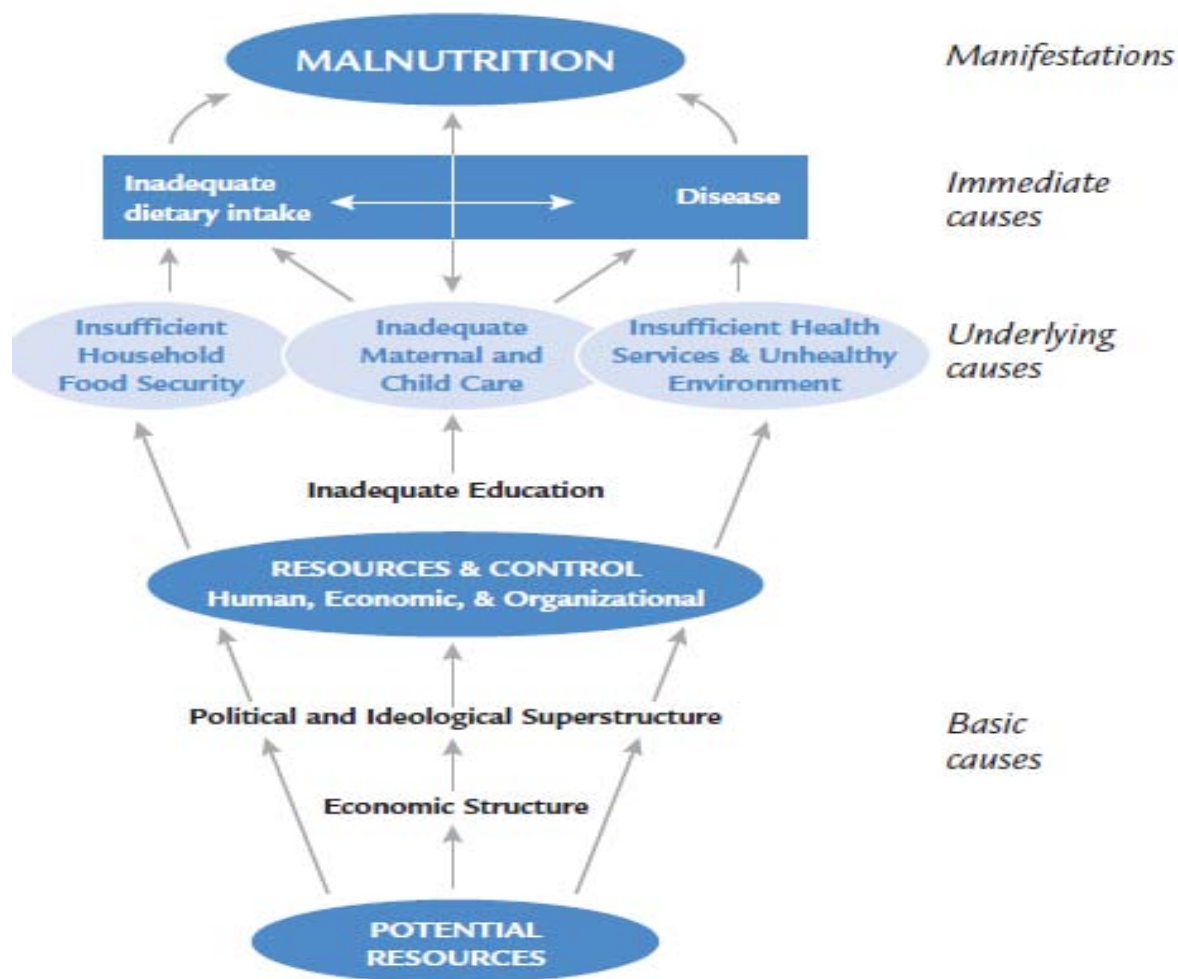

Figure 1: Coneptual frame work adopted from UNICEF, 1990

The immediate causes of malnutrition are inadequate food intake and infectious diseases, which in turn, result from a combination of three underlying causes that relate to the nutrition, social and health environment of the child. Inadequate household food security, inadequate maternal and child care, insufficient services and unhealthy environment are the underlying causes, which in turn, result from basic causes; Formal and non-formal institutions, political and ideological superstructure economic structure and potential resources (Figure 1) (31).

III. STUDY OBJECTIVES

a) General objective

To assess the nutritional status and associated factors of orphans and vulnerable preschool children on care and support from a nongovernmental organizations in Hawassa town, Southern Ethiopia.

b) Specific objectives

➤ To estimate prevalence of malnutrition among orphans and vulnerable children on care and support from nongovernmental organization based on anthropometric measurement

➤ To identify associated factors for malnutrition among orphans and vulnerable children on care and support from nongovernmental organization

IV. METHODS AND MATERIALS

a) Study period and Setting

The study was conducted from January 27 to April 1, 2014 in Hawassa town administrative council, which is the capital of Southern Nation Nationalities People"s Regional state. Hawassa town is found 273 km south of Addis Ababa, the capital city of Ethiopia. Its boundaries are Shashmene town in the north, Wondo-Genet district in the east, Malga district in the South-east and Hawassa Lake in the west. It has a projected population for 2013/14 was 316,842 people, out of this 163,039 are males and 153,803 are females with the annual population growth rate 4.02. The municipality has 8 sub-city and 32 kebeles (32).

The potential health coverage of the City administration was 92 % in 2013 G.C. There are one referral hospital, one district hospitals, three private hospitals, 10 health centers, 15 health posts, 47 private clinics, 49 drug stores, 11 diagnostic laboratories and 12 pharmacies in the City administration. The total number of OVC on care and support in Hawassa Town was 10,693 and out of this 3000 was under five and there were 28 NGO"s that provide care and support for OVC (33).
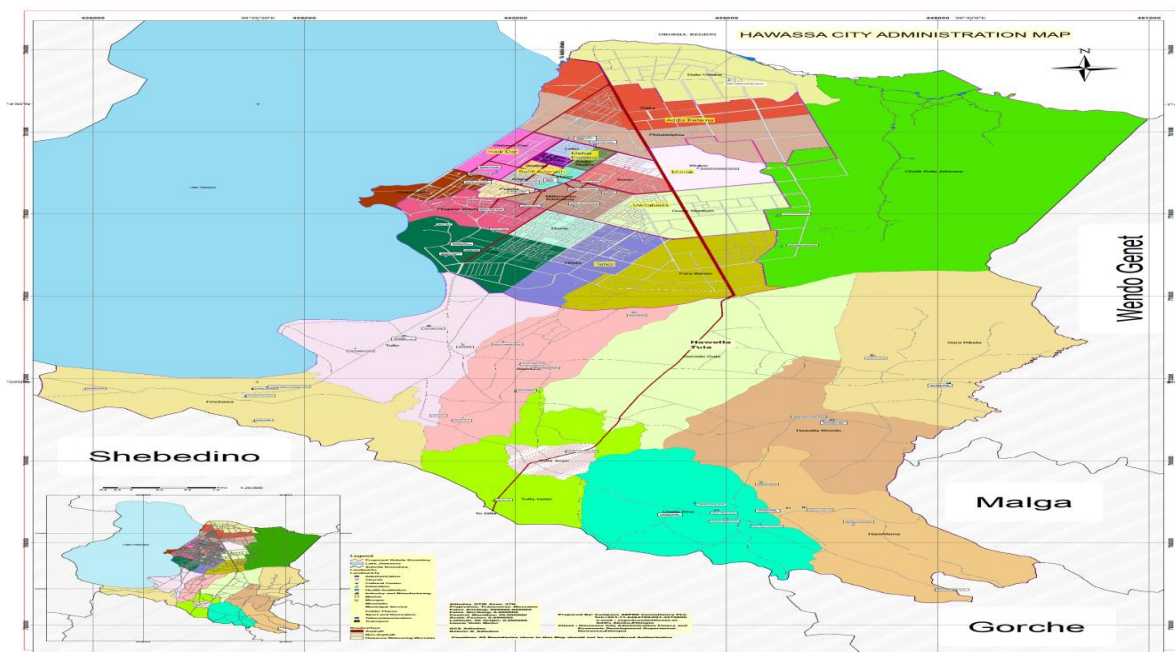
Figure 2: Map of the study area

b) Study Design

Community based cross-sectional study was conducted.

c) Source population

All Orphans and vulnerable children aged 6-59 month on care and support from Nongovernmental Organizations in Hawassa town.

i. Study population

orphan and vulnerable children aged 6-59 month on care and support in selected sub cities

Exclusion criteria

➤ Orphans 6-59 month of age who were severely ill or with disability

d) Sample size determination

To determine the number of Orphan and Vulnerable Children to be included in the study, the single population formula was used to calculate the sample size for first specific objective and for this proportion the most prevalent form of malnutrition (44.1%) was taken from EDHS 2011GC (8).

Assumption

n= sample size

Z α /2=1.96 level of significant

P= Proportion of the most prevalent form of malnutrition 44.1%

D= degree of precision 0.05

The sample size is calculated using

$$\frac{(Z1-\alpha/2)^2*P*(1-P)}{d^2} = \frac{(1.96)^2*0.441(1-0.441)}{0.05^2} = \frac{3.84\ 16*0.44\ 1*0.559}{0.0025} = \mathbf{372}$$

372 with 90% response rate =372*1/0.9= **414**

❖ Since the total population is < 10,000 correction was made and n final was calculated as n final=n/(1+n/N)=414/(1+414/3000)=364

This is the Main Factors affecting the nutritional status of Children from literature and then the second objective is calculated using OPEN EPI 2.3 version as follows with the (20,21).

Sample size for second objective

Assumption 95% CI, power of 80% and Ratio of case to controls 1:4

	HH income in birr	Employment status	Education of mother/caretaker	N^o orphan children cared for
% cases exposed	≥ 1000 33.4%	Employed 38%	Literate 13%	17% caring for 3 or more orphans
% controls exposed	<1000 66.6%	Unemployed 62%	No Education 44%	54% caring for 1
Sample Size for cases	25	47	25	19
Sample size for controls	98	186	99	75
Total Sample size with 90% RR	137	264	137	104

❖ Since the sample size calculated for the first objective could accommodate the second objectives 364 was selected due to representativeness.

e) Sampling procedures

All sub cities in Hawassa town were identified by name and then using simple random sampling technique by lottery Methods 4 sub city was selected and the sample size for the sub city was proportionally allocated. The sampling frame was prepared for selected sub city in Hawassa town depending on registration from sub city and then by using simple random sampling method using random number table subjects were identified and household survey was conducted using mother child supporting groups as a guiders at each sub cities who know house the selected orphan and vulnerable children were located.
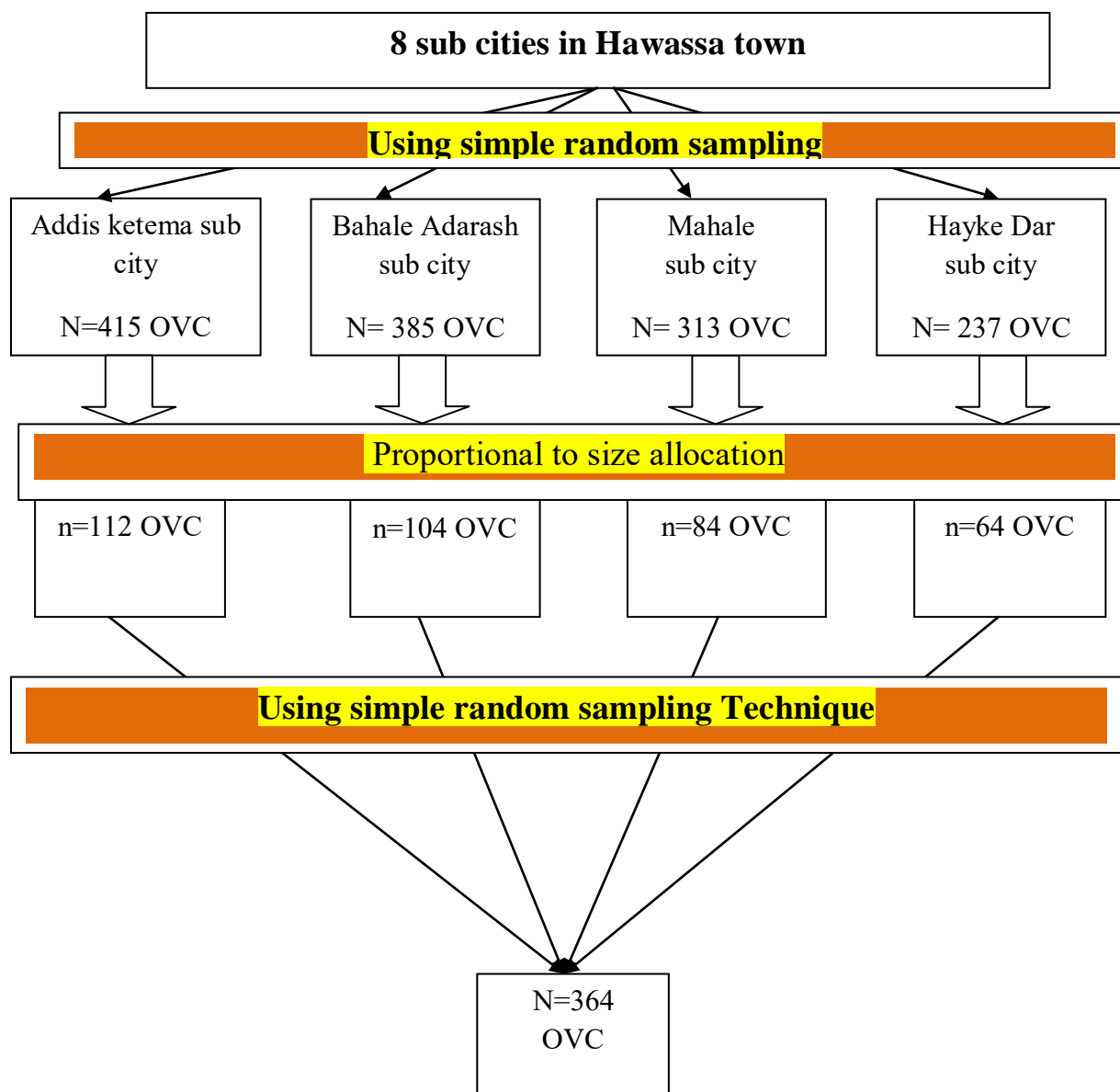
Proportional to size allocation =total number of OVC in each selected sub cities/ the sum of OVC in selected sub cities *total sample size

Figure 3: Schematic presentation of sampling procedure

f)   Study variable

Dependent Variable

Nutritional status– stunting, wasting, underweight
Independent Variables

Demographic factors (age, sex, ethnicity, education, number of children in HH, marital status), Socio economic variables (income, employment), Child health care (immunization, sickness), Environmental /sanitation factors (source of water, latrine, domestic waste disposal), food in security variables, Dietary intake (breast feeding, Individual Dietary Diversity Score).

g)   Data collection procedures and measurements

The data collection instruments were a structured pretested interviewer administered questionnaire, 24 hours dietary recall measurements at individual level and anthropometric measurements. Questionnaire was adapted from different relevant studies and standards and was prepared originally in English language and then it was translated into Amharic language for data collection purpose and then back to English for reconsideration. Data were collected from mother/caretakers and measurements from orphan and vulnerable preschool children aged 6-59 month who were on care and support in NGO"s during the time of data collection

h)   Data quality control

The pretest of the instrument was carried out in Tula Sub city which was not selected for this study in 5%

of the sample and pretest ensures validity of the instrument. The pre-tested data were not included as part of the main data of the study.

The data collections were facilitated by 4 enumerators who are diploma nurses and two health officers as supervisors. Enumerators and supervisors were given one day training by principal investigator in Hawassa health center on the objectives of the study, on the contents of the questionnaire, on the methodology of the study, on the issues of the confidentiality of the responses, on the use of instruments and on the procedures how to take anthropometric measurement. All measurements were carried out using standard procedures by explaining the procedure to the mother /care takers.

Anthropometric measurements

Age: were collected from the mother/caretakers and looking up age in official registers for counter check. The 15th day of the month was used when the date of birth is unknown and if the month of birth was unknown, the midpoint of the year of birth was used.

Sex: Was recorded as male and female.

Length/Height: were measured using Wooden board in recumbent position while the child barefooted and free of head wearing in children <2 years old to the nearest 0.1 cm and height was measured using Wooden board in standing-up position while the child being barefooted and free of any head wearing in children >2 years old and was recorded to the nearest 0.1 cm.

Weight: weight was measured using a 25 kg hanging spring scale graduated by 100 g for children while clothes are removed and was recorded to the nearest 0.1 kg. The scale was calibrated immediately before and during each session by placing standard calibration weights of 5 kg iron on the scale to ensure accuracy.

MUAC: were measured for children >65cm in height using color coded standard MUAC tape meter by calculating the midpoint of the child"s left upper arm by first locating the tip of the child"s shoulder and the tip of the elbow through right angle position and measurement was taken in the mid point by straighten the child"s arm and read the measurement to the nearest 0.1cm.

Households Food Insecurity Access scale

Based on the responses given to the nine questions and frequency of occurrence over the past 30 days, households are assigned a score that ranges from 0 to 27. A higher HFIAS score is indicative of poorer access to food and greater household food insecurity. The lower the score, the most food secured a household was. A score of<17 was classified as food secured and a score of≥17 classified as food insecure. Household Food Security was assessed during the site assessment using the household level component of HFIAS (34).

Individual dietary diversity score

Was Calculated on the basis of the number of food groups consumed within the 24 hours recall period from the total of 11 food groups. The food group consumption frequency score (FGFS) was calculated by assigning a score of 0 if not consumed during the previous 24 hours, 1 if consumed. For children 6 months to 5 years was scored as high DDS if the score out of the 9 is ≥4 and as low DDS if < 4 (35).

i) *Data Management*

First code was given to the completed questionnaire and then data was entered and clean up using EPI-info version 3.5.1 software and SPSS analysis statistical package to cheek for frequencies, accuracy, outliers, and consistencies and missed values and variables and Anthropometric measurement data was entered, clean up using ENA SMART. Any error was identified and corrected.

j) *Data Analysis and Presentation*

The prevalence of malnutrition among OVC was assessed by calculating the percentages of children 6–59 months using ENA SMART and analysis was made using WHO Standard cut off point below - 2 S.D to determine nutritional status as stunting, wasting or underweight using z-scores. Odds ratio with 95%confidence interval was computed to assess the strength of the association and statistical significance and Data were then exported to SPSS and Binary and multivariate Logistic analysis was carried out to see the effect of each independent variable on nutritional status. Logistic regression was used to control any confounders at p value 0.05. . Bar graph was used for diagrammatic summarization of categorical variables and tables were used for summarization variables.

k) *Operational Definition*

An orphan: was a child aged 6 to 59 month whose mother, father, or both have died.

Social orphans: one or both their parents may still be alive but who have been unable to perform parental duties because of illness or acute poverty among other reasons.

Vulnerability: high probability of a negative outcome which results from risky or uncertain events and lack of appropriate means to deal with them.

Vulnerable children: were defined as being under the age of 59 month and currently at high risk of lacking adequate care and protection.

Standard Definition

Stunting which is below -2 S.D from median height for age of reference population,

Wasting which is below -2 S.D from median weight for height of reference population

Underweight which is below -2 S.D from median weight for age of reference population.

l) Ethical consideration

The study proposal was submitted to the ethical Review Board of Mekelle University College of health sciences and was approved. Following the endorsement by ethical Review Board, official permission was secured from Hawassa city administration department Women"s, children and youth Affairs through a support letter from the department of public health, college of health sciences, Mekelle University. Selected Sub cities were informed about the objective and purpose of the study through a support letter from the Hawassa city administration department Women"s, children and youth Affairs. The participants were informed about the objective and purpose of the study and parental informed written consent was obtained from each participant during data collection and anthropometric measurement. Introduction of the study, method of the questioning and confidentiality letters was attached to the cover page of the questionnaires.

The participants were informed that they have a full right to participate or decline from participating in the study and information was collected anonymously. There was no serious harm to the participants and children who were malnourished using MUAC tape meter during data collection was notified to the respected service providing organization for management of malnutrition and the result of this study will help us to know the nutritional status of orphans and vulnerable preschool children and to improve services provided by NGO"s for Orphans and vulnerable preschool children.

m) Dissemination of Information

The results of this study will be communicated to Mekelle University, college of health sciences, the Bureau of women"s affairs, the Regional Health Bureau and other concerned bodies through hard copy and presentation. Publication on an appropriate journal will also be attempted.

V. Results

a) Socio-demographic characteristics

A total of 359 aged OVC 6-59 months were participated in the study with an age category 48-59 month accounted 161(44.8%) followed by 36-47 month 85(23.7%) with a mean age of 39.25 month and the response rate was 98.6%.Non response rate was due to refusal to participate in the study. Regarding sex, 187(52.1%) of the study participants were males and 172 (47.9%) were females. The number of Under five children in household two and above was 65(18.1%). protestant and orthodox was the dominant religion in this study which was 181(50.4%) and 161(44.8%), respectively. Wolyita was the dominant ethnicity 249(69.4%). Double orphan in this study was 22(6.1%) and maternal orphan was about 69(19.2%) and paternal orphan 7(1.9%).over half of the respondents 281(78.3%) were parents and female parents constituted 347(96.7%) were females and among the care takers 168(46.8%) were attended primary education. Concerning the occupation of care takers, majority 216(60.2%) was unemployed and majorities of the care takers were married 323(90%) and almost half of them the households were headed by husband/wife of husband (Table-1).

Table 1: Socio demographic characteristics of the study participants in selected sub city, Hawassa town, Ethiopia, 2014

Variable	Frequency	Percent (%)
Age of the child in Month (n= 359)		
6 - 11	10	2.8
12 - 23	42	11.7
24 - 35	61	17.0
36 - 47	85	23.7
48 - 59	161	44.8
Mean age (SD)		
Sex of the child (n=359)		
Male	187	52.1
Female	172	47.9
Under 5 children in HH (n=359)		
<2	294	81.9
>=2	65	18.1
Family members in HH (n=359)		
<5	162	45.1
>= 5	197	54.9

Religion of parents/care takers (n=359)		
Orthodox	161	44.8
Muslim	12	3.3
Protestant	1 81	50.4
Others *	5	1.4
Ethnic group (n=356)		
Welyta	249	69.9
Sidama	27	7.6
Amhara	41	11.5
Guragie	25	7.0
Others **	14	3.9
Parental status (n=359)		
Alive	337	93.9
Not alive	22	6.1
Orphan status (n=337)		
Alive	261	20.5
Paternal	7	2.1
Maternal	69	77.4
Sex of the caretaker (n=359)		
Male	12	3.3
Female	347	96.7
Education of mothers (n=359)		
Illiterate	140	39
Primary	168	46.8
Secondar y and above	51	14.2
Occupation of care takers (n=359)		
Unemployed	143	39.8
Employed	216	60.2
Marital status (n=359)		
Single	36	10
Married	323	90
Head of the family (n=359)		
Care taker	160	44.6
Husband/wife of care takers	186	51.8
Others ***	1 3	3.6

*Catholic
 **Kenbata, Hadiya
 **** uncles, Grand parents

b) *Water, sanitation and hygiene characteristics*

The source of water for the 200(55%) of the OVC is public stand and the amount of water used per day which was >15L was 323(90%).Majorities 344(95.8%) used Container as a means of water storage. Furthermore, 355(98.9%) of the household had hand washing facilities. Three hundred and forty four (95.8%) of the house hold had access for latrine and nearly half (47%) of latrine was pit type. Over half (57.1%) used municipality service for domestic waste. Hundred and one (28.1%) of the house hold have separated kitchen for cooking (Table 2).

Table 2: Water, sanitation and hygiene characteristics of the study subject sin the selected sub city, Hawassa town, Ethiopia, 2014

Variable	Frequency	Percent (%)
Source of water (n= 359)		
Pipe	157	43.7
Public stand	200	55.7

Protected spring /well	2	0.6
Amount of water used per day (n= 359)		
<=15L	36	10.0
>15L	323	90.0
Method of water storage n= 359)		
Pot	15	4.2
Container	344	95.8
Hand washing while feeding (n= 359)		
Yes	355	98.9
No	4	1.1
Latrine availability(n=359)		
Yes	344	95.8
No	15	4.2
Type of latrine(n=344)		
Pit	162	47.0
VIP	143	41.6
Water carriage	24	7.0
Others	15	4.4
Waste disposal system (n= 359)		
Pit	104	29.0
Open	18	5.0
Municipality service	205	57.1
Separated Kitchen (n= 359)		
Yes	101	28.1
No	258	71.9

c) Feeding practice and Dietary intake

Three hundred and forty two (95.3%) of the OVC were ever breast fed. Similarly, 285(81.9%) initiated BF within the first hour after delivery. Majorities 91.7% of the OVC exclusively breast feeding. And 249 (69.4%) introduced complementary food within 6 to 12 month. Furthermore, 74(74.4%) of the OVC first complementary food was milk and over half 56.3 % used cup and spoon as means of child feeding. (Table 3)

Table 3: Feeding practice and Dietary intake reported by mothers/caretakers of 359 under five children subjects in selected sub city, Hawassa town, Ethiopia, 2014

Variable	Frequency	Percent (%)
Ever breastfeed(n = 359)		
Yes	342	95.3
No	11	3.1
Not known	6	1.7
Initiation of breast feeding (n = 348)		
Within First hour	285	81.9
Within eight hour	29	8.3
2-3 day	26	7.5
Not known	8	2.3
Age ceased breast feeding (n = 339)		
< 6 month	14	4.1
6-11 month	76	21.2
>=12 month	249	69.4

Age Started complementary feeding (n = 359)		
Immediately after birth	20	5.6
Within 1-6 month	75	20.9
6-12 month	249	69.4
12 month latter	15	4.2
First complementary food child received (n = 359)		
Milk	74	74.4
Adult food	30	8.4
Porridge	54	15
Means of child feeding (n = 359)		
Hand	49	13.6
Cup and spoon	202	56.3
Bottle	108	30.1

Table 4: Proportion of the OVC who consumed specific food items within 24 hours reported by mothers/care takers of 359 under five children subjects in selected sub city, Hawassa town,Ethiopia, 2014

Food item consumed	frequency	Percentage (%)
Cereals		
Yes	291	81.1
No	68	18.9
Roots and Tubers		
Yes	210	58.5
No	149	41.5
Vegetables		
Yes	224	62.4
No	135	37.6
Fruits		
Yes	134	37.3
No	225	62.7
Meat		
Yes	19	5.3
No	340	94.3
Eggs		
Yes	37	10.3
No	322	89.7
Poultry		
Yes	22	6.1
No	337	93.9
Pulses and Nuts		
Yes	155	43.2
No	204	56.8
Milk and Milk product		
Yes	90	25.1
No	269	74.9
Oils and Fats		
Yes	186	48
No	173	52

Table 5: Distribution of responses to household's food security module items reported by mothers/caretakers of 359 under five children subjects in selected sub city, Hawassa town, Ethiopia, 2014

Variable	Frequency	Percent (%)
Worry that not hav ing enough food (n= 359)		
Never	69	19.2
Rarely	206	71. 0
Sometimes	38	13.0
Often	46	15. 8
Not able to eat the kinds of food you preferred		
Because of lack of resources? (n= 359)		
Never	59	16.4
Rarely	19 1	63. 6
Sometimes	56	18. 6
Often	53	17. 6
Eat just a few kinds of food day after		
Day due to lack of resources ? (n= 359)		
Never	45	12.6
Rarely	21 2	67. 5
Sometimes	55	17. 5
Often	47	15.0
Eat food that you preferred not to eat		
Because Of lack of resources? (n=359)	81	22.6
Rarely	192	69 .0
Sometimes	53	19 .0
Often	43	15 .4
Eat a smaller because there were not enough		
Food? (n=359)		
Never	63	17.5
Rarely	20 1	67 .9
Sometimes	62	20. 9
Often	33	11.1
Eat a fewer meal because there W ere not		
enough food? (n= 359)		
Never	43	12.0
Rarely	22 2	70. 2
Som etimes	54	17.0
Often	40	12.6
No food at all because there were Not enough		
resources? (n= 359)		
Never	147	69.3
Rarely	151	71.2
Sometimes	49	22.2
Often	12	5.6

Sleep at night hungry because There was not enough food? (n= 359)		
Never	97	27.0
Rar ely	183	69.8
Sometimes	54	20.6
Often	25	9.5
Whole day eating anything because There was not enough food? (n= 359)		
Never	174	48.5
Rarely	133	71.8
Sometimes	42	22 .7
Often	10	5.4

d) *Maternal and child care characteristics*

Three hundred and sixteen (88%) care takers had ANC follow up during their previous pregnancy and Two hundred and seventy three (76%) of them gave birth at health facilities. over three fourth (76.6%) of the OVC had ever received vaccination and majorities (86.2%) of the OVC were received vitamin A supplementation (Table 4).

Table 6: Maternal and child care characteristics in selected sub city, Hawassa town, ethiopia, 2014

Variable	Frequency	Percent (%)
ANC follow -up (n= 359)		
Yes	316	12 .0
No	43	88 .0
Place of deliver (n= 359)		
Home	86	24 .0
Health facility	273	76 .0
Who attend the deliver (n= 359)		
TBA	84	23.4
Health personnel	275	76.6
Child received Vaccination (n= 359)		
Yes	333	92.8
No	25	7.0
Not known	1	.3 0
Type Vacci nation received (n= 315)		
BCG	62	19.7
Polio	64	20.3
Measles	11	3.5
All	178	56.5
Vitamin A supplementation (n= 359)		
Yes	311	13.4
No	48	18.6
Measles Infection(n= 359)		
Yes	52	14 .5
No	307	85. 5
Cough in 2 weeks (n= 359)		
Yes	78	21.8
No	281	78.3

Diarrhea in 2 weeks (n= 359)		
Yes	54	15.0
No	305	85.0
Fever in 2 weeks (n= 359)		
Yes	98	27.3
No	261	72.7

e) OVC care and support characteristics in selected sub city, Hawassa town, Ethiopia, 2014

Seventy one percent of the OVC get nutritional support from nongovernmental organization through direct assessment and supplementation as a main means of support which was 51%. About 170 (47.4%) of the OVC had health care support from the supportive organization through free health care access as a main means of support (52.6%). About 170 (46.5%) of the OVC had economic strengthening support from supportive organization through income generating activities and access to credit 111(30.9%) and 86 (24%) respectively .About 221(61.6%) of the OVC had educational support from supportive organization through direct assistance 99 (27.6%). About 232(64.6%) of the OVC had Psychological support from supportive organization through Training of Psycho social support 86 (24.0%). About 153(42.6%) of the OVC had legal support from supportive organization and About 142(42.6%) of the OVC had shelter and care support from supportive organization (Table 5).

Table 7: OVC care and support characteristics in selected sub city, Hawassa town, Ethiopa, 2014

Variable	Frequency	Percent (%)
Food and nutrition support (n= 359)		
No	103	28.7
Through Assessment and supplementation	183	51.0
Through Link to health center	59	16.4
Through Training	31	8.6
Health care (n=359)		
No	189	52.6
Through free access	131	36.5
Through Home visit	48	13.4
Through Training	18	5.0
Economic strengthening (n=359)		
No	192	53.5
Through Vocational training	79	22
Through Income g enerating activities	111	30.9
Through Access to credits	86	24.0
Education support (n=359)		
No	138	38.4
Through Direct assistance	99	27.6
Through Identifying and promoting	91	25.3
Through Training	61	17
Psychological support (n=359)		
No	127	35.4
Through Training of Psycho social support	86	24.0
Through Support	129	35.9
Through Parenting	41	11.4

Legal protection (n=359)		
No	206	57.4
Through Protection	82	22.8
Through Link to	89	24.8
Shelter and care (n=359)		
No	218	60.7
Through Support family	98	27.3
Through Assessment	29	8.1
Through Improve shelter	29	8.1
Duration of support (n=359)		
< 6 month	41	11.4
>= 6 month	318	88.6
Adequacy of support (n=359)		
Adequate	108	30.1
Not adequate	248	69.1
No answer	3	.8

f) Prevalence of malnutrition among OVC

The prevalence of stunting 35.1% (95%CI: 30.3, 40.2), wasting 7.5 % (95% C.I 5.2 - 10.7) and underweight 8.9 % (95% C.I 6.4 - 12.3) among orphans and vulnerable children. (Figure 3)
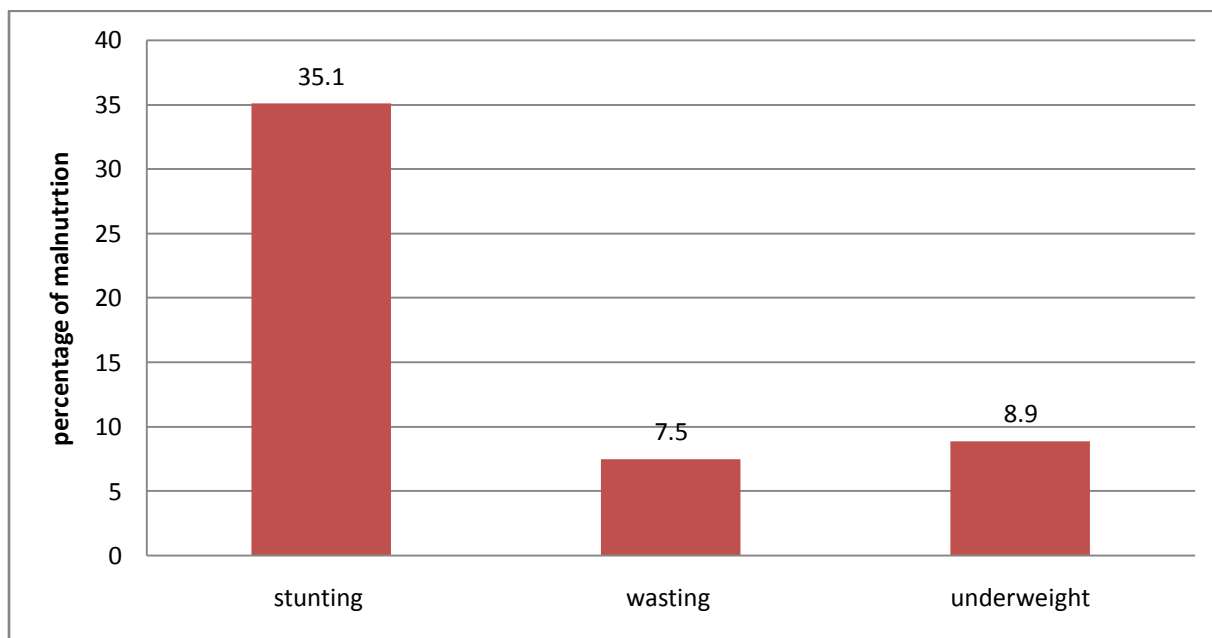
Figure 4: Magnitude of Malnutrition among orphans and vulnerable children aged 6-59 month on care and support from NGO in Hawassa town, Ethiopia, 2014.

g) Factors associated for malnutrition among OVC

i. Factors associated for stunting

The multivariate logistic regression analysis identified children who have parents, marital status and educational status of the care takers, first complementary food the child received as determinant factors for stunting. The odds of stunting among OVC whose either parent were alive were 3.717 times an increased risk than those who were not alive (AOR 3.717; 95% CI 1.405, 9.804).OVC of married care taker were 74.1% at reduced risk to be stunted than those OVC of single care takers (AOR .259; 95% CI .751,.089). The odds of stunting among OVC care takers whose educational status primary were 2.777 times at an increased risk when compared to OVC of care takers their educational status were secondary and above (AOR 2.777; 95% CI 1.272, 6.063). The odds of stunting among OVC whose first food porridge were 2.463 times an increased risk than OVC whose first complementary food were milk (AOR 2.463; 95% CI 1.328, 4.568) (Table 8).

Table 8: Logistic regression analysis showing associated factors for stunting among OVC aged 6-59 month on care and support from NGO in Hawassa town, Ethiopia, 2014

Variable	stunting		COR (95% CI)	AOR (95% CI)
	Yes N (%)	No N (%)		
Parental status				
Alive	123 (97.6 %)	214 (91.8%)	1	1
Not alive	3 (2.4%)	19 (8.2%)	3.861(1.332,11.234) *	3.717(1.405 ,9.804) **
Marital status				
Married	121 (96 .0%)	202(86.7%)	1	1
Single	5 (4.0%)	31(13.3%)	.269(.711, .102) *	.259(.751, .089) **
Educational status				
Illiterate	45 (35.7 %)	95 (40 .8%)	1.942 (.893 , 4.224) *	1.804 (.813 , 4.003)
Primary	71(56.3%)	97(41.6%)	3.001(1.409, 6.391) *	2.777 (1.272, 6.063) **
Secondary and above	10 (7.9 %)	41 (17 .6%)	1	1
First comp. Food				
Milk	84 (66.7%)	191 (81.9%)	1	1
Adult food	14 (11.1 %)	16 (6.9%)	2.082(.970, 4.470) *	1.671(.758, 3.683)
Porridge	28(22.2%)	26(11.2%)	2.563(1.414, 4.646) *	2.463(1.328,4.568) **
Health and care supp				
Ye s	53(31.2%)	117(68.8%)	1	1
No	73(38.6%)	116(61.4%)	1.05 3 (0.74 2,1.48 3) *	1.00 (0.91 ,1.10)
Vitamin A supp				
Yes	113(36.3%)	198(63.7%)	1	1
No	13(27.1%)	35(72.9%)	6.750(3.212,14.187)*	2.558 (.923, 7.090)
IDDS				
Highly DD	34(29.8%)	153(62.4%)	1	1
Less DD	92(37.6%)	80(70.2%)	1.67 1 (0.78 2, 3.613) *	1.09 4 (0.52 1,2.28 3)
HFIAS				
Food secured	113(36.8%)	194(63.2%)	1	1
Food in secured	13(25%)	39(75%)	2.09 2 (0.67,6.52) *	2.07 5 (0.74 4,5.74 1)
Eco . strengthen supp				
Yes	52(31.1%)	115(68.9%)	1	1
No	74(38.5%)	118(61.5%)	1.01 6 (0.99 6,1.03 2) *	0.99 1 (0.97 7,1.01 9)

* P-value < 0.25 in the bivariate analysis ** P-value < 0.05 in the multivariate analysis

ii. *Factors associated for Wasting*

The multivariate logistic regression analysis identified cough prior to 2 weeks of this survey, HH food security and food and nutritional support from NGO were identified as determinant factors for Wasting. The odds of wasting among OVC who were have cough prior to 2 weeks of this survey were 2.272 times an increased risk than OVC who were not have cough (AOR 2.272; 95% CI 1.997, 5.181) The odds of wasting among OVC from food in secured HH were 2.667 at increased risk than to be those who were from food secured (AOR 2.667; 95% CI 1.072, 6.667). The odds of wasting among OVC who have no food and nutritional support from NGO were 6.251times at increased risk to be wasted when compared to OVC who do have food and nutritional support.(AOR.6.251; 95% CI. 1.427, 9.778).(Table 9).

Table 9: Logistic regression analysis showing associated factors for wasting among OVC aged 6-59 month on care and support from NGOS in Hawassa town, Ethiopia, 2014

Variable	Wasting		COR (95% CI)	AOR (95% CI)
	Yes N (%)	No N (%)		
Cough				
Yes	10(37.0%)	68(20.5%)	2.421(1.072, 6.667) *	2.272(1.997,5.181) **
No	17(63.0%)	263(79.5%)	1	1
HH food security				
Food secured	19(70.4%)	287(86.7%)	1	1
Food in secured	8(29.6%)	44(13.3%)	2.747(1.134,6.667) *	2.667(1.072,6.667) **
Food and nut. Supp.				
Yes	25(92.6%)	230(69.5%)	1	1
No	2(92.6%)	101(30.5%)	5.495(1.277,13.811)*	6.251(1.427,9.778) **
Fever				
Yes	10(10.2%)	88(89.8%)	2.917(1.314,6.468) *	1.62(0.93,2.82)
No	17(6.5%)	244(93.5%)	1	1
Diarrhea				
Yes	6(11.1%)	48(88.9%)	1.334 (0.662,2.682) *	0.997 (0.966,1.014)
No	21(6.9%)	284(93.1%)	1	1
IDDS				
High DD	16(6.5%)	229(93.5%)	2.079 (0.745,5.744) *	1.014(0.873,1.175)
Less DD	11(9.6%)	103(90.4%)	1	1
Measles Vaccination				
Yes	6(11.5%)	46(88.5%)	1.018 (0.982,1.038) *	0.824(0.492,1.355)
No	21(6.8%)	286(93.2)	1	1

iii. *Factors associated for under weight*

The multivariate logistic regression analysis identified family size and duration of breast feeding as determinant factors for underweight. The odds of underweight among OVC from >= 5 family size were 2.78 times at an increased risk than those who family size < 5(AOR 2.778; 95% CI 1.148, 6.721). the odds of underweight among OVC who were breast feed for 6-12 month were 3.26 times at an increased risk when compared to OVC who were breast feed for >=12 month (AOR 3.257; 95% CI 1.344, 7.891). (Table10).

Table 10: Logistic regression analysis showing associated factors for underweight among OVC aged 6-59 month on care and support from NGO in Hawassa town, Ethiopia, 2014

Variable	Under weight		COR (95% CI)	AOR (95% CI)
	Yes N (%)	No N (%)		
Family size				
<5	21(65.6%)	141(43.1%)	1	1
>=5	11(34.4%)	186(56.9%)	2.518(1.176, 5.394) *	2.256 (1.148, 6.721) **
Duration breast feeding				
< 6 month	2(7.1%)	12(3.9%)	3.285(.623,17.317) *	3.026(.612, 14.952)
6-11 month	13(46.4%)	63(20.3%)	3.746(1.654, 8.484) *	3.257(1.344, 7.891) **
>=12 month	13(46.4%)	23.6(75.9%)	1	1
Vitamin A supp				
Yes	2(4.2%)	46(95.8%)	1	1
No	30(9.6%)	281(90.4%)	1.47 5(0.52,4.21) *	1.37 2(0.39 1,4.87 6)

cough				
Yes	1(1.3%)	77(98.7%)	1.55 2 (1.05 ,2.30 2) *	1.28 3(0.85 5,1.90 2)
No	31(11.0%)	250(89.0%)	1	1
Food and Nut. Support				
Yes	27(10.5%)	229(89.5%)	1.63 4(1.07 2,2.49 3) *	1.27 3(0.87 6,1.87 4)
No	5(4.9%)	98(95.1%)	1	1

* P-value < 0.25 ** P-value < 0.05

VI. DISCUSSION

The present study investigated nutritional status and associated factors of orphans and vulnerable preschool children on care and support from nongovernmental organizations in Hawassa town, southern Ethiopia 2014GC.

a) Stunting

In this study, the prevalence of stunting is a bit higher than the studies done among orphans and vulnerable children in Zambia (29%), Nigeria (23.1%), Mongolia (15.6%), Gumberiti (24%) respectively (15,16,36,37).This might be due to the difference in study period, socioeconomic characteristics, health service delivery, study area and age difference.

However, the magnitude of stunting in the present study was found to be a bit lower than a studies conducted among similar age groups in west Gojiam (43.2%), Tigray (42.7%), Haramaya (42.7%), EDHS (44% & 44.1%) Bangladesh (42%) respectively (8, 17, 18, 21, 38). The variation might be due to involvement of special segments of the study subject who are on care and support.

The magnitude of stunting was found to be consistent with the regional prevalence of Dire Dawa (36.3%), Harari (29.8%), Nepal (37%) (8, 39).This might be due to similarities in socio economic characteristics and age categories.

The analysis of this study indicated that children who have parents, marital status and educational status of the care takers, first food the child received were identified as determinant factors.

Although it is generally held that maternal orphans are at greater risk for health problems because of the loss of their primary caregiver, children who had lost a father were more likely to be malnourished than non-orphans, indicating that loss of a father may be at least as significant as loss of a mother. As to the finding of this result, The odds of stunting among OVC whose either parent were alive were 3.717 times an increased risk than those who were not alive (AOR 3.717; 95% CI 1.405, 9.804).More ever, A study conducted on poorer health and nutritional outcomes in orphans and vulnerable young children not explained by greater exposure to extreme poverty in Zimbabwe showed that OVC aged 6-59 months were more likely to be stunted than non-OVC (36).

Concerning marital status, there was significant association between the marital status of the care takers and stunting, OVC of married care taker were 74.1% at reduced risk to be stunted than those OVC of single care takers (AOR .259; 95% CI .751,.089).This could be due to the reason that married caretaker have an opportunities to have economic strengthen and other support from their partner than single caretakers and will have an impact on nutritional status of orphan and vulnerable children.

Regarding educational status, there was significant association between the educational status of the care takers and stunting, The odds of stunting among OVC care takers whose educational status primary were 2.777 times at an increased risk when compared to OVC of care takers their educational status were secondary and above (AOR 2.777; 95% CI 1.272, 6.063).Similar findings are reported in other studies in Garhiwali Himalyas and Bostwana respectively (40, 41).This could be due to the reason that as the educational level of the caretakers of OVC increase their knowledge to different nutritional program and adherence to nutritional education given by supportive NGOs will increase. So, they can apply it to their children in order to make their children well nourished.

Concerning first complementary food the child received, there was significant association between first complementary food the child received and stunting, The odds of stunting among OVC whose first food porridge were 2.463 times an increased risk than OVC whose first complementary food were milk (AOR 2.463; 95% CI 1.328, 4.568). This could be due to the fact that in the first six month of life, all the infant"s nutritional needs are met by the mother"s breast milk, but from the age of six month onwards breast milk alone can"t provide the entire nutrient. In the current study it was found that children who began complementary feeding with milk were significantly at reduced risk to being stunted compared to those children who began complementary feeding with porridge. This could be due to the reason that the process of making porridge make the porridge less content in nutrients and May exposes the Orphan and vulnerable children to the risk of infection and malnutrition than making milk.

b) Wasting

The magnitude of wasting was found to be more or less consistent with the regional prevalence of

Amhara (9.9%),oromia (9.7%) and Harar (9.1%) and among orphans and vulnerable children in Zambia (5%) were wasted respectively (8,32). The figure in this study is however bit higher than Addis Ababa (4.6%). And lower than Dire Dawa (12.3%),Gambella (12.3%), Afar (22.2%)and Southern Sudan (22%) (8,42). This difference also probably the difference in due to study period, study area, study subjects, socioeconomic characteristic. There was significant association between children who have cough prior to 2 weeks of this survey and wasting, The odds of wasting among OVC who were have cough prior to 2 weeks of this survey were 2.272 times an increased risk than OVC who were not have cough (AOR 2.272; 95% CI 1.997, 5.181). This is due to the fact that Infection and nutritional status of children are interrelated where malnutrition can accelerate disease progression, and Infection worsens malnutrition by weakening the immune system and hindering nutrient intake, absorption, and storage which further affect the nutritional status of the child according to the vicious cycle of malnutrition. There was also a significant association between Household food insecurity and Wasting, The odds of wasting among OVC from food in secured HH were 2.667 at increased risk than to be those who were from food secured (AOR 2.667; 95% CI 1.072, 6.667).This could be due to the reason previously demonstrated that household food insecurity is increased among orphans living in households (43).And that orphans are more vulnerable to food insecurity than non-orphans (44). It has also been demonstrated that orphaned children in sub-Saharan Africa tend to have more malnutrition compared to non orphans (45). A similar study conducted in Nigeria revealed that food-insecure households were five times more likely to have wasted children than food secure households (46). Concerning food and nutrition support from NGO, there was significant association between food and nutrition support from NGO and Wasting. The odds of wasting among OVC who have no food and nutritional support from NGO were 6.251times at increased risk to be wasted when compared to OVC who do have food and nutritional support.(AOR.6.251; 95% CI.1.427, 9.778). This could be due to the reason that food and nutrition support along with other support from the supportive organization make Orphan and Vulnerable Children will have the access for food and nutrition which enable them to have adequate intake and prevent from being wasted.

c) Underweight

The prevalence of Underweight in OVC was 8.9 % (95% C.I 6.4 - 12.3) in this survey. The magnitude of Underweight was found to be consistent with the regional prevalence of DireDawa, Harari and Somalia and significantly varied from other region and might be due similarities in the nature of study setting and involvement of special segments of the study subject

respectively (8). The prevalence of Underweight in the current study is lower than a study done among orphans and vulnerable children in Zambia prevailed (19%) (36). this could be due to the difference in the study subject. Where use study was from a town, receiving care and support form NGO and this might contributed to lower underweight compared to national and regional figure by EDHS 2011. . Regarding associated factors of malnutrition, analysis of this study indicated that family size in households and duration of breastfeeding were identified as determinant factors for Underweight. There was also a significant association between family size households and Underweight. The odds of underweight among OVC from >= 5 family size were 2.778 times at an increased risk than those who family size < 5(AOR 2.778; 95% CI 1.148, 6.721). This could be due to the reason that Orphans and Vulnerable Children could not be able to get adequate and balanced food required for their growth and development as the number of family size increase. Thus, larger family sizes have adverse effect on the nutritional status of a child. Moreover when economically inactive members in a household increases relative to the number of economically active members of a household, the limited available food resources will be depleted without satisfying the required nutrition (47).

As to duration of breast feeding, there was significant association between duration of breast feeding and Underweight. the odds of underweight among OVC who were breast feed for 6-12 month were 3.257 times at an increased risk when compared to OVC who were breast feed for >=12 month (AOR 3.257; 95% CI 1.344,7.891).Currently recommended and preferred infant feeding option in the context of HIV by WHO as well as national ministries of health of most developing countries is; during the first six months of life exclusive breastfeeding; then after appropriate complementary foods should be introduced at six months of age with continued breastfeeding until nutritionally adequate diet without breast milk can be provided. Early cessation and abrupt weaning of breastfeeding should be avoided (48). In line with this facts, in this study the longer the OVC on breast feeding, the decreased risk to be underweight.

VII. Strength

✓ using standardized, validated tools
✓ community based study

VIII. Limitation

✓ Cross sectional nature of the study
✓ Not triangulated with qualitative study

IX. Conclusion

This study revealed that, 1. Prevalence of malnutrition was high and it was the top list among the

health problems in orphans and vulnerable children who were in care and support from nongovernmental organization in Hawassa town, Ethiopia. 2. Parental status, marital status, educational status, and first food the child received was significantly associated with stunting. 3. Identified cough prior to 2 weeks of this survey, HH food security and food and nutritional support from NGO Households were associated with wasting. 4. Having more ($>=5$) Family size in the household and duration of breast feeding Orphan of Vulnerable children were associated with underweight among orphans and vulnerable preschool children in this study.

X.   RECOMMENDATION

1. Community based nutrition program targeting Orphan and Vulnerable Children should be established to tackle the problem of malnutrition at community level depending on the severity of malnutrition identified
2. Nutrition education by supportive organization in coordination with health extension workers should be strengthening to improving the child feeding practice of parents on appropriate infant and young children feeding.
3. Nutritional Intervention by supportive organization to improve the food security and care of orphaned and vulnerable children in the community
4. Integrating Food and nutrition support by supportive organization is mandatory to improve the nutritional status of orphans and vulnerable children
5. Continued attention should mandatory to duration of breast feeding practices mothers/caretakers to avoid malnutrition among orphans and vulnerable by supportive organization.

6. Woreda health office should be collaborated with BOWA and supportive organization to improve access health facilities having special attention to Orphan and Vulnerable Children.
7. Further comparative study should be done to see OVC from other segments of population in the study setting that were not included in the present study

XI.   ACKNOWLEDGMENTS

XII.   ACRONYMS/ABBREVIATIONS

AIDS Acquired immune Deficiency Syndrome
CSA Central Statistics Agency
DHS Demographic and Health Survey
EDHS Ethiopia Demographic and Health Survey
ENA Emergency Nutritional Assessment
ETB Ethiopian Birr
FANTA Food and Nutrition Technical Assistance
FAO Food and Agricultural Organization
MDG Millennium Development Goal
MPH Master of Public Health
MSF Medicines Sans Frontiers
MUAC Mid Upper Arm Circumference
NGO Non Governmental organization
NNP National Nutritional Programme
OR Odds Ratio
OVC Orphan and vulnerable children
SMART Standardized Measuring Assessment for Relief and Transition
SNNPR Southern Nation Nationality People Regional State
UNICEF United nation children Fund
WHO World Health Organization

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Annex I: Information sheet

Hello my Name is -------------------------- I am a data collector for public health nutrition master‟s student project at Mekelle University .The objective of this study is to assess the nutritional status and associated factors among orphans and vulnerable preschool children on care and support from nongovernmental organization in Hawassa town. The information collected from you will be useful for the health care provider, Bureau of women‟s affairs, the Regional Health Bureau and other concerned bodies to improve the service given to OVC. An interview question will present to you and anthropometric measurement will be taken from your child. Your child has been selected randomly in this study and you and your child name will not be mentioned in the questionnaire and the information you have given will be kept in confidence. You can quit at any point of interview or you can skip questions. We would like to thank you in advance for participating in our study. For additional information you can contact the principal investigator with the following address: Name of principal investigator: Mr.Bisrat Getaneh Mobile. Cell phone: +251(0)911881252. Email:bisratlove@gmail.com.

Annex I: Consent form

As to the information given ahead, I have been informed that the objective of this study is to assess the nutritional status and associated factors among orphans and vulnerable preschool children aged 6-59 months. I have understood that participation in this study is entirely voluntarily and study has no any risk. My name will not be written on this form and the information I give will never be shared to others. I may not answer any questions that I don't want to answer and I may end this interview at any time I want therefore I am giving my written consent to participate in this study in titled "Nutritional status and associated factors among Orphans and vulnerable preschool children aged 6-59 months on care and support from nongovernmental organization Hawassa town..

Please *Check box (✓) to that* show the respondent‟s commitment to participate in this study

☐ 1. Agree

☐ 2. Disagree (End the interview)

Signature of Participant _____Date _____

Annex III: Interviewer Administered Questionnaire in English

Data collection tools for Mekelle University, college of health sciences, department of public health MPH/PHN research project on Nutritional status and associated factors among Orphans and vulnerable preschool children on care and support from nongovernmental organization Hawassa town. The following Questionnaire classified in to 5 parts as socio Demographic factors, Socio economic variables, Child health care, Environmental /sanitation factors, food

insecurity variables, Dietary intake and anthropometric measurements.

CODE OF THE QUETIONNAIRE_____

Name of the interviewer_____

Signature_____

Date of interview (dd/mm/yyyy) _____

Result of interview:

1-	Completed	3-	Refused
2-	Partially completed	4-	Respondent not available

Checked by supervisor;

 Name ………………….……….. Signature ………………… Date ………………...

Part I: Demography and socioeconomic characteristics

Code	Questions	Coding categories	Sk ip to
Q. 101	Address of the child	sub -city _____ Kebele _____	
Q. 102	Age of the child in Month	_____month	
Q. 103	Sex of the child	☐ 1. Male ☐ 2. Female	
Q. 104	Number of children in under Five in HH	_____ _____	
Q. 105	How many members are present in the HH now	_____	
Q. 106	Religion of parents/caretaker	☐ 1. Orthodox ☐ 2. Muslim ☐ 3. Catholic ☐ 4. Protestant ☐ 5. Other, Specify_____	
Q. 107	Ethnicity of the mother/caretakers	☐ 1. Sidama ☐ 2. Wolayita ☐ 3. Gurage ☐ 4. Amhara ☐ 5. Tigre ☐ 6. Other, Specify_____	
Q. 10 8	Are the parents of the child alive	☐ 1. Yes ☐ 0. No	If no, skip to Q. 1 10
Q. 10 9	If, yes, who is alive	☐ 1. Mother ☐ 2. Father ☐ 3. Both ☐ 4 . Not Known	

Q.110	The respondent ‚s relation with the child	☐ 1. Parents ☐ 2. Brother ☐ 3. Sister ☐ 4.Grand parents ☐ 5.other relative ☐ 6.neighbour hood	
Q.111	Age of the care tak ers/ Guardian	_____ _____ years	
Q.112	Sex of the care taker / Guardian	☐ 1.male ☐ 2.female	
Q.113	Educational status of care taker	☐ 1. No formal education ☐ 2. Read & write ☐ 3. Grade 1 -8 ☐ 4. Grade 9 -10 ☐ 5. Grade 10+2 & above	
Q.114	Oc cupation of the care taker/Guardian	☐ 1. House wife ☐ 2. Private employee ☐ 3. Government employee ☐ 4. Daily laborer ☐ 5. Merchant ☐ 6. Other, Specify_____	
Q.115	Marital status of the care taker/Guardian	☐ 1. Single ☐ 2. Married ☐ 3. Divorced ☐ 4. Widowed ☐ 5. Separated	If not married, skip to Q.117
Q.116	Occupation of the husband /wife	☐ 1. Private employee ☐ 2. Government employee ☐ 3. Daily laborer ☐ 4. Merchant ☐ 5. Other, Specify____	
Q.117	Who is the head of the family?	☐ 1. car etakers ☐ 2. Husband /wife of care takers ☐ 3.other ,Specify_____	
Q.118	What is your family total monthly income?	_____ Eth. Birr	

Part II: Sanitation and Hygiene related question

Q.119	Where is your Source of water?	☐ 1. Pipe ☐ 2. publ ic stand ☐ 3. protected spring /well ☐ 4. Other, Specify _____	
Q.120	How much water used per day ?	_____L/day	
Q.121	What Method used for water storage ?	☐ 1. Pot ☐ 2. Jeri Can ☐ 3. Bucket ☐ 4. Other, Specify _____	
Q.122	Do you was h your hands with soap and water Whenever you feed your child?	☐ 1. Yes ☐ 0. No	
Q.123	Do you have latrine?	☐ 1. Yes ☐ 0. No	If no skip to Q.125
Q.124	What type of latrine available	☐ 1. pit latrine ☐ 2. Ventilated Improved latrine ☐ 3. Water carriage type ☐ 4. Other	
Q.125	Where do You dispose domestic waste	☐ 1. Pit ☐ 2. Open ☐ 3. MunicipalityService ☐ 4. Other, Specify_____	
Q.126	Do you have separated kitchen	☐ 1. Yes ☐ 0. No	

Part III: Dietary intake

| Q. 201 | Did the child Brest feed | ☐ 1. Yes
☐ 2. No
☐ 3. Don"t know | If not yes, skip to Q.206 |
| Q.202 | When did mother/caretakers first put the child on breastfeeding? | ☐ 1. With in first hour of delivery
☐ 2. Within the firs t 8 hour of delivery
☐ 3. After 2 -3 days
☐ 4. Don"t Know | |

Q.203	Did the child exclusively breas tfeed ?	☐ 1. Yes ☐ 2. No ☐ 3. Don''t know	
Q.204	For how long the childe Breastfeed?	_____ month	
Q.205	At what age did you start to give food in Additio n to your breast milk?	☐ 1.Immediately after birth ☐ 2.Within 1 to 6 months ☐ 3.Within 6 to 12 months ☐ 4.Twelve month later	
Q.206	What is the first food mother/caretakers used to feed the child?	☐ 1. Milk ☐ 2. Adult food ☐ 3. Pourage ☐ 4.Other specif y	
Q.207	What do Mother/caretakers use d to feed the child?	☐ 1. Hand ☐ 2. Cup and spoon ☐ 3. Bottle ☐ 4. Don''t know	
Q.208	Did the child eat any flat bread, biscuits, or any other foods made from cereal (maize, sorghum, millet, wh eat, barely or teffe) yesterday?	☐ 1. Yes ☐ 0. No	
Q.209	Did the child eat any pumpkin, carrots, y/orange flesh sweet potatoes irish potato, white sweet potato, onion, white yam, cassava, enset and other foods made from roots yesterday?	☐ 1. Yes ☐ 0. No	
Q.210	Did the ch ild eat any dark green leafy vegetables (kale, Swiss chard, cabbage) and other vegetables (tomato, onion) yesterday?	☐ 1. Yes ☐ 0. No	
Q.211	Did the child eat any fruits like ripe mango, papaya, banana, avocado and lemon and orange…) and other fr uits yesterday?	☐ 1. Yes ☐ 0. No	
Q.212	Did the child eat any flesh meat (beef, lamb, goat, chicken) and any organ meat (liver, kidney, heart) yesterday?	☐ 1. Yes ☐ 0. No	
Q.213	Did the child eat any eggs yesterday?	☐ 1. Yes ☐ 0. No	
Q.214	Did the child eat any fresh or dried fish yesterday?	☐ 1. Yes ☐ 0. No	

Q.215	Did the child eat any food made from beans like kidney beans, haricot beans, field peas, cow peas, chick peas, nuts, lentils or others yesterday?	☐ 1. Yes ☐ 0. No	
Q.216	Did the child drink milk and milk products yesterday? (milk, cheese, yogurt or other milk products)	☐ 1. Yes ☐ 0. No	
Q.217	Did the child eat any food with oil, fat or butter yesterday?	☐ 1. Yes ☐ 0. No	
Q.218	Did the child eat any sugar or honey, sweet/soft drink yesterday?	☐ 1. Yes ☐ 0. No	
Q.219	Did the child eat any spices (black pepper, salt), condiments (soy sauce, hot sauce), and coffee, tea yesterday ?	☐ 1. Yes ☐ 0. No	

Part IV: Morbidity variables

Q.301	Has mother ever attended ANC during her pregnancy of this child?	☐ 1.Yes ☐ 0.No	
Q.302	Where did mother give birth to the child?	☐ 1. Home ☐ 2. Health facility ☐ 3.Other ,Specify_____	
Q.303	Who attended the delivery ?	☐ 1. TBA ☐ 2. Health personnel ☐ 3.Other,Specify_____	
Q.304	Did the child receive vaccination?	☐ 1. Yes ☐ 2. No ☐ 3. Don''t know	
Q.305	Do you have vaccination card	☐ 1. Yes ☐ 0. No	If no skip to Q.307
Q.306	What is the vaccination status?	☐ 1.BCG ☐ 2. Polio ☐ 3.Me sales ☐ 4 Other, Specify_____	
Q.307	Is the child received vitamin A supplementation? (in the last 6 months)	☐ 1. Yes ☐ 0. No	
Q.308	Had the child suffer from infections like measles?	☐ 1. Yes ☐ 0. No	
Q.309	Fever for the past 2 weeks ?	☐ 1. Yes ☐ 0. No	
Q.310	Cough for the past 2 weeks ?	☐ 1. Yes ☐ 0. No	

Q.311	Diarrhea for the past 2 weeks?	☐ 1. Yes ☐ 0. No	
Q.312	Bilat eral pitting oedema on clinical examination	☐ 1. 0 ☐ 2. + ☐ 3. ++ ☐ 4. +++	

Part IV: Food insecurity and malnutrition

Q.401	Did you worry that your household would not have enough food?	☐ 1. Yes ☐ 0. No	If no, skip to Q.40 3
Q.402	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	
Q.403	Were you or any household member not able to eat the kinds of food you preferred because of lack of resources ?	☐ 1. Yes ☐ 0. No	If no, skip to Q.40 5
Q.404	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	
Q.405	Did you or any household member eat just a few kinds of food day after day due to lack of resources ?	☐ 1. Yes ☐ 0. No	If no, skip to Q.40 7
Q.4 06	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	
Q.407	Did you or any household member eat food that you preferred not to eat because of lack of resources to obtain other types of food?	☐ 1. Yes ☐ 0. No	If no, skip to Q.40 9
Q.408	If yes , how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	
Q.409	Did you or any household member eat a smaller meal than you felt you needed because there was not enough food?	☐ 1. Yes ☐ 0. No	If no, skip to Q.4 11
Q.410	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	
Q.411	Did you or any household member eat fewer meals in a day because there was not enough food?	☐ 1. Yes ☐ 0. No	If no, skip to Q.41 3
Q.412	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes	

Q.413	Was there ever no food at all in your household because there were not enough resources to get more ?	☐ 1. Yes ☐ 0. No	If no, skip to Q.41 5
Q.414	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	
Q.415	Did you or any household member go to sleep at night hungry because there was not enough food ?	☐ 1. Yes ☐ 0. No	If no, skip to Q.41 7
Q.416	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	
Q.417	Did you or any household member go a whole day without eating anything because there was not enough food?	☐ 1. Yes ☐ 0. No	If no, skip to Q.501
Q.418	If yes, how frequent?	☐ 1.rarely ☐ 2.sometimes ☐ 3.often	

Part V: OVC Care and support

Q.501	What type of Support Provided by NGO? (more than one answer is possible)	1.Food and Nutrition 2.Health Care 3.Economic Strengthening 4.Education 5.Psychosocial Support 6.Legal Protection 7.Shelter and Care
Q.502	If Food and Nutrition, what services provided?	☐ 1.Nutritional assessment and supplementary feeding ☐ 2.links to other health and nutrition interventions ☐ 3.training on nutrition, diet, and food preparation for care takers
Q.503	If Health Care, what services provided?	☐ 1.free access to health services for OVC and guardians ☐ 2.Regular home visits to assess health status of the child ☐ 3.training to caregivers on the importance of immunization, malaria prevention, hygiene and sanitation, optimal nutrition

Q.504	If Economic Strengthening, what services provided?	☐ 1.vocational training for caregivers ☐ 2.income-generating activities involving small business ☐ 3.access to credit
Q.505	If Education ,what services provided	☐1.Direct assistance to subsidize school costs ☐2.Identifying and promoting educational opportunities ☐3.Training caregivers to OVC who are not enrollment in school
Q.506	If Psychosocial Support, what services provided?	☐ 1. providing regular training to care givers on psychosocial support for OVC ☐ 2.develope psychosocial support groups to provide support to OVC and caregivers ☐ 3.parenting and communication skills for caregivers, support during illness
Q.507	If Legal Protection, what services provided?	☐ 1. protect children about the legal rights of children ☐ 2.link OVC and caretakers to legal services and child protection bodies when required
Q.508	If Shelter and Care, what services provided?	☐ 1.Supporting families with home visits ☐ 2.Regularly assess and identify the shelter and care needs of OVC ☐ 3.Improve shelter and care for OVC in the community
Q.509	For how long supported by the NGO?	--------------------month
Q.510	Please, express your View as to the adequacy of support?	☐ 1.Adequate ☐ 2.Not adequate ☐ 3.No answer

Part VI: Anthropometric Measurements

Q.601	Age the child	-------------------- (month)
Q.602	Weight of the child (if no bilateral pitting edema)	-------------------- (kg)
Q.603	Height of the Child	-------------------- (cm)
Q.604	MUAC of the child (Ht>65cm)	-------------------- (mm)

Annex IV: Information sheet in Amharic version

የመረጃ ቅጽ

ሰላምታ! ስሜ ………………………………………………እኔ ቃለ መጠይቅ አድራጊ በመቐለ ዩኒቨርሲቲ በማህበረሰብ ጤና የስነ ምግብ የድህረ-ምረቃ ተማሪ የመመረቂያ ጽሑፉን በመሰብሰብ ላይ እገኛለሁ:: ይህ ጥናት ሊሰራ የታቀደው በሀዋሳ ከተማ ሲሆን የጥናቱ ዋና ዓላማም ወላጅ ያጡና ተጋላጭ የሆኑ ሕጻናትን የምነ-ምግብ ሁኔታ ይተመልከተ ጥናት ሲሆን፣ ከዚህ ጥናት የሚገኘው ውጤት በእነኚህ ወላጅ ያጡና ተጋላጭ ህጻናት ድጋፍና እንክብካቤ ላይ ለተሰማሩ ድርጅቶች ስራቸውን እንዲገመግሙና ማሻሻል ያለባቸውን ለማሻሻል፣ ለወደፊቱ ስልት እንዲነድፉ ያስችላቸዋል:: የአንትሮ ፓሞትሪክ ልኬት የሚወሰደው ከህጻኑ/ና ሲሆን ቃለ-መጠየቅ የሚቀርበው ለርስዎ ነው:: በዚህ ጥናት ላይ የእርሶ እና የህጸኑ ተሳትፎ በጣም ጠቀሚታ አለው:: እርስዎም ሆኑ ህጻን ወደ ጥናቱ የተካተታችሁት በእጣ ሲሆን የምታደርጉትም ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ ነው:: ያልተስማማዎት ጥያቄ ሲኖር አለመመለስ፣ በማንኛውም ሰዓት ቃለ መጠይቁን የማቋረጥ መብትዎ የተጠበቀ ነው:: የሰጡኝ መረጃ በሙሉ በሚስጥር ስለሚያዝ ማንም ሰው ሊያገኘው አይችልም:: ለበለጠ መረጃ ጥናቱን የሚያካሂደው ስም፣ ብስራት ጌታነህ ፣ የሞ.ስልክ ቁ. +251(0)911881252 ፣ ኤሜል: bisratlove@gmail.com.

የመተማመኛ ቅጽ

እኔ ከለይ በተሰጠኝ መርጃ መሰርት ፣ የጥናቱ ዋና ዓላማም ወላጅ ያጡና ተጋላጭ የሆኑ ሕጻናትን የምነ-ምግብ ሁኔታ ይተመልከተ ጥናት መሆኑ የተገለጸልገኝና የምናደርገው ተሳትፍ በፈቃደኝነት ላይ የተመሰረተ መሆኑን ፣ያልተስማማኝ ጥያቄ ሲኖር አለመመለስ፣ በማንኛውም ሰዓት ቃለ መጠይቁን የማቋረጥ መብቴ የተጠበቀ መሆኑ:: የምስጠው መረጃ በሙሉ በሚስጥር ስለሚያዝ ለማንም ሰው ሊያገኘው አንነደማይችል በ መርዳት ለ ወላጅ ያጡና ተጋላጭ የሆኑ ሕጻናትን የምነ-ምግብ ሁኔታ ለሚመልከተው ጥናት እኔ የጥናቱ ተሳታፊ ለመሆን የመተማመኛ ፎርማ ከዚህ በታች አኑሪያለሁ ::

ይህን ምልክት (✓) በሳጥን ውስት በማስይት ይጥናት ተሳታፊው ፈቃደኝነታቸውን ያመልክቱ፡

☐ 1. እስማማለሁ

☐ 2. አልተስማማሁም (አመስግነህ/ሽ መጠይቁን አቋርጥ/ጪ)

ቃለ መጠይቅ ተድራጊው ፌርማ _____ቀን_____

የሥነ-አመጋገብ ሁኔታ ለማጥናት የተዘጋጀ ቃለ መጠይቅ በአማርኛ

በመቐለ ዩኒቨርሲቲ በማህበረሰብ ጤና የስነ ምግብ የድህረ-ምረቃ ክፍል ስር የመመረቂያ ጽሑፌን ሲሆን ይሄ ጥናት ሊሰራ የታቀደው በሀዋሳ ከተማ ነው የጥናቱ ዋና ዓላማም ወላጅ ያጡና ተጋላጭ የሆኑ ሕጻናትን የሥነ-ምግብ ሁኔታ ይተመልከት ጥናት ሲሆን፤ ከዚህ በታች ይሚገኙ መጠይቆች በ 6 ክፍል የተከፋፈሉ ናቸው፡ እንዚህም የማህበራዊና የሥነ-ምጣኔ ፤ የአካባቢና የግል ንጽህናን በተመለከተ፤ የአመጋገብ ሁኔታን ሚመለከት ጤና ነክ፤ የቤተሰብዎ የምግብ ዋስትና የሚፈትሹ መጠይቆችና የአንትሮ ፖሞትሪክ ልኬትናቸው፡፡

መለያ.ቁ _____

የቃለ መጠይቅ አድራጊው ስም _____

ፊርማ _____

ቀን _____

የቃለ መጠይቅ ውጤት:

1- የተጠናቀቀ   3- ያልተስማሙ

2- በክፊል የተጠናቀቀ   4- በቃለ መጠይቅወቅት ያልተገኙ

የተቆጣጣሪው ስም _____ፊርማ_____ቀን_____

ክፍል I: ማህበራዊና የሥነ-ምጣኔ መጠይቆች			
ተ.ቁ	ጥያቄ	መልስ	ይለፍ
Q.101	የህፃኑ/ፃ አድራሻ?	ክ/ከ _____ ቀበሌ _____	
Q.102	የህፃኑ/ፃ እድሜ (በወራት) ?	_____ወር	
Q.103	የህፃኑ/ፃ ጾታ?	☐ 1. ወንድ ☐ 2. ሴት	
Q.104	በቤት ውስጥ ከአምስት አመት በታች ያሉ ህጻናት ስንት ናቸው?	_____	
Q.105	በቤት ውስጥ ስንት ሰው ይኖራል?	_____	
Q.106	የህፃኑ/ፃ ሃይማኖት?	☐ 1.ኦርቶዶክስ ☐ 2.ሙስሊም ☐ 3.ካቶሊክ ☐ 4.ፕሮቴስታንት ☐ 5.ሌላ ካለይጥቀሱ	

Q.107	የህፃኑ/ኗ ብሔር?	☐ 1. ሲዳማ ☐ 2. ወላይታ ☐ 3. ጉራጌ ☐ 4. አማራ ☐ 5. ትግሬ ☐ 6. ሌላ ካለይጥቀሱ	
Q.108	የህፃኑ/ኗ ወላጆች በሕይወት አሉ?	☐ 1. አዎ ☐ 2.የለም	የለም ከሆነ ወደ ጥያቄ ቁጥር110
Q.109	አዎ ካሉ ማን?	☐ 1.እናት ብቻ ☐ 2.አባት ብቻ ☐ 3.ሁለቱም በሕይወት አሉ ☐ 4.አይታወቅም	
Q.110	ቃለ መጠየቅ የሚደረግለት ሰው ከህፃኑ/ኗ ጋር ያለው ዝምድና?	☐ 1. ወላጅ ☐ 2. ወንድም ☐ 3. እህት ☐ 4. አያት ☐ 5. ሌላ ዘመድ ☐ 6.ሌላ ካለ ይጥቀሱ	
Q.111	የሞግዚቱ/ቷ እድሜ?	_____ዓመት	
Q.112	የሞግዚቱ/ቷ ጾታ?	☐ 1.ወንድ ☐ 2.ሴት	
Q.113	የሞግዚቱ/ቷ የትምህርት ደረጃ?	_____	
Q.114	የሞግዚቱ/ቷ ስራ?	☐ 1. የቤት እመቤት ☐ 2. የግል ተቀጣሪ ☐ 3. የመንግስት ሰራተኛ ☐ 4. የቀን ሰራተኛ ☐ 5.ነጋዴ ☐ 6. ሌላ ካለ ይጥቀሱ	
Q.115	የሞግዚቱ/ቷ የጋብቻ ሁኔታ?	☐ 1.ያላገባ/ች ከሆነ ጥ.ቁ 117 ☐ 2.ያገባ/ች ☐ 3. የተፋታ/ች ☐ 4. ባል/ሚስት የሞተባት ☐ 5.የተለያየ/ች	ያላገባ/ች ከሆነ ጥ.ቁ 117

Q.116	የባል/የሚስት ስራ?	☐ 1.የግል ተቀጣሪ ☐ 2. የመንግስት ሰራተኛ ☐ 3. የቀን ሰራተኛ ☐ 4. ነጋዴ ☐ 5. ሌላ ካለ ይጠቀስ	
Q.117	የቤተሰቡ ሃላፊ ማን ነው?	☐ 1.ሞግዚቲ/ቱ ☐ 2.የሞግዚቲ/ቱ ባል/ሚስት ☐ 3.ሌላ ካለ ይጠቀስ	
Q.118	የቤተሰብ የገቢ መጠን በወር ስንት ይሆናል?	_____በብር	

ክፍል ‖ ፡ የአካባቢና የግል ንጽህናን በተመለከተ

Q.119	የመጠጥ ውሃ ከየት ታገኛላችሁ?	☐ 1. ከቧንቧ ☐ 2. ከህዝብ ቧንቧ ☐ 3. ከምንጭ ☐ 4. ሌላ ካለ ይጠቀስ	
Q.120	በቀን ምንያህል ውሃ ይሆናል?	_____ሊ/በቀን	
Q.121	ውሃ የማስቀመጫ መንገድ?	☐ 1. እንስራ ☐ 2. ጀሪካን ☐ 3. ሳፋ ☐ 4. ሌላ ካለ ይጠቀስ	
Q.122	የህፃኑ/ሪ ምግብ ከመስጠትሽ/ህ በፊት እጅሽን በሳሙናና ውሃ ትታጠቢያለሽ/ህ?	☐ 1.አዎ ☐ 2.የለም	
Q.123	መፀዳጃ ቤት አላችሁ?	☐ 1.አዎ ☐ 2.የለም	
Q.124	አዎ ካሉ ምን አይነት?	☐ 1. የግል ጉድጉዋድ ☐ 2. ጉድጉዋድ ☐ 3. በውሃ የሚሰራ ☐ 4. ሌላ ካለ ይጠቀስ	
Q.125	የቤት ቆሻሻ የት ታስውግዳላችሁ?	☐ 1.ጉድጉዋድ ☐ 2.ሜዳ ☐ 3.የመዘጋጃ አገልግሎት ☐ 4.ሌላ ካለ ይጠቀስ	
Q.126	ለብቻዉ የተለየ የምግብ ማብስያ አላችሁ?	☐ 1.አዎ ☐ 0.የለም	

ክፍል II ፤ የአመጋገብ ሁኔታን በሚመለከት

Q.201	ህፃኑ/ኗ ጡት ጠብቷል/ታለች?	☐ 1.አዎ ☐ 2.የለም ☐ 3.አላውቅም	
Q.202	ለመጀመርያ ጊዜ ህፃኑ/ኗ ጡት የጠባው/ችው ከተውለደ/ች በስንት ጊዜ ውስጥ ነበር?	☐ 1. በአንድ ሰዓት ውስጥ ☐ 2. በስምንት ሰዓት ውስጥ ☐ 3. 2-3 ቀን ☐ 4. አላውቅም	
Q.203	ህፃኑ/ኗ ለመጀመሪያ 6 ወር ጡት ብቻ ጠብቷል/ታለች?	☐ 1.አዎ ☐ 2.የለም ☐ 3.አላውቅም	
Q.204	ህፃኑ/ኗ ጡት ለምን ያህል ጊዜ ጠብቷል/ታለች?	_____ በወር	
Q.205	ተጨማሪ ምግብ ለህፃኑ/ኗ መመገብ የተጀመረው መቼ ነበር?	☐ 1.ልክ አንደተወለደ/ች ☐ 2.ከ 1አስክ 6 ወር ባለው ግዜ ☐ 3.ከ 6 አስክ12 ወር ባለው ግዜ ☐ 4.ከ 12 ወር በኃላ ☐ 5.አላውቅም	
Q.206	ህፃኑ/ኗ ለመጀመርያ ጊዜ የተመገበው ምግብ ምን ነበር?	☐ 1.ወተት ☐ 2.የአዋቂ ምግብ ☐ 3.በስሎ የተዘጋጀ ገንፎ ☐ 4.አላውቅም	
Q.207	ህፃኑ/ኗ ለመመገብ የሚጠቀሙበት ምን ነበር?	☐ 1.በእጅ ☐ 2.በሲኒና ማንኪያ ☐ 3.በጡጦ ☐ 4.ሌላ ካለ ይጠቀስ	
Q.208	ትናንት ዳቦ(ቂጣ)፣ ከበቆሎ፣ ከማሽላ፣ ከዳጉሳ፣ ከስንዴ፣ ከገብስ ወይም ከጤፍ የተሠራ ምግብ በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.209	ትናንትከአትክልቶች፣ደባ፣ከሮት፣ቢጫስኩዋርድንች፣ብ ርቱካናማ ድንች ፣ከድቡልቡል ድንች፣ ከስኳር ድንች፣ ከሽንኩርት፣ ከሀረግ ቦየ፣ ኮባ፣ ከአንስትና ከሌሎች ስራስሮች የተሠራ ምግብ በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.210	ትናንት ከአረንጓዴ አትክልቶች የተሠራ ለምሳሌ ጎመን፣ ቆስጣና ጥቅልል ጎመን፣ ቲማቲም፣ሽንኩርት የተሠራ ምግብ በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.211	ትናንት ከፍራፍሬ ለምሳሌ ማንጎ፣ ፓፓያ፣ ሙዝ፣ ዘይቶን፣አቮካዶ፣ ሎሚ፣ ብርቱከን በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	

Q.212	ትናንት ከስጋ ዉጤቶች ለምሳሌ የበሬ ስጋ፣ የበግና ይፍየል ስጋ፣ የዶሮ ስጋ እና ከሆድ ዉስጥ ስጋ ምሳሌ ጉበት፣ ኩላሊት፣ ልብ በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.213	ትናንት ዕንቁላል በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.214	ትናንት ጥሬ ወይም የበሰለ ዓሣ በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.215	ትናንት ቦሎቄ፣ ከባቄላ፣ ከአተር፣ ከአኩሪ አተር፣ ከሽምብራ እና ከመሳሰሉት በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.216	ትናንት ወተትና የወተት ወጤቶች ለምሳሌ አይብ፣አርጎ በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.217	ትናንት በዘይት/በቅቤ የተሰራ ምግብ/ ሌሎች ቅባት ነገሮችን በልቶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.218	ትናንት ከጣፋጭ ነገሮች ለምሳሌ ስኳር፣ ማር፣ ሸንኩራ አገዳ፣ ለስላሳ መጠጦችን ወስዶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
Q.219	ትናንት ቅመማ-ቅመም፣ ቡና፣ ሻይ፣ ወስዶል/ለች?	☐ 1.አዎ ☐ 0.የለም	
ክፍል III: ጤና ነክ ጥያቄዎች			
Q.301	የህፃኑ/ኗ እናት የቅድመ ወሊድክትትል ነበራቸው?	☐ 1.አዎ ☐ 0.የለም	
Q.302	ህፃኑ/ኗ የተወለደው/ችው የት ነበር?	☐ 1.ቤት ውስጥ ☐ 2.በጤና ተቋም ☐ 3.ሌላ ካለ ይጠቀስ	
Q.303	ህፃኑ/ኗ ያዋለደው ማነ ነበር?	☐ 1.የልምድ አዋላጅ ☐ 2.የጤናባለሙያ ☐ 3.ሌላ ካለ ይጠቀስ	
Q.304	ህፃኑ/ኗ ክትባት ወስዶል/ለች?	☐ 1.አዎ ☐ 0.የለም⟶	ጥ.ቁ 307
Q.305	የህፃኑ/ኗ የክትባት ካርድ አለ?	☐ 1.አዎ ☐ 0.የለም	
Q.306	ህፃኑ/ኗ የወሰዳቸው የክትባት ዓይነት?	☐ 1.ቢሲጂ ☐ 2.ፖሊዮ ☐ 3.ሚዚልስ ☐ 4.ሌላ ካለ ይጠቀስ	
Q.307	ህፃኑ/ኗ የ ቫይታሚን ኤ እንክብል ባለፈት 6 ውራት ወስዶል?	☐ 1.አዎ ☐ 0.የለም	
Q.308	ህፃኑ/ኗ በ ኩፍኝ ተጠቅቶ/ታ ነበር?	☐ 1.አዎ ☐ 0.የለም	

Q.309	ባለፉት ሁለት ሳምንታት ህፃኑ/ኗ ትኩሳትነበረው/ራት?	☐ 1.አዎ ☐ 0.የለም	
Q.310	ባለፉት ሁለት ሳምንታት ህፃኑ/ኗ ሳል ያለው ህመም ነበረው/ራት?	☐ 1.አዎ ☐ 0.የለም	
Q.311	ባለፉት ሁለት ሳምንታት ህፃኑ/ኗ ተቅማጥ ይዞት/ይዟት ነበር?	☐ 1.አዎ ☐ 0.የለም	
ክፍልIV: የቤተሰቡዓ የምግብ ዋስትና የሚፈትሹ ጥያቄዎች			
Q.401	ባለፉ 30 ቀናት ቤታችን በቂ ምግብ የለም ብለው ተጨንቀው ያውቃል?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 403
Q.402	መልሶ አዎ ከሆነ ፤ምንያህል ግዜ? አልፎአልፎ፤(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ(በወር ከ 3 እስከ 10 ግዜ) ፤አብዛኛው ግዜ (በወር ከ10 ግዜበላይ)	☐1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
Q.403	ባለፉ 30 ቀናት ገቢያ በማነሱ ምክንያት እርስዎና የቤተሰቦዓ አባል የሚፈልጉትን የምግብ አይነት ያልበሉበት ግዜ አለ?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 405
Q.404	መልሶ አዎ ከሆነ ፤ምንያህል ግዜ? አልፎአልፎ፤(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ(በወር ከ 3 እስከ 10 ግዜ) ፤አብዛኛው ግዜ (በወር ከ10 ግዜበላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
Q.405	ባለፉ 30 ቀናት ገቢያ በማነሱ ምክንያት እርስዎና የቤተሰቦዓ አባል ትንሽ ምግብ የበሉበት ግዜ አለ?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 407
Q.406	መልሶ አዎ ከሆነ ፤ምንያህል ግዜ? አልፎአልፎ፤(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ(በወር ከ 3 እስከ 10 ግዜ) ፤አብዛኛው ግዜ (በወር ከ10 ግዜበላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
Q.407	ባለፉ 30 ቀናት የሚፈልጉትን የምግብ አይነት ለማግኘት ገቢዎብ ማነሱ ምክንያት እርስዎና የቤተሰቦዓ አባል የማይፈልጉትን የምግብ አይነት የበሉበት ግዜ አለ?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 409
Q.408	መልሶ አዎ ከሆነ ፤ምንያህል ግዜ? አልፎአልፎ፤(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ(በወር ከ 3 እስከ 10 ግዜ) ፤አብዛኛው ግዜ (በወር ከ10 ግዜ በላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
Q.409	ባለፉ 30 ቀናት በቂ ምግብ ባለመኖሩ እርስዎና የቤተሰቦዓ አባል መአድ ላይ ምግብ ያነሰበት ጊዜ ነበር?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 411
Q.410	መልሶ አዎ ከሆነ ፤ምንያህል ግዜ? አልፎአልፎ፤(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ (በወር ከ 3 እስከ 10 ግዜ) ፤አብዛኛው ግዜ (በወር ከ10 ግዜ በላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
Q.411	ባለፉ 30 ቀናት በቂ ምግብ በለመኖሩ እርስዎና የቤተሰቦዓ አባል ትንሽ ምግብ የበላችሁበት ቀን ነበር?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 413
Q.412	መልሶ አዎ ከሆነ ፤ምንያህል ግዜ? አልፎአልፎ፤(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ (በወር ከ 3 እስከ 10 ግዜ) ፤አብዛኛው ግዜ (በወር ከ10 ግዜ በላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	

Q.413	ባለፉ 30 ቀናት ገቢዎ በማነሱ ምክንያት ቤታችሁ ውስጥ ፈፅሞ ምግብ ያልነበረበት ጊዜ ነበር?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 415
Q.414	መልሶ አዎ ከሆነ ፧ምንያህል ግዜ? አልፎአልፎ፦(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ (በወር ከ 3 እስከ 10 ግዜ) ፣አብዛኛው ግዜ (በወር ከ10 ግዜ በላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
Q.415	ባለፉ 30 ቀናት በቂ ምግብ ባለመኖሩ እርስዎና የቤተሰበዎ አባል የተራበበት ጊዜ ነበር?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 417
Q.416	መልሶ አዎ ከሆነ ፧ምንያህል ግዜ? አልፎአልፎ፦(በወር1 ወይም 2ግዜ)፣ የተወሰነ ግዜ (በወር ከ 3 እስከ 10 ግዜ) ፣አብዛኛው ግዜ (በወር ከ10 ግዜ በላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
Q.417	ባለፉ 30 ቀናት በቂ ምግብ ባለመኖሩ እርስዎና የቤተሰበዎ አባል ሙሉ ቀን ምግብ ያልበሉበት ጊዜ ነበር?	☐ 1.አዎ ☐ 0.የለም ⟶	ጥ.ቁ 501
Q.418	መልሶ አዎ ከሆነ ፧ምንያህል ግዜ? አልፎአልፎ፦(በወር1ወይም 2ግዜ)፣ የተወሰነ ግዜ (በወር ከ 3 እስከ 10 ግዜ) ፣አብዛኛው ግዜ (በወር ከ10 ግዜ በላይ)	☐ 1.አልፎ አልፎ ☐ 2.የተወሰነ ጊዜ ☐ 3.አብዛኛው ጊዜ	
	ክፍልIV: እንክብካቤና ድጋፍን የተመለከተ		
Q.501	ከእርዳታ ድርጅቶች የተደረገልዎት ድጋፍ ምን ምን ነበር?	☐ 1.የምግብ ☐ 2.የሕክምና ☐ 3.የገንዘብና የገቢ ማስገኛ ☐ 4..የትምህርት ቁሳቁስ ☐ 5 የምክር አገልግሎት ☐ 6. የህግ ክለላ ☐ 7.የመጠልያና እንክብካቤ	
Q.502	የምግብ የሚያገኙ ከሆነ፧በምን መንገድ ድጋፉን ያግኙ ነበር?	☐1 የምግብ እጥረት በመዳሰስ የአልሚ ምግብ አርዳታ በ ማድረግ ☐2.በሕክምናምግብ ድጋፍ ግንኙነት በማጠናከር ☐3.ለ አሳዳጊወቸ የስነምግብ አያያዝና አዘገጃጀት ስልጠና መስጠት
Q.503	የሕክምናድጋፍየሚያገኙ ከሆነ፧በምን መንገድ ድጋፉን ያግኙ ነበር?	☐1.ነፃ የሕክምና አገልግሎት በመስጠት ☐2.ቤት ለቤት በ መሄድ የህፃኑ/ኗ ጤንነት በማየት ☐3.ለአሳዳጊው ስለ ክትባት፧ውሃ መከላከልና ስለግል ንጽና ስልጠና በመስጠት
Q.504	የገንዘብና የገቢ ማስገኛ ድጋፍ የሚያገኙ ከሆነ፧በምን መንገድ ድጋፉን ያግኙ ነበር?	☐ 1.አጫጭር ስልጠና በ መስጠት ☐ 2.የገቢ ማስገኛ ስራወችን በ መፍጠር ብድር በማመቻቸት
Q.505	የትምህርት ቁሳቁስ ድጋፍ የሚያገኙ ከሆነ፧በምን መንገድ ድጋፉን ያግኙ ነበር?	☐ 1.በቀጥታ የትምህርት ውጭን በመቻል ☐ 2.የትምህርት እድል በመፍልግና በማመቻቸት ☐ 3.ለአሳዳጊው ህፃኑ/ኗ በትምህርት ገበታ ላይ እንዲገኙ ስልጠና በ መስጠት

Q.506	የምክር አገልግሎት የሚያገኙ ከሆኑ፣በምን መንገድ ድጋፉን ያግኙ ነበር?	□ 1.በቀሚንት ስነልቦናዊና ማህበራዊ ድጋፍ ስልጠና በመስጠት □ 2.ድጋፍ ስጭ አካላትን በማቀቀም ለህፃኑ/ሷ እና ለ አሳዳጊወቸ .የምክር አገልግሎት በመስጠት □ 3..ቤተሰባዊ ግንኙነት እንዲኖር በማድረግ
Q.507	የህግ ክለላ ድጋፍ የሚያገኙ ከሆኑ፣በምን መንገድ ድጋፉን ያግኙ ነበር?	□ 1.የህፃኑ/ሷ ህጋዊ መብት በመጠበቅ □ 2..ለ ህፃኑ/ሷ እና ለአሳዳጊው የህግ ክለላ አገልግሎት አደረጊ አካላት በማገናኘት አገልግሎት በመስጠት
Q.508	የመጠልያና እንክብካቤ ድጋፍ የሚያገኙ ከሆኑ፣በምን መንገድ ድጋፉን ያግኙ ነበር?	□ 1.ቤት ለቤት በ መሄድ የቤተሰብ ድጋፍ □ 2.በቀሚነትየመጠልያ እንክብካቤ ፍላጎትን በማየት
		□ 3.በህብርተስቡውስጥ የመጠልያ እንክብካቤ በማሻሻል
Q.509	ለምን ያህል ጊዜ ድጋፍ ተደረገሎት?	_____ በወር
Q.510	በእርሶ አስተያየት የሚደረግሎት ድጋፍ እንዴት ያዩታል?	□ 1. በቂ ነው □ 2. በቂ አይደለም □ 3. መልስ የለም

<div align="center">አንትሮ ፖሞትሪክ ልኬት</div>

Q.601	የህፃኑ/ሷ አሁን የደረሰችበት/በት እድሜ	_____ በወር
Q.602	የሆለትዮሽ ወደ ውስጥ የሚሰረጎድ የግር እብጠጥ ሆኔታ በምርመራ?	□ 1. 0 □ 2. + □ 3. ++ □ 4. +++
Q.603	የህፃኑ/ሷ አሁን ያለው/ላት ቁመት	_____ (ሴ.ሜ)
Q.604	የህፃኑ/ሷ አሁን ያለው/ላት ክብደት	_____ (ኪ.ግ)
Q.605	የህፃኑ/ሷ አሁን ያለው/ላት የክንድ ልኬት	_____ (ሚ.ሜ)

Effect of Various Parameters of Carbon and Nitrogen Sources and Environmental Conditions on the Growth of Lactobacillus Casei in the Production of Lactic Acid

By Jale Mohseni, Mohammadreza Fazeli & Alireza Shahab Lavasani

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Abstract- Lactic acid is widely used in food and pharmaceutical industries and is produced by two biological (fermentative processes) and synthetic methods. Due to the production capacity of isomer $L(+)$, the highest global production of lactic acid is done by fermentation. *Lactobacillus casei* strain was used in this study that is the homofermentative bacteria producing $L(+)$-lactic acid. Two nitrogen sources of yeast extract and corn steep liquor and two sugar sources of sucrose and molasses at temperatures of 37 C°, 40 C° and 42 C° as well as two pHs 5 and 7 were used in the study. The results showed that higher lactic acid is produced by increasing the amount of yeast extract compared to the use of corn steep liquor. Moreover, *Lactobacillus casei* using sucrose produced more lactic acid than the molasses. The optimal temperature for lactic acid produced by the bacterium was 37 C° in these experiments. In addition, it was found that *Lactobacillus casei* could produce more lactic acid at pH = 5 than at pH = 7, and the production of the acid reached the maximum value at pH = 5. In optimum conditions, 60/90g/L Lactic acid was obtained after 24 hours incubation of samples (pH = 5 and 37 C°).

Keywords: (+) l-lactic acid, lactobacillus casei, corn steep liquor, yeast extract, molasses.

GJMR-L Classification: NLMC Code: QU 145

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Strictly as per the compliance and regulations of:

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Jale Mohseni [α], Mohammadreza Fazeli [σ] & Alireza Shahab Lavasani [ρ]

Abstract- Lactic acid is widely used in food and pharmaceutical industries and is produced by two biological (fermentative processes) and synthetic methods. Due to the production capacity of isomer L(+), the highest global production of lactic acid is done by fermentation. *Lactobacillus casei* strain was used in this study that is the homofermentative bacteria producing L(+)-lactic acid. Two nitrogen sources of yeast extract and corn steep liquor and two sugar sources of sucrose and molasses at temperatures of 37 Cº, 40 Cº and 42 Cº as well as two pHs 5 and 7 were used in the study. The results showed that higher lactic acid is produced by increasing the amount of yeast extract compared to the use of corn steep liquor. Moreover, *Lactobacillus casei* using sucrose produced more lactic acid than the molasses. The optimal temperature for lactic acid produced by the bacterium was 37 Cº in these experiments. In addition, it was found that *Lactobacillus casei* could produce more lactic acid at pH = 5 than at pH = 7, and the production of the acid reached the maximum value at pH = 5. In optimum conditions, 60/90g/L Lactic acid was obtained after 24 hours incubation of samples (pH = 5 and 37 Cº).

Keywords: (+) l-lactic acid, lactobacillus casei, corn steep liquor, yeast extract, molasses.

I. Introduction

Lactobacillus microorganisms are known as the favorite residents of digestive tract. Lactic acid is the only or main product of the microorganisms, by which they can reinforce an acidic environment. These conditions are not suitable for many pathogenic bacteria in digestive tract and thus destroy the bacteria (Kandler & Weiss, 1985). The bacteria obtain their required energy through metabolism of sugars during the fermentation process, and produce lactic acid as the main and final product (Champagne *et al*., 2005). Lactic acid is widely used as a flavoring and preservative in food, pharmaceutical, leather and textile industries. The acid is also polymerized into a biodegradable poly lactic acid (PLA) that is used in the manufacture of sutures to close wounds as well as prostheses in the pharmaceutical industry. In addition, it is applied to make products with chemical base (Chooklin *et al*.,

2011). In today's industry, this acid is produced both as fermentative and chemical form. With today's improvement in fermentative processes, fermentative production of lactic acid is 90% and its chemical production is only 10% (Chiarini *et al*., 1996).

Lactobacillus casei is a strain of lactic acid bacteria with a remarkable phenotype and variabile genotype having a variety of bacterial colonies, some of which are present in the human digestive duct. Homofermentative L. casei can produce L(+)-lactic acid. Lactic acid, which is found in both optically active form of D(-) and L(+), is produced by a chemical reaction through hydrolysis of lactonitriles and microbial fermentation processes (Altiok *et al*., 2006). One of the features of the industrial microorganisms to produce lactic acid is their ability of quick and complete fermentation of cheap raw materials, the need for a minimum amount of nitrogen material and high yield of production (Narayanan *et al*., 2004).

Nabi Bidhendi and Bani Ardalan (2004) studied continuous and discontinuous production of lactic acid from whey using immobilized lactobacillus, compared the use of wooden laminates, the use of brick and glass with adsorption and eggshell with glutaraldehyde with covalent bonding and observed that wood can have the maximum amount of adsorption and is the best stabilizer for production. Parmjit *et al*. (2010) explored whey fermentation and production of L(+)-lactic acid by *Lactobacillus casei*. The effects of various parameters such as pH, medium, temperature, inoculation, age of inoculation, mixing and incubation time were examined on the increase of lactose into lactic acid. Optimization of vaporization conditions resulted in the reduced fermentation time and increased conversion rate of lactose into lactic acid. Chooklin *et al*. (2011) investigated the palm syrup fermentation by *Lactobacillus casei* TISTR 1500 as the microorganism producing lactic acid. They observed that physical and chemical properties determine the amount of lactic acid production, and total palm syrup concentrate, dry cell weight and lactic acid increase by increasing sugar. Lactobacillus have complex food requirements because they are microorganisms that can have a limited

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bioproductivity and cannot make the growth factors needed by their body. They cannot just grow on the carbon source and mineral salts of nitrogen.

Therefore, they need a different set of amino acids and vitamins. The growth factors are usually provided by nitrogen sources such as yeast extract, peptone and ammonium sulfate. In particular, yeast extract has the greatest effect due to the presence of purines, pyrimidine and vitamins B (Hujanen et al., 2001; Narayanan et al., 2004; Yu et al., 2008). According to all the scientific resources available, this study tried to optimize suitable culture medium to produce a high concentration of the bacterium and the resultant metabolites (Typically, lactic acid) by considering the minimum facilities at a laboratory scale so that the results could be used in the food and pharmaceutical systems.

II. Materials and Methods

Microstructures: Lactobacillus casei donated by the Laboratory of Pharmacy faculty, Tehran University was used in this study.

Medium: in order to select a suitable sugar source, 100cc ingredients of the medium MRS (except for the sugar source) were first prepared in a 250cc Erlenmeyer flask in six separate flasks, then the studied sugars (sucrose, and then sugar beet molasses) were added to the two mixes. Three flasks were set to pH 7, the remaining flasks were set to pH 5 by adding some hydrochloric acid (HCl), and then an equal amount of the intended bacterium (Lactobacillus casei) was added to each. Flasks were incubated at temperatures 37 C°, 40 C° and 42 C° with the stirring speed of 150 rpm. To determine the effect of different nitrogen sources, 100cc media were prepared in the 250cc flask with the exception that the two nitrogen sources of yeast extract (YE) and corn steep liquor (CSL) (0.2% yeast extract and the remaining corn steep liquor were added to the medium) replaced with nitrogen sources of MRS medium. The provided media were sampled at intervals of 8, 12 and 24 hours.

Measurement of final lactic acid: the exact amount of lactic acid was measured based on the chromatography methods using HPLC. Thus, it was necessary to obtain a standard curve of lactic acid after defining the method properties. Therefore, various concentration samples were prepared from pure lactic acid and 20 microliters of it were injected into the device. Of course, three injections of each concentration were conducted on the intra day and between three different days in order to consider the coefficient of intra day and between days variations. After obtaining the standard curve of lactic acid, test samples were injected that included injecting 20 ml of the freshly prepared supernatant (normal test), the supernatant plus volumetric 1% of lactic acid (test 1%) and the supernatant plus volumetric 2% of lactic

acid (test 2%). Each of the test samples were injected three times into the device. Lactic acid was added to the supernatant with the aim of the preparation of internal standard so that the existence of lactic acid could be ensured by increasing the height and area under the intended peak curve in the test sample. The concentration of lactic acid in the supernatant was obtained after obtaining areas under the curve and height of the test sample and data compliance with the standard curve.

Experimental design: repeated measures design was used to evaluate the effects of time, source, temperature and pH on lactic acid with the within group factor of time and between group factors of source, temperature and pH in this study. Bonferroni post hoc test was used to detect significant differences for mutually between different levels of time. As well as to detect significant differences between different levels. Moreover, Tukey post hoc test was used to determine significant differences between different levels of source and temperature. SPSS 16 was used to analyze data, and statistical significance was considered at 0.05.

III. Discussion and Conclusion

a) Source of nitrogen

The nitrogen source is the most important factor affecting for Lactobacillus growth (Wood & Holzapfel, 1995). However, high levels of nitrogen in the extract can cause cell death (De Lima et al., 2009). Examining Figures 1, 2 and 3, it can be seen that maximum production of lactic acid in three samplings was obtained by adding raw material of yeast extract. The results indicate that the maximum production of lactic acid is 60/90g/L in the comparison between the two nitrogen sources of yeast extract and corn steep liquor that is related to yeast extract at a temperature of 37 °C and pH = 5. As seen, yeast extract had the highest production of lactic acid after 8 hours, 12 hours and 24 hours of incubation of media. Due to the high yield production of lactic acid by *Lactobacillus casei* using the raw material of the yeast extract compared to corn steep liquor, in all media conditions studied and with regard to the fact that the better treatment with the production of 60.90g/L lactic acid was associated with an increase in the raw material, it could be found that since yeast extract is one of the raw materials necessary for the growth of the microorganism and the best source of nitrogen for growth and lactic acid production, decrease in the use of the yeast extract and replacement of the nitrogen source with corn steep liquor would result in a significant reduction in lactic acid production. Hujanen et al. (2001) also provided yeast extract and corn steep liquor as nitrogen sources with *Lactobacillus casei* and observed that the bacterium could use both nitrogen sources and produce lactic acid. However, evaluation of the results reported in these researchers research

indicated that production of lactic acid by *Lactobacillus casei* in the same medium conditions using the nitrogen source of yeast extract was more than that of the condition when corn steep liquor was provided as nitrogen source with the bacterium. Our results is consitent with these findings. Furthermore, *Lactobacillus casei* production was increased from the beginnig of the fermentation to 24 after wars by both nitrogen sources of corn steep liquor and yeast extract that is compatible with the results obtained in our study (Hujanen *et al*., 2001).

b) Carbohydrate source

As a sugar source, molasses was compared with sucrose and the effect of each on the lactic acid produced by *Lactobacillus casei* were studied. Table 1 shows the results. As seen, maximum production of lactic acid is 35/53g/L that is related to the sugar sucrose in the medium after 24 hours of incubation at 37 C° and initial pH 5. The process of increasing the amount of lactic acid produced by sucrose compared to molasses was observed in three samplings (8 hours, 12 hours and 24 hours) and in all conditions ranging from

pH = 7 and pH = 5 as well as at temperatures of 37, 40, 42 C°.

After studying and observing the results obtained in the test medium conditions, it can be found that lactic acid production in media containing sucrose was significantly higher than that of when molasses was used by *Lactobacillus casei*. Since sucrose is simpler than molasses, it could be concluded that the more simpler the sugar available the bacteria, the more ability the microorganism will have to use it and produce lactic acid.

Chooklin *et al*. (2011) studied the level of lactic acid using the raw material of palm produced by *Lactobacillus casei* and observed that lactic acid production increases within 24 hours of the onset of the activity of bacteria. Moreover, they could observe that *Lactobacillus casei* using glucose had the maximum production of lactic acid. The researchers concluded that maximum production of lactic acid is related to the use of glucose, fructose and sucrose, respectively. The results indicated that the microorganism used simple sugars easier and produced more lactic acid. This result is compatible with our results (Chooklin *et al*., 2011).
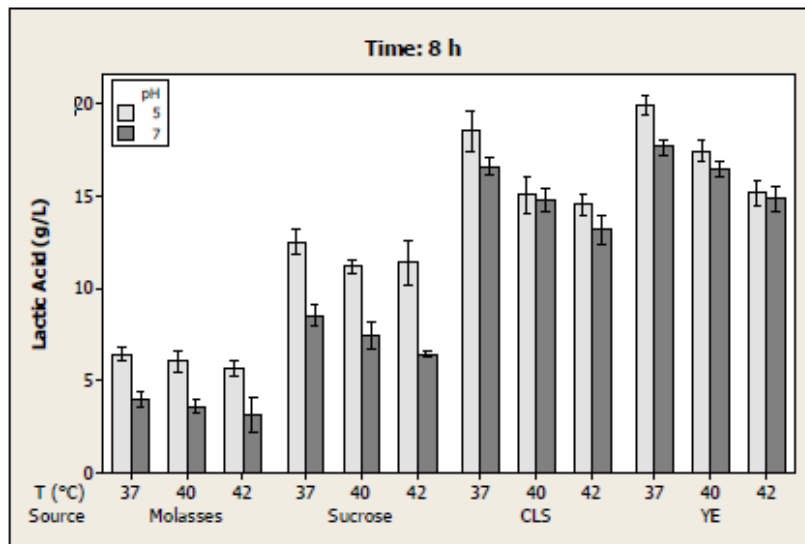

Figure 1: Comparison of lactic acid produced by *Lactobacillus casei* in nitrogen sources of yeast extract and corn steep liquor and sugar sources of sucrose and molasses after 8 hours of bacterial growth.
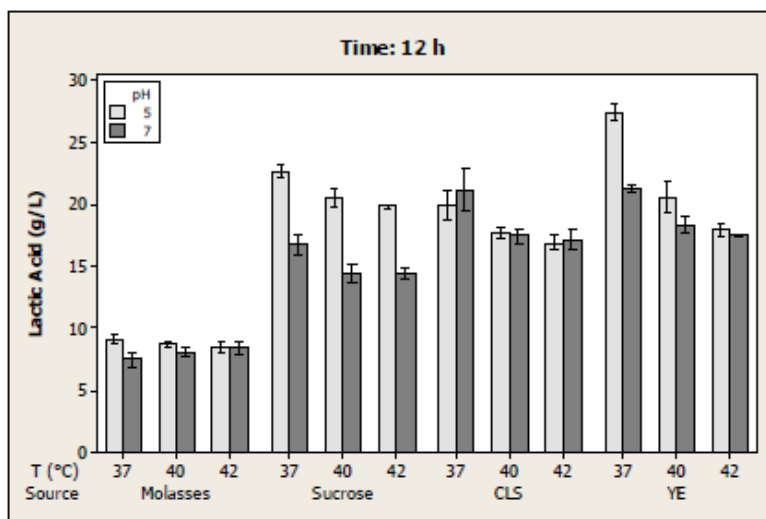
Figure 2: Comparison of lactic acid produced by *Lactobacillus casei* in nitrogen sources of yeast extract and corn steep liquor and sugar sources of sucrose and molasses after 12 hours of bacterial growth.
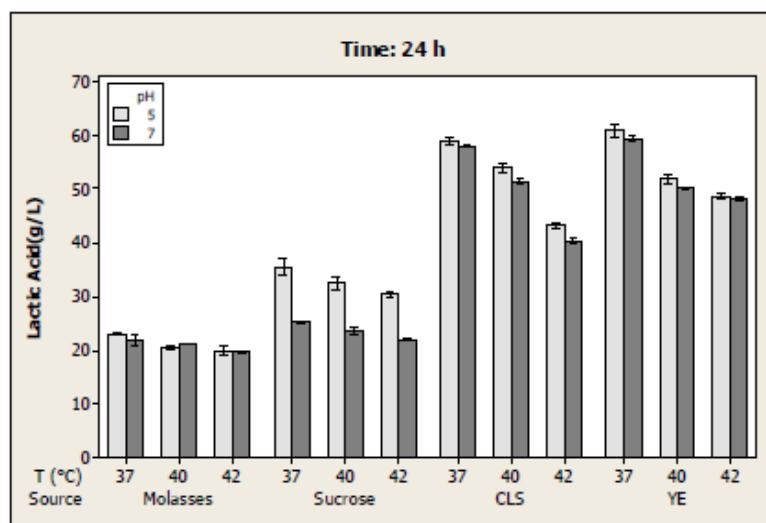
Figure 3: Comparison of lactic acid produced by *Lactobacillus casei* in nitrogen sources of yeast extract and corn steep liquor and sugar sources of sucrose and molasses after 24 hours of bacterial growth.

c) Temperature and pH

After analyzing the results from the lactic acid produced by *Lactobacillus casei* at temperatures of 37, 40 and 42 C°, it was seen that the maximum production of lactic acid was at 37 C° and acid production decreases with increasing temperature. As seen in Figures 4, 5 and 6, and this decreased process with the raw material of corn steep liquor is 58/93g/L, 54/03g/L and 43/36g/L after 24 hours at pH = 5 and temperatures of 37, 40 and 42 C°. Among the two pHs used in this study, maximum production of lactic acid was at pH = 5. For example, the production of lactic acid using the nitrogen source of yeast extract at 42 C°, pH = 7 and pH = 5 and equals 48/16g/L and 48/70g/L, respectively.

In the case of better treatments related to the use of the raw material of yeast extract and temperature of 37 C°, the production of lactic acid with pH = 7 and pH = 5 equals 59/43g/L and 60/90g/L, respectively. Hujanen *et al.* (2001) also provided yeast extract and corn steep liquor as nitrogen sources with bacteria. and observed that the bacterium could use both nitrogen sources and produce lactic acid. Using statistical method of surface response, they found optimum temperature around 35 C° as the optimum temperature to produce the greatest amount of acid produced by the above-mentioned bacteria. Moreover, Qi and Yao (2007) reported the optimum temperature of 37 C° (Hujanen *et al.*, 2001; Qi and Yao., 2007). Rincon *et al.* (1993) also reported the optimum pH of 4.5 that indicates acidophility of the microorganism. This is very close to the optimized pH obtained in our study (Rincon et al., 1993).

Table 4: The lactic acid produced by *Lactobacillus casei* after 24 hours of incubation at 37 C˚ and pH 5 and 7

Table 5: The lactic acid produced by *Lactobacillus casei* after 24 hours of incubation at 40C˚ and pH 5 and 7

Table 6: Lactic acid produced by *Lactobacillus casei* after 24 hours of incubation at 42C° and pH 5 and 7

IV. Conclusion

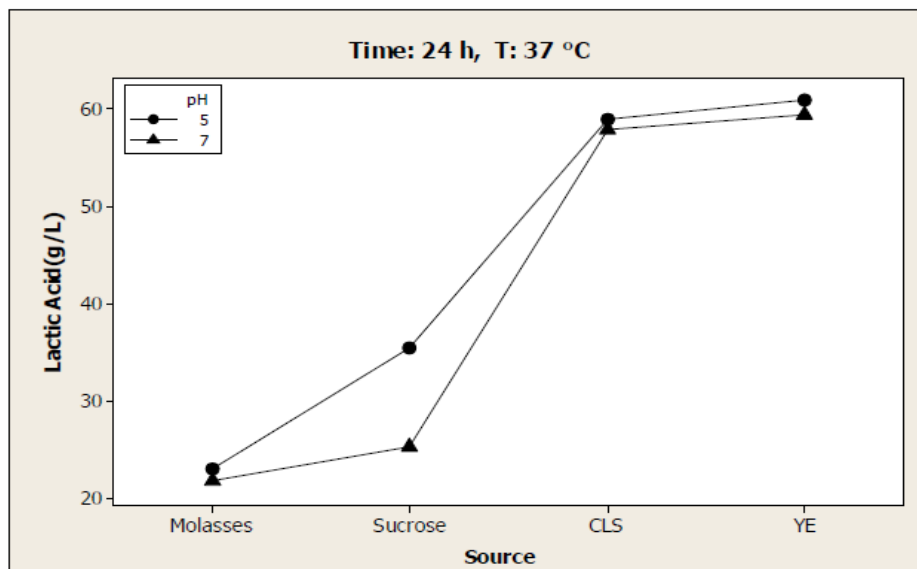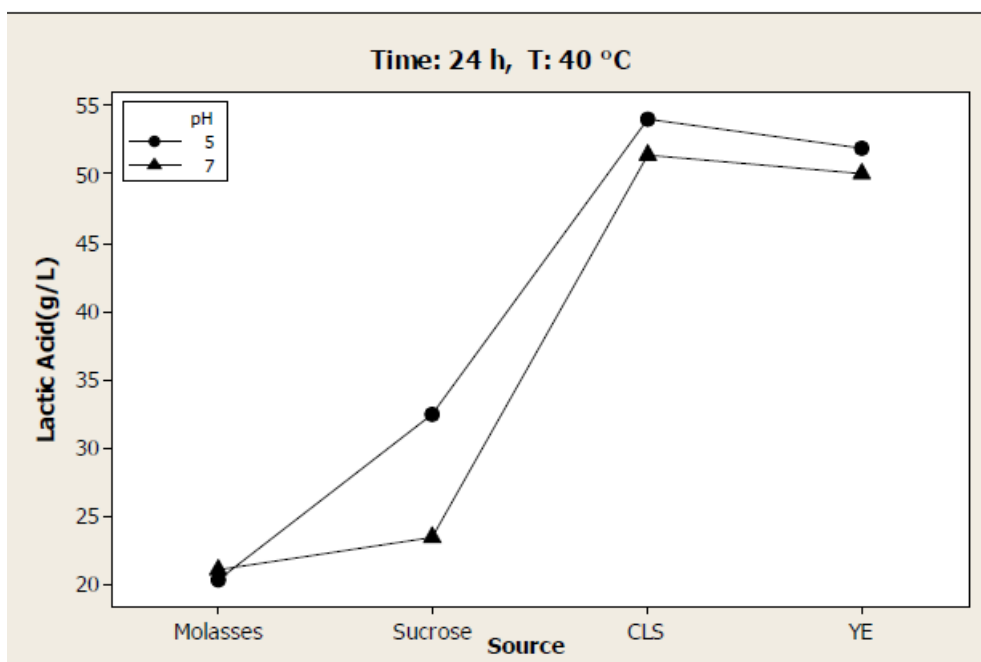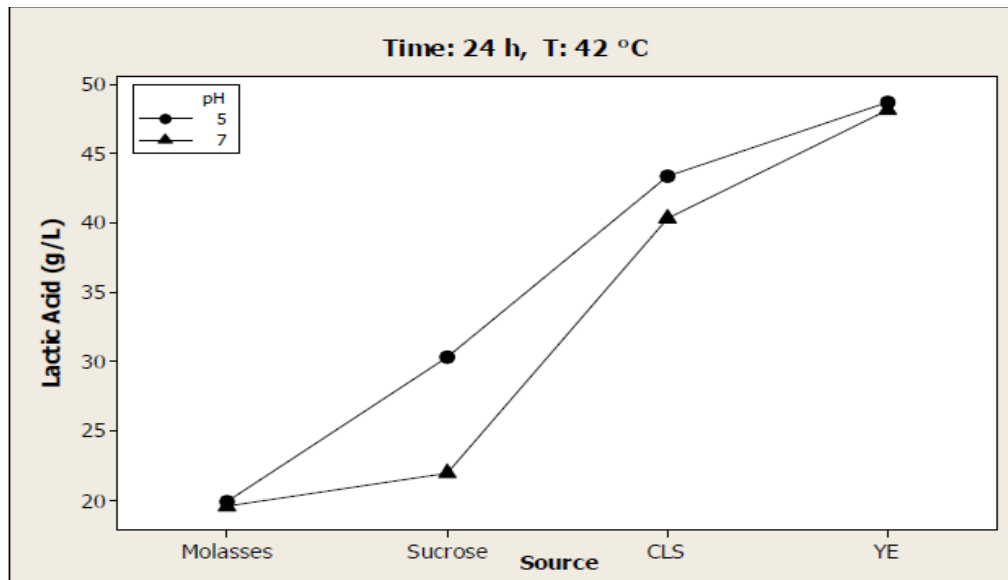This research findings showed that the best source of sugar from the two sources tested for the growth of the bacterium *Lactobacillus casei* and lactic acid produced by the bacterium is the sugar source of sucrose. Since other studies in this area also represents a better use of sugar sources as our study, it could be concluded that *Lactobacillus casei* consumed the simple sugars better. The bacteria using yeast extract could produce more Lactic acid compared to the existence of nitrogen source of corn steep liquor in the medium that results from the raw materials necessary for the growth of microorganism in yeast extract. *Lactobacillus casei* at temperature 37 C̊ and pH = 5 could produce the highest lactic acid. It should be noted that although there was no significant difference in the amount of lactic acid produced by *Lactobacillus casei* when using high levels of yeast extract compared to its decrease and replacement with corn steep liquor, with regard to the significant price difference between the two nitrogen sources and given that more lactic acid was produced by corn steep liquor, economic production could be expected by increasing the amount of corn steep liquor and declining the amount of yeast extract. Furthermore, in the case of sugar sources used in the study, although there was a significant difference in the amount of lactic acid produced by *Lactobacillus casei* after using sucrose and molasses, replacement of molasses with sucrose could be justified with regard to appropriate production of lactic acid by molasses and a lower price than sucrose.

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> The "FARSM" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall,Ph.D., FARSS or William Walldroff, M.S., FARSM.

FARSM accrediting is an honor. It authenticates your research activities. After recognition as FARSM, you can add 'FARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSM designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

Once FARSM title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSM can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSM, you will be given a renowned, secure and free professional email address with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.
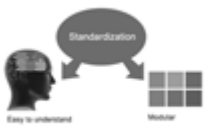
The FARSM will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSM member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSM members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSM member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.

The FARSM is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSM member can decide its price and we can help in making the right decision.

The FARSM member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

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The ' MARSM ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The "MARSM" is a dignified ornament which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., MARSM or William Walldroff, M.S., MARSM.

MARSM accrediting is an honor. It authenticates your research activities. Afterbecoming MARSM,you can add 'MARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSM designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSM, you willbe given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSM member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

Auxiliary Memberships

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.
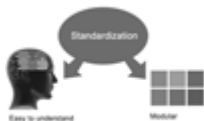
After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.

The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

➤ The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"
- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.

- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.

- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.
"

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The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.<u>Online Submission</u>: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

   (II) Choose corresponding Journal.

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Page Size: 8.27" X 11'"

- Left Margin: 0.65
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- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
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- Two Column with Equal Column with of 3.38 and Gaping of .2
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- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
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You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

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Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

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Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

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Please mention proper reference and appropriate acknowledgements wherever expected.

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3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

 Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than $1.4 \times 10\text{-}3$ m3, or 4 mm somewhat than $4 \times 10\text{-}3$ m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

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6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including <u>definite statistics</u> - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

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- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)

- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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