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The role of black tea and *berbere*-spiced food consumption on appetite and energy intake of pre-school children in an orphanage in Addis Ababa

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Declaration

I the undersigned, declare that this thesis is my original work and all the source of data's used for the thesis has been duly acknowledged.

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Dedication

This work is dedicated to God who allows me to start and finish this journey. I thank you, God for being by my side all the time even though I do not deserve any of it.

Acknowledgment

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Hilu, thanks sis you are a great friend and a loving sister thanks for all your support through those tough time and for all your efforts to relax me and for making life a lot easier. I thank you sincerely.

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Abbreviation

5-HT- 5^{th} Sertoinin receptor

AgRP - Agouti gene-related peptide

CNS- Central nervous system

HPLC-High Pressure Liquid Chromatography

EC - Epicatechin

ECG – Epicatechin gallate

EGC- Epigallatecatechin

EGCG - Epigallatecatechin gallate

EI-Energy intake

GI- gastrointestinal

GLP-1 Glucagon-like peptide-1

HR – Heart rate

MJ - Mega joule

NPY Neuropeptide Y

PNS - Parasympathetic nervous system

PYY Peptide tyrosine tyrosine

SNS – Sympathetic nervous system

VAS-Visual Analog Scale

VLT-very light tea where the concentration of caffeine and catechin below the detection level.

Abstract

The effect of drinking habitual concentration of black tea or habitual consumption of red pepper on appetite and food intake of children remains unknown.

The objective of this study was to determine the role of habitually consumed black tea or *berbere*-spiced food on the appetite and energy intake of children less than five years of age in an orphanage in Ethiopia.

With-in subject crossover design was used to which 21 preschool children (24- 54 months) participated. The effect of black tea or *berbere*-spiced food on energy intake and appetite was investigated by evaluating intake of the test meals. Anthropometric measurements, caffeine and catechin analysis in tea, and proximate composition of the test meals were also determined.

Although fewer children (n= 5) had anthropometric values of <-2 SD, more than half of the children were at the border of Z score for stunting, underweight, and wasting. The consumption of habitual concentrations of black tea had no significant effect on satiation (*P=0.07)* and satiety (P=0.22*)*. Similarly, the consumption of *berbere*-spiced foods had no significant effect on satiation *(P=0.55)*. This suggests that both the consumption of berbere-spiced foods and black tea had no effect on food/energy intake (P> 0.05). In contrast, increasing portion size had a significant effect on appetite and energy intake *(P< 0.001)*. Therefore age-dependent increase in portion size may be required to improve the nutritional status of the children.

Keywords: Black tea, *berbere,* food/energy intake (EI), appetite, Satiety, satiation, portion size

Chapter 1- *Introduction*

1. Introduction

1.1. Background

Malnutrition is any condition caused by excess or deficient food energy. Deficiency of energy causes under-nutrition whereas excess causes obesity (Ronquillo *et al.*, 2008). Under-nutrition is one of the leading causes of morbidity and mortality on children in developing countries and it is the cause of more than half of all infant and child mortality in developing countries (Mathers *et al.*, 2009). Furthermore, obesity has become an epidemic in many parts of the world and is on the rise in some developing countries (Wang *et al.*, 2002)

Ethiopia has a very high prevalence of stunting, underweight, and wasting (Teshome *et al.*, 2009). Some of the risk factors for under-nutrition were identified as the region of residence, education of mother, age of the child, inappropriate child feeding practice either due to insufficient quantity and, or low energy and nutrient density of complementary foods (Amsalu & Tigabu, 2008; Girma & Genebo, 2002; Mulugeta *et al.*, 2010). For instance, the median energy and nutrient intakes of young children in North Wollo, northern Ethiopia was reported to be low (Baye *et al.*, 2013). This was partly because the nutrient densities of the complementary foods were low, but also because the amount of foods consumed per day and per meal was very low. Tea and spicy foods were commonly consumed by these young children (Baye *et al.*, 2013; Gibson *et al.*, 2009).

Capsaicin, caffeine, and tea catechins have been reported to be effective in weight management by enhancing dietary fat oxidation and inducing thermogenesis (Diepvens *et* *al.*,2007; Westerterp-Plantenga, 2010). However, these results are not consistent as other studies stated that green tea extract do not have significant effect on weight reduction (Hsu *et al.*, 2008). In addition, theophylline attenuates 5-HT-2C receptor dependent anorexia and anxiety suggesting that tea may not produce adverse effect on appetite since it contains both caffeine and theophylline (Alam *et al.*, 2011).

1.2 Statement of the problem

Several studies suggest that bioactive ingredients which have significant effects on appetite regulation and energy intake could lead to reductions in body weight if used in conjunction with low energy diet (Halford & Harrold, 2012). Even though the effect of each bioactive ingredient on appetite and energy intake reduction is not clearly understood, the combined effect of caffeine and green tea catechins; caffeine and capsaicin as well as red pepper containing different mix of spices may have the potential of reducing appetite and energy intake in humans (Carter & Drewnowski, 2012; Kovacs & Mela, 2006; Roberts, 2005). However, most studies on the component of tea and red pepper were conducted on obese adults in developed countries. In Ethiopia, tea and *berbere*, a red pepper with mix of spices often used as condiment, are daily consumed across all age groups. However, the effect of habitual concentrations of black tea or *berbere*-spiced food on appetite and food intake of children remains unknown. Given that low food and energy intakes are associated with child under nutrition in developing countries, the role of black tea and *berbere*-spiced foods needs to be evaluated so as to devise guidelines and recommendations to improve child nutrition.

1.3. Objectives

1.3.1 General objective

To determine the role of black tea or *berbere*-spiced food consumption on appetite and energy intake of Ethiopian children less than five years of age

1.3.2. Specific objectives

- To investigate the effect of habitually consumed concentrations of black tea on appetite and energy intake in children less than five years of age.
- To investigate the effect of habitually consumed *berbere*-spiced food on appetite and energy intake in children less than five years of age.
- To investigate the effect of portion size of meals on food and energy intake
- To investigate whether increasing portion size leads to higher macronutrient intakes

Chapter 2- *Literature review*

2. Literature Review

2.1. Appetite regulation

Appetite is defined as a natural desire to satisfy a bodily need, especially for food. The counterpart of appetite is satiety, which refers to the state of further eating is inhibited and occurs as a consequence of having eaten. Satiation is the satisfaction of appetite that develops during the course of eating and eventually results in cessation of eating. Appetite sensations are formed in the brain, through the integration of a multitude of neural and hormonal signals involved in the regulation of food intake. The hypothalamus and brain stem are involved in appetite and feeding regulation. Neuronal projections from hypothalamus integrate signals from the brain, the peripheral circulation and the gastrointestinal tract to regulate energy intake and expenditure while the brain stem plays a role in individual meal size determination (Neary *et al.*, 2004).

Control of food intake involves not only the central nervous system (CNS), but also the adrenals and the gastrointestinal (GI) tract. Furthermore, adipose tissue has been found to play an important role in regulation of food intake by producing several endocrine and pancreas mediators, including insulin, leptin, adiponectin, resistin and tumour necrosis factor-α, which are known to influence food intake (Näslund & Hellström, 2007; Neary *et al.*, 2004). Besides to these appetite is also regulated by the gut hormones ghrelin and Peptide tyrosine tyrosine (PYY3-36), and signaled to the hypothalamus. In addition; agonists at the 5-HTreceptors and drugs that inhibit the re-uptake of serotonin reduce feeding (Neary *et al.*, 2004).

For instance studies reveled that ghrelin endogenous ligand which is synthesized predominantly in the stomach and found in the circulation of healthy humans has been shown to promote increased food intake, the effects of administering intravenous ghrelin on appetite and food intake on human indicated that energy intake increase when ghrelin injection was adminstred (Wren *et al.*, 2001). Other studies show that CCK and GLP-1 are meal-related satiety signals that are released from the gastrointestinal tract during food intake. Both peptides promote a sense of fullness that encourages an end to the meal. Therefore both peptides are factors that trigger the termination of eating, participating in a meal-to-meal control system (Gutzwiller *et al.*, 2004). Furthermore orally administered GLP-1 and PYY3-36 have effect on appetite which enhanced fullness at meal onset and reduced energy intake (Steinert *et al.*, 2010).

There are many peripheral signals that can contribute to feeding behavior; short-term signals are primarily from the GI tract (e.g., CCK and GI stretch receptors) and are involved in promoting sensations of satiety that lead to meal termination. These short-term signals by themselves are not sufficient to regulate energy balance and body adiposity. The long-term signals insulin and leptin are produced and circulate in proportion to recent energy intake and body adiposity. Together, the short- and long-term signals interact to regulate energy balance in that insulin and leptin appear to determine the sensitivity of the brain to the satiety-producing effects of the short-term signals from the GI tract (Havel, 2001).

Regulation of food intake involves dual mechanisms. The short-term control of hunger ultimately leading to meal taking is primarily governed by gut-derived signaling to the brain, and of which ghrelin is stimulatory, whereas glucagon-likepeptide-1 (GLP-1) and PYY are inhibitory. Low circulating insulin, PYY3-36, and elevated ghrelin levels are observed in case of fasting or starvation lead to an increase in neuropeptide Y (NPY) and Agouti gene-related peptide (AgRP) neuronal activity and increased appetite, whereas the opposite is seen post-prandially (Neary *et al.*, 2004). The long-term control is of metabolic origin where the endocrine action of adipose tissue comes into focus, with peptide hormones such as leptin, are released in the periphery to act on centers in the brain regulating appetite and metabolic control (Näslund & Hellström, 2007).

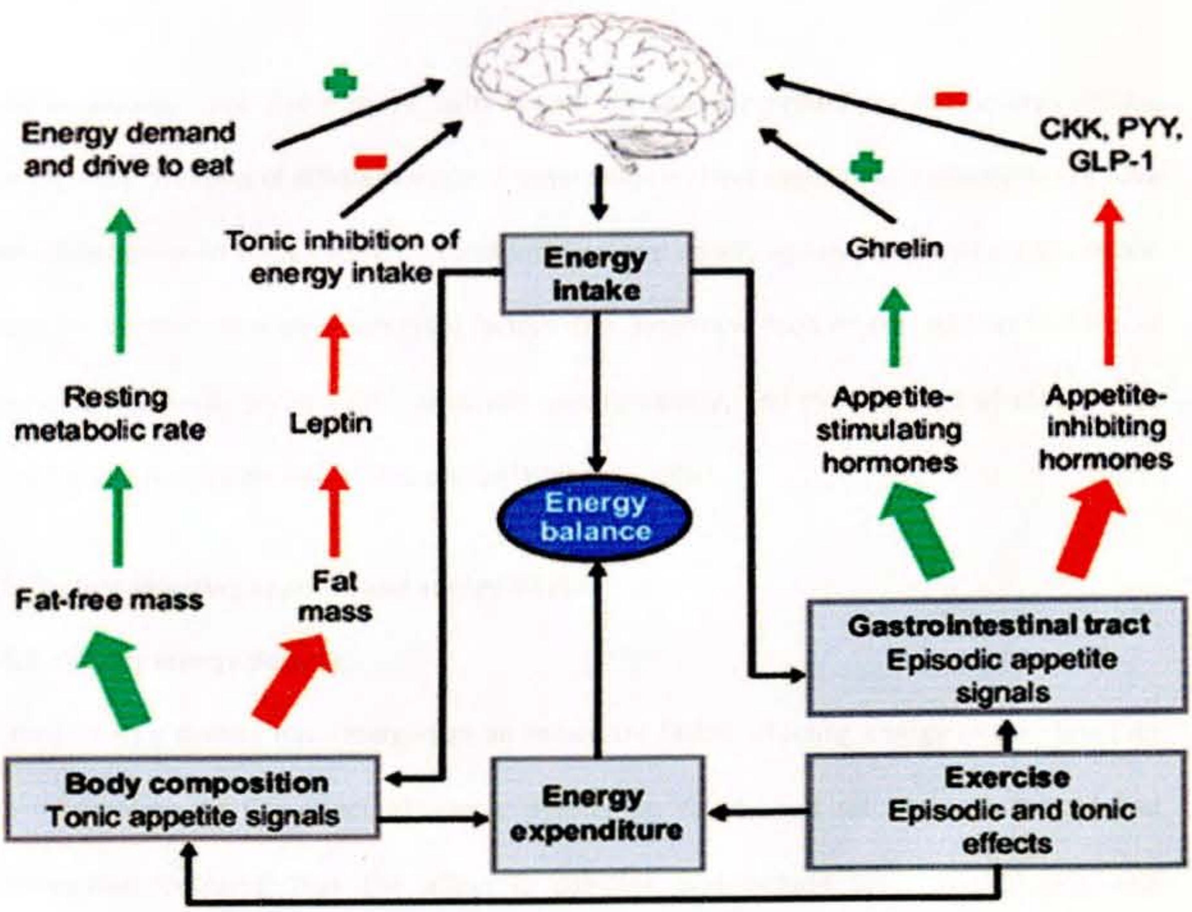

Figure 2.1, Dual mechanism of appetite control by gut hormones and central nervous system

Therefore appetite can be considered as a balance between excitatory and inhibitory processes. The excitatory processes arise from bodily energy needs and constitute a drive for food (which in humans is reflected in the subjective experience of hunger). The most obvious inhibitory processes arise from post-ingestive physiological processing of the consumed food, and these are reflected in the subjective sensation of fullness and a suppression of the feeling of hunger. However, the sensitivity of both the excitatory and inhibitory processes can be modulated by signals arising from the body's energy stores. Consequently, the balance between the excitatory and inhibitory processes has implications for body weight regulation (Blundell *et al.*,2001).

Several studies have been made with regard to appetite regulation and energy intake. Furthermore the roles of different internal factors which affect appetite and energy intake have been determined to some extent. Beyond satiation and satiety signals felt physically by people, there are several other environmental factors that determine food intake; such as visibility of foods, convenience, portion size, perceived dietary variety, and the presence of others have substantial influence on food consumption (Wansink, 2004).

2.2. Factors affecting appetite and energy intake

2.2.1. Dietary energy density

Dietary energy density has emerged as an important factor affecting energy intake; however the information on the effect of energy density on satiety and satiation are limited and investigation indicated that the effect is complex and include both psychological and physiological mechanisms (Rolls, 2009). An investigation made on pre-school children with regard to energy intake showed that decreasing the energy density of multiple foods served over the course of two days leads to a reduction in the ad-libitum energy intake (Leahy *et al.*,2008). Other study indicates that energy density influences energy intake independently of macronutrient (fat) content and palatability. Energy intake was significantly greater from the diets of high energy density than from the diets of lower energy density. Although energy intake was greatly reduced in the diet of low energy density as compared with the diet of high energy density, individuals reported no differences in feelings of hunger or fullness (Bell *et al.*, 1998). In addition findings suggest that frequent consumption of energy dense foods, especially those that are typically consumed at a fast rate may promote excess energy intake (Karl *et al.*, 2013).

Studies show that humans have a weak innate ability to recognize foods with a high energy density and to appropriately down-regulate the bulk of food eaten in order to maintain energy balance. This induces so called 'passive over-consumption'. Most fast foods have an extremely high energy density and that is more than twice the energy density of recommended healthy diets. The study concludes that the high energy densities of many fast foods challenge human appetite control systems with conditions for which they were never designed. Among regular consumers they are likely to result in the accidental consumption of excess energy and hence to promote weight gain and obesity (Prentice & Jebb, 2003).

According to Van Kleef *et al.* (2012) conclusion; food with high energy density have a lower satiating capacity but, are considered to be tastier. Furthermore de Castro (2006) stated that short term energy intake was controlled on the basis of consumed food weight and volume rather than food energy content. Besides (Blatt *et al.*, 2012) revealed that reduction in both the energy content and energy density of pre-portioned entrées added together to decrease daily energy intake. Adding fruits and vegetables to decrease the energy density of entrées allowed the portion size to be maintained while energy content was reduced, which contributed to enhanced satiety.

Stubbs *et al.* (2000) concluded that energy density is a factor, which at high levels can facilitate excess energy intake, and at low levels constrains energy intake. However, the effects that dietary energy density may exert on appetite and energy intake should be considered in a context of other nutritional and non nutritional determinants of energy intake rather than as a substitute for those considerations (Stubbs *et al.*, 2000).

2.2.2. Portion size

People would be more susceptible to the influence of portion size. Studies show that substantial increase in food intake in response to larger portion size in both men and women was observed with a range of values for BMI (body mass index). Thus, the ready availability of foods in large portions is likely to be facilitating the overconsumption of energy in many people (Rolls *et al.*, 2002).

Researchers observed that serving size and opportunity to eat was a powerful determinant of children energy intake than the amount of food they previously consumed (Mrdjenovic & Levitsky, 2005). Fisher *et al.* (2003) stated that repeated exposure to a large portion size results in consistent increases in preschool-aged children's intake at a meal. The changes occurred without recognition of increasing portion size or compensatory decreases in the intake of other foods at the meal. A tendency of greater intakes was also observed for children who ate more when served large portions than other children without the opportunity for large portion size these suggests that children who show poor satiety responses may also have a greater behavioral susceptibility to this environmental cue.

Other studies demonstrated that chronic exposure to larger portion sizes can induce sustained increases in energy intake (Rolls *et al.*, 2007) and suggests that the effects of portion size may be powerful enough to affect rate of weight gain over time (Jeffery *et al.*, 2007). In addition, Fisher *et al.* (2007) revealed the effects of portion size on young children's energy intake, beyond individual meals, on daily energy intake. As a result doubling the portion size of several entrée and an afternoon snack served during a 24-h period increased children's total energy intake. Moreover findings showed that when both the energy density and the portion size of a food are increased, both factors act independently to affect energy intake. Thus, when increased simultaneously, the effects of energy density and portion size add together to affect energy intake (Kral *et al.*, 2004).

2.2.3. Dietary fiber

The role of dietary fiber in enhancing the perceived satiety and reducing the desire to eat was illustrated in Lyly *et al.* (2009) research when comparing a beverage containing different type of fiber with a beverage without fiber. In addition Inulin-type fructans consumption leads to an increase in proglucagon–GLP-1 (7-36) amide synthesis, with consequences of increasing the portal concentration of GLP-1 (7-36) amide and decreasing the peripheral concentration of ghrelin. It is thus able to modulate endogenous production of incretins and anorexigenic–orexigenic peptides through the ingestion of dietary fiber in rats (Cani *et al.*, 2004). Slavin and Green (2007) concluded that increasing dietary fiber intake promotes satiety, decrease hunger and helps provide a feeling of fullness. However Willis *et al.* (2010) argued that increasing the dose of mixed fiber did not influence satiety, gut hormone level or food intake in dose dependant manner.

Fiber diets have benefits on decreasing the incidence of obesity. The relevance of fiber in obesity development is centered on fiber's role in food intake control. Inclusion of fiber in the diet promotes satiation and prolongs satiety, aids in long-term compliance to low energy diets, and encourages healthy food choices and eating habits (Burton-Freeman, 2000). Furthermore studies indicated that increasing consumption of dietary fiber with fruits, vegetables, whole grains, and legumes across the life cycle is a critical step in stemming the epidemic of obesity found in developed countries. The addition of functional fiber to weight-loss diets are considered as a tool to improve the prevalence of obesity (Slavin, 2005).

Studies demonstrates that the potential of inulin and lupin-kernel fibre as effective fat replacers in the formulation of palatable foods with high satiating powers that may be of value in diets designed to reduce long term energy and fat intake (Archer *et al.*, 2004). In addition a review regarding the effect of fiber treatments on Visual analog score (VAS) satiety ratings and food or energy intake conducted by Clark & Slavin (2013) declared that 39% of fiber treatments had a benefit on VAS satiety while 22% of fiber treatments significantly reduced energy and food intake. However the authors reported that all fiber types did not affect appetite equally. B - Glucan, lupin kernel fiber, whole grain rye, rye bran, and a mixed fiber diet improved VAS ratings. Besides, fiber treatments that had a benefit on satiety response often had little impact on energy intake and vice versa. Moreover, inconsistent results were seen for fiber dose independent of fiber type, suggesting that fiber type may influence the optimal dose that is required to reduce appetite.

2.2.4. Macronutrients and energy intake

There is a hierarchy in the satiating efficiency of macronutrients such that per mega joule (MJ) of energy ingested, protein exerts a greater effect than carbohydrate that exerts a greater effect than fat. Alcohol appears enigmatic and there is evidence that in moderate doses it may actually stimulate energy intake (Stubbs *et al.*, 2000).

Investigation as regards to the effect of dietary protein on satiety show that, increase in energy from dietary protein at constant carbohydrate intake produces a sustained decrease in ad libitum caloric intake that may be mediated by increased central nerves system leptin sensitivity (Weigle *et al.*, 2005). Moreover Veldhorst *et al.* (2008) concluded that different proteins cause different nutrient related responses of (an) orexigenic hormones. GLP-1 release evoked by a high protein meal is stimulated by the carbohydrate content. Also PYY release is stimulated by a high protein meal. Ghrelin does not seem to be affected by a high protein meal or diet, and little information is available on CCK. Although an orexigenic hormones support satiety nutrient specifically, usually they are not mathematically related to satiety. Other research indicated that high protein breakfast led to greater increases in daily perceived fullness and greater reductions in corticolimbic activation compared with the non protein breakfast. Furthermore, only the high protein breakfast led to daily reductions in the hunger-stimulating hormone ghrelin, increases in the satiety hormone PYY, and reductions in evening snacking, particularly of high fat foods, compared with skipping breakfast (Leidy *et al.*, 2013).

Studies confirmed that the carbohydrate supplement suppressed ratings of hunger, desire to eat and prospective consumption as well increased ratings of fullness whereas the fat supplement failed to produce such effects. Furthermore the investigators deduced that the rapid digestion of carbohydrate would lead to a prompt rise in blood glucose concentrations and hence initiate the post absorptive phase of satiety. The slower physiological processing of fat would not promote such action. In addition other studies indicated that energy intakes after the normal and fat-supplemented breakfasts did not differ from each other, but intake after the carbohydrate supplement was significantly lower. This high intake of fat induces only a weak effect on satiety relative to the number of joules consumed (and compared with a smaller number of joules of carbohydrate). Therefore studies suggest that the appetite-control system may have only weak inhibitory mechanisms to prevent the passive overconsumption of dietary fat. The results indicate how this action could induce a positive energy balance and lead to a gradual upward drift in body mass index (Blundell *et al.*, 1993).

2.2.5 Other factors affecting appetite and food /energy intake

Evidence shows that direct breastfeeding during early infancy is related to greater appetite regulation later in childhood. A study made by DiSantis *et al.* (2011) showed that children who were fed in a bottle during the first three months of life were less likely to have high satiety responsiveness at the age of 3-6 years when compared to children who were breastfed.

In addition to the above factors which affect appetite and satiety; there are some food products which assumed to have appetite suppression effect and lowering energy intake. A research made by Roberts (2005) declared that herbal supplement containing black tea and caffeine increased metabolic rate as well promote weight loss in sixteen human subjects. The authors suggested that the possible mechanism for weight loss in addition to thermogenesis might be reduction in energy intake. In addition Yoshioka *et al.* (2001) stated that combination of caffeine & red pepper has the potential to considerably reduce energy intake.

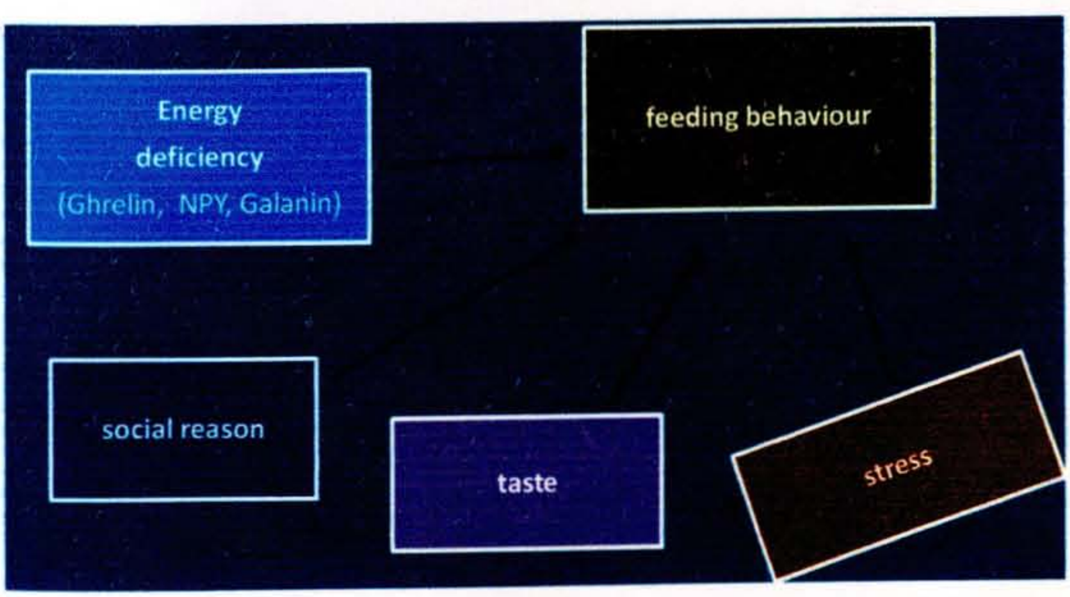

Figure 2.2, Factors affecting feeding behavior

In general appetite and energy intake is affected by different factors and therefore feeding occurs for various reasons, including energy deficiency following fasting or physical exercise, when palatable food is present, stress following isolation of pain and social reasons. Each of these reasons is probably mediated by different signal and normal mechanisms regulating food intake (Konturek *et al.*, 2004).

2.3. Black tea

Tea began as a medicine and grew into a beverage, in China, in the eighth century; it entered the realm of poetry as one of the polite amusements. The fifteenth century saw Japan ennoble it into a religion of aestheticism-teaism. Teaism is a cult founded on the adoration of the beautiful among the sordid facts of everyday existence. It inculcates purity and harmony, the mystery of mutual charity, the romanticism of the social order. It is essentially a worship of the Imperfect, as it is a tender attempt to accomplish something possible in this impossible thing we know as life (Okakura, 2008).

Tea is one of the most widely consumed beverages in the world; black tea represents approximately 72% of total consumed tea in the world, whereas green tea accounts for approximately 26% (Dwight Sato, 2007). Tea can be grouped into three types, i.e., green, black and oolong tea. Green tea is not fermented and is a major beverage consumed in Asian countries. Black tea generally refers to the fermented tea that is more popular in North America and Europe. Oolong tea is a partially fermented product whose production and consumption are confined to China. The major components of green tea leaves are catechins. In black tea, they are oxidized and dimerized during fermentation to the yellow-orange pigments, theaflavins, or polymerized to the red "pigments" called thearubigins. In contrast, oolong tea contains a mixture of catechins, theaflavins and thearubigins (Leung *et al.*, 2001).

There are several varieties of tea; however, all tea comes from the Camellia sinensis bush, a small flowering evergreen, native to China and India. The plant Camellia sinensis is an evergreen shrub of the Theaceae family. Tea is an extract of the dried leaves from Camellia Sinensis and it is usually prepared by infusing the leaves in hot water. The composition of the tea leaves depends on a variety of factors, including climate, season, horticultural practices, and the type and age of the plant. The primary polyphenols found in tea are called catechins, which account for 30% - 40% of dry tea weight (Cabrera *et al.*, 2006; Wang *et al.*, 2000). Catechins are strong antioxidants and thus are recognized as able to prevent the tissue damage caused by free radicals. This has led to the suggestion that they have beneficial actions regarding the prevention of various chronic diseases such as atherosclerosis and the suppression of glucose levels seen with diabetes. It is also suggested that tea has great potential to help reduce the incidence of major diseases worldwide, especially when combined with a healthy lifestyle (Weisburger, 1997).

There are four major catechins in tea: epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC) and epigallocatechingallate (EGCG), of which EGCG is the most abundant. The components of each type of tea are slightly different, due to the different production methods. Green tea comes from fresh steamed leaves, which contain more catechins, including EGCG, ECG and EC. Black and oolong tea contain more theaflavins and thearubigins (Vuong *et al.*, 2010). Tea infusions also contain variable amounts of potentially active food constituents, such as caffeine, theanine or theogallin (EFSA, 2008).

Capsaicin, caffeine and tea catechins have been effective in long-term weight management (Diepvens *et al.*, 2007; Westerterp-Plantenga, 2010). Anti- obesity effect of tea catechins in human was observed and authors suggested that the possible mechanism could be increase energy expenditure and decrease energy intake via inhibiting absorption (Hase *et al.*, 2001). Harada *et al.* (2005) finding confirmed that long term ingestion of tea catechins enhance dietary fat oxidation and diet induced thermogenesis. Combination of caffeine and green tea catechins intake was also associated with increased energy expenditure due to high thermogenesis and fat oxidation along with weight gain reduction (Lopez-Garcia *et al.*, 2006; Rudelle *et al.*, 2007; Westerterp-Plantenga *et al.*, 2005). In contrary other study stated that green tea extract does not have significant effect on weight reduction (Hsu *et al.*, 2008).

A research made by Roberts (2005) declared that herbal supplement containing black tea and caffeine increased metabolic rate as well promote weight loss in sixteen human subjects. The authors suggested that the possible mechanism for weight loss in addition to thermogenesis might be reduction in energy intake. Even though the effect of each bioactive ingredient on appetite and energy intake was not clearly stated the combination of Caffeine and green tea catechins as well caffeine and capsaicin reduce appetite and energy intake in human (Carter & Drewnowski, 2012; Reinbach *et al.*, 2009).

In addition Yoshioka *et al.* (2001) stated that combination of caffeine & red pepper has the potential to considerably reduce energy intake. In this study also the effect of caffeine or red pepper on energy intake was not separately studied. Belza *et al.* (2007) examined the effect of caffeine on thermogenesis and energy intake in twelve human subject and discovered that it increase thermogenesis but the reduce energy intake is not significant and the authors declared that the small sample size might adversely affect the outcome regard to energy intake. A review made by Westerterp-Plantenga *et al.* (2006) suggested that tea, caffeine & spices have the potential to produce significant effects on metabolic targets such as satiety, thermogenesis, and fat oxidation, when they are studied in a standardized laboratory context.

2.4: Red pepper

Red pepper is a fruit of the plants 'Capsicum annuum' and 'Capsicum frutecens' that come from the genus 'Capsicum,' belonging to the family of 'Solanaceae' family (Rajendra, 2009). *Berbere* which is a combination of spices and red pepper is one of the favorite spices in Ethiopia and is commonly used for flavoring, seasoning and imparting aroma or coloring of many traditional foods. The hotter the red pepper, the more capsaicin it contains (Rajendra, 2009).

Red pepper or chilli peppers have a group of bioactive unique molecules called capsaicinoids. They are responsible for the fruit's pungent sensation and display potentially valuable pharmacological properties (Thiele *et al.*, 2008). This sensation occurs as capsaicin binds to the same group of nociceptors which also leads to the sensation of pain from heat and acid (Sanatombi & Sharma, 2008). Even though there are more than 10 chemical structures of capsaicinoids, the most prominent forms are capsaicin and dihyrocapsaicin, accounting for almost 90% of capsaicinoids (Meghvansi *et al.*, 2010).

The health benefit of red pepper and its components is being examined in different countries. The possible effect on weight management through its effect on appetite, satiety and diet-induced thermogenesis has got a great attention.

An investigation made on Japanese women to assess the effects of red pepper added to high fat and high carbohydrate meals on postprandial energy and substrate metabolism indicate that red pepper increases diet-induced thermogenesis and lipid oxidation in addition increase in lipid oxidation is mainly observed when foods have a high fat content (Yoshioka *et al.*, 1998). Furthermore Whiting *et al.* (2012) concluded that capsaicinoids and capsinoids can play an active role in increasing energy expenditure. These compounds may help to promote lipid oxidation but the effects on appetite are less clear. And the authors suggested that Capsaicinoids and capsinoids may have a role to play in weight management problems. Reinbach *et al.* (2009) conclude that thermogenic food ingredients have energy intake reducing effects when used in combinations, and in positive energy balance. Energy balance did not affect possible treatment induced energy intake, but did affect appetite, thereby supporting negative energy balance. These results suggest that bioactive ingredients (capsaicin, green tea, CH-19 sweet pepper; a non pungent cultivar of red pepper) may be helpful in reducing energy intake to prevent body weight gain and may support body weight loss by relatively sustaining satiety and suppressing hunger.

In other study,Yoshioka *et al.* (2001) demonstrated that the combination of caffeine and red pepper has the potential to considerably reduce energy intake when this condition is compared with a control day without these compounds. The authors suggest that these effects are mediated by an increase in the Sympathetic nerves system (SNS): Parasympathetic nerves system (PNS) activity ratio as measured by power spectral analysis of heart rate (HR). In addition in the short term, both oral and gastrointestinal exposure to capsaicin increased satiety and reduced energy and fat intake; the stronger reduction with oral exposure suggests a sensory effect of capsaicin (Westerterp-Plantenga *et al.*, 2004). Soups containing red pepper induced a dose-dependent decrease in subsequent energy intake, and the dose responsible for the maximal tolerable feeling of spiciness reduced fat intake in Japanese males. It is suggested that the effect of red pepper on food intake behavior occurs after passing through the mouth and involves activation of the SNS (Yoshioka *et al.*, 2004).

A study indicates that the capsaicin supplementation of one meal in a postprandial state has no effects on substrate utilization, appetite feelings and PYY responses. However, the observed effects of capsaicin on GLP-1 and ghrelin responses warrant more intensive research on the possible eating behavior-related effects of capsaicin (Smeets & Westerterp-Plantenga, 2009). Capsaicin has been shown to be effective on body-weight management, yet when it is used clinically; it requires a strong compliance to a certain dosage, which has not been shown to be feasible yet (Diepvens *et al.*, 2007). However, study show that capsaicin and its pungent principles have significant effect on energy intake and energy expenditure, for instance around 23% increase on energy expenditure was observed after meal (Kovacs & Mela, 2006).

Chapter 3- *Materials and Methods*

3. Materials and Methods

3.1. Study protocol

3.1.1. Subjects

Human subjects were used to evaluate the role of black tea and *berbere*-spiced food consumption on the appetite and energy intake of preschool children. Study eligibility were: age of the child (1-5 years), child without any serious medical condition or food allergies, child with anthropometric values > -2 SD of the WHO multi-growth reference, children residing under similar environment (i.e. in the same orphanage) as well routine consumers of black tea and *berbere*- spiced foods.

A total of twenty six children, twelve boys and fourteen girls, were available for this study and the age of the children were between two and five years (24-59 months). Out of these children, four were under-weight (WAZ<-2), among which two were wasted, (WHZ<-2), and one was stunted (HAZ<-3) and thus were excluded (n= 5) from the analyses. This resulted in a total sample size of twenty one children, twelve girls and nine boys. This sample size is often considered adequate for within-subject, cross-over design and the study was in line with other similar type of studies (Brennan *et al.*, 2012; Leidy *et al.*, 2013).

3.1.2. Location

The study site was an orphanage in Addis Ababa. The name and details of the orphanage is not given for privacy reasons. The orphanage is a surrounded compound under which the children are living in similar condition depending on their age group and health status.

3.1.3. Experimental design

The thesis is constituted of two studies that evaluated the effect of black tea and *berbere*-spiced food consumption on the appetite and energy intake of preschool children. In both studies, with-in subject crossover design was used.

Study 1: *Treatment*: Bread with tea in an opaque plastic cup.

Control: Bread with very light tea infusion with no detectable caffeine/catechin served in an opaque plastic cup.

Very light tea was prepared by adding a cup of boiled black tea which is 150ml in a kittle of 2.5L which had hot water with sugar. The basement of the kittle was radish and when cup of tea added it had the color of tea. Since the plastic cup was opaque it was difficult to differentiate the tea and very light tea.

Study 2: *Treatment*: Pasta/ rice with a sauce prepared with *berbere*.

Control: Macaroni/ pasta with a sauce prepared without *berbere*

The control test meal contains the entire ingredient used during the preparation of treatment test meal in the similar amount except berbere, however as a colorant turmeric root (Curcuma Longa) was used. 3 g of turmeric root was added during the preparation of 4kg of pasta or macaroni.

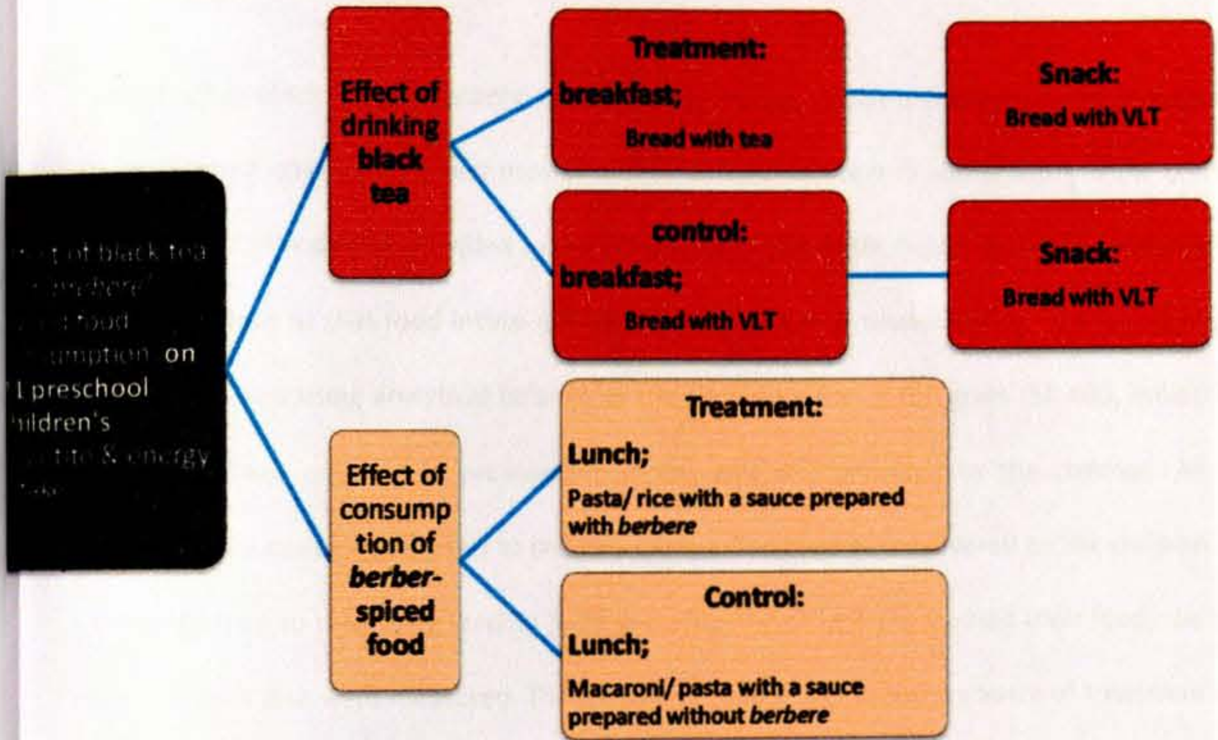

Figure 3.1, Schematic representation of the experimental design of the studies

3.1.4. Food Sample collection

Samples of the test meals were collected before it was provided to the children. Two separate samples were collected for moisture and biochemical analyses using air tight polyethylene bags and were placed in an ice box. The samples were transported to Addis Ababa University, Center for Food Science and Nutrition. Moisture content was immediately determined. Samples were stored at -20^{0} C until further biochemical analysis.

3.1.5. Food intake assessment procedure

The effect of black tea and *berbere*-spiced food on energy intake and appetite was investigated by evaluating intake of the test meal and the subsequent meal in comparison to the control. The amount of test meal provided to the children was 50%-100% higher than the normal intake of the children so that food intake will not be limited due to unavailability. The food samples were measured using analytical balance to the decimal place of 0.1 gram (SF-400, India). The measured food was put in pre-numbered dish and was provided to the children. All the necessary measures were taken to prevent mix-up. The food was delivered to the children in a similar fashion to their daily feeding habit and after the children consumed their food, the left-overs in each dish were measured. The amount of food intake in the presence of treatment was compared with the control and the difference was used to evaluate the effect of the consumption of habitual concentrations of black tea or *berbere*- spiced food on energy intake and appetite.

3.2. Anthropometry

All anthropometric measurements were made by the same person to avoid inter-examiner errors. The height and weight of the children were measured twice using standardized techniques with the children wearing light clothing and no shoes. Z-scores for height-for-age (HAZ), weight-for-age (WAZ) and weight-for-height (WHZ) were calculated using the WHO multicentre growth reference (WHO, 2006) data using the software ENA 2007. None of the children had unacceptably extreme anthropometric values (De Onis *et al.*, 1997; WHO, 2006).

Underweight and wasting were defined respectively as WAZ or WHZ,−2 and Stunting were defined as HAZ,-3.

3.3. Biochemical Analysis

The collected food samples were freeze dried and homogenized prior to biochemical analyses.

3.3.1. Moisture content

The moisture content was analyzed by following AOAC (1995), method No 923-09 (DHG-9055A, China).The food samples were placed in a container and the weight of the sample was determined before and after drying at 105^0C to constant mass. The moisture content of the sample was calculated as follow.

$$Moisture\ (\%) = \frac{Mi-Md}{Mi} \times 100 \quad \text{.............. Equation (1)}$$

Where, Mi = Weigh before drying

Md =Weigh after drying

3.3.2. Crude protein analysis

The solid food sample was homogenized before analysis. Protein was determined by the Kjeldahl method, AOAC (1995) No 925-09 (K9840, china). In this method, proteins and other organic food components in a sample was digested with sulfuric acid in the presence of catalysts. After this, the total organic nitrogen was converted to ammonium sulfate. The digest then neutralized with alkali and distilled into a boric acid solution. The borate anions formed was titrated with standardized Sulfuric acid (H_2SO_4), which is converted to nitrogen in the sample. The crude protein was determined by multiplying the Nitrogen (N) content by 6.25 (Chang, 2010).

Addition of reagent: The sample was weighed in a tecator tube and placed in the tecator rack. After this 6ml of acid mixture will be added by using bird or oxford pipette carefully and mixed with the sample immediately. Then 3.5 ml of hydrogen peroxide was added step by step and watched out for violent reactions in fume hood. As soon as the most violent reaction has ceased, then the tube was shaken by hand and put it back into the rack. Then after 3gm of catalyst (a mixture of Potassium Sulphate (K_2SO_4) and selenium in a ration of 100 to 0.5) was added and stand for 5 – 15 min before digestion.

Digestion: The tube in the rack was put into the digester in the fume hood at 370^0C. The exhaust manifold will be located on top of tubes and the digestion was continued until clear solution appeared (3hrs) in the fume hood.

Distillation and titration: The running water was checked for condensation before starting the analysis, and then the analyzer was checked with distilled and de-ionized water until it stabilizes to ≤ .05%. The blank tube containing only the reagents was put and its value recorded in the space provided, which is used for subtraction from the sample with reagents.

Titration: The distillated solution was titrated with 0.1N Hydrochloric acid to a reddish color.

$$Total\ Nitrogen\ (\%) = {[(V - Vb) \times N \times 14]}/_{w}$$

$$Crude\ Protein\ (\%) = Total\ Nitrogen\ (\%) \times 6.25$$ .... Equation (2)

Where, V = volume of sulfuric acid consumed to neutralized the sample

V_b= the volume of acid consumed to neutralize the blank

N = normality of the acid

14=Eq. wt of nitrogen

6.**25** = conversion factor from total nitrogen to crude protein

3.3.3. Crude fat analysis

Crude fat content was determined by using soxhlet method, AACC (2000)-No 30-10 (Huaye, China). The solvent builds up in the extraction chamber for 5 – 10min and completely surrounds the sample and then siphons back to the boiling flask. Fat content is measured by weight loss of the sample or by weight of the fat removed.

Two gram sample was weighed into an extraction thimble and covered with absorbent cotton. A 50 ml solvent (petroleum ether) will be added to a pre-weighed cup. Both thimble and cup will be attached to the extraction unit. The sample was subjected to the extraction with solvent for 30 min followed by rinsing for 1.5 h. The solvent will then be evaporated from the cup to the condensing column. Extracted fat in the cup will be placed in an oven at 110°C for an hour to evaporate the residual solvent and the cup with the crude fat allow to cool and then the crude fat was calculated using following formula:

$$Crude\ fat, persent\ by\ weight = \frac{W2-W1}{W} \times 100$$ .... Equation (3)

Where, W1 = Weight of the extraction cup (g)

W2 = Weight of the extraction cup plus the crude fat (g)

W = Weight of sample food (g)

3.3.4. Crude fiber analysis

Crude fiber content was analyzed by following AACC (2000) No 32-10 (Foss Fibertec 2010, Sweden). One gram of sample was transferred to 600 ml beaker. The sample was digested with 1.25% sulfuric acid and it was washed with distilled water. And then it was digested by 1.25% sodium hydroxide. The digested sample was filtered in coarse porosity 75-76 µm crucible in apparatus at a vacuum of about 25 mm. The residue left after reflux was washed away with 1.25% sulfuric acid near boiling point. The residue was dried at 110^{0}C for one hour, and was allowed to cool in desiccators. Then the weight was determined, that was (M1). Then after the sample was turn in to ash at 550C, it allowed cool in desiccators and the weight was be determined that was (M2). The total crude fiber was calculated as follows.

$$Total\ crude\ fiber(\%) = \frac{M1-M2}{M3} \times 100$$ .... Equation (4)

Where, M1 is the dried sample

M2 is the dried ashes and weighted sample and

M3 is the weight of sample

3.3.5. Ash content

Ash content of samples was determined according to AOAC (1995) method No. 923-09 (CSF 1200, England). Clean porcelain dish was ignited at 550 ^{0}C for 3 hrs and will cooled in desiccators and weighed as (M1). Then about 3g of food sample was put in to the porcelain dish and weighed as (M2). This sample was dried at 120 ^{0}C for 1h and was carbonized on hot plate. The dish with its contents was transferred to a muffle furnace and ignited at about 550 ^{0}C until ashing completed. The residue was weighed as (M3). The total ash was expressed as percentages on dry matter basis as follows:

$$Total\ ash(\%) = \frac{M3-M1}{M2-M1} \times 100$$ .... Equation (5)

Where, M2 –M1 is sample mass in gm on dry base

M3 –M1 is mass of ash in gm

3.3.6 Carbohydrate content

Carbohydrate content was determined by difference.

% C = 100 – [%M + % P + %F +% Fib + % A] .... Equation (6)

Where, %C = Percent Carbohydrate content

%M = Percent Moisture content

% P= Percent Protein content

% F = Percent Fat content

% Fib = Percent Fiber content

% A = Percent Ash content.

3.3.7. Energy content

Energy content of the test meals were calculated using fat, carbohydrate and protein contents of the meals that accounts for 17 kJ or 4 Kcal per gram for protein and available carbohydrate, and 37 kJ or 9 Kcal per gram for fat, according to

$$\left[\%\, carbohydrate \times {gram\ of sample\ food}/_{100}\right] \times 4kcal \cdots EC$$

$$\left[\%\, Protein \times {gram\ of sample\ food}/_{100}\right] \times 4kcal \cdots EP$$

$$\left[\%\, fat \times {gram\ of sample\ food}/_{100}\right] \times 9\ kcal \cdots EF$$

$$\boldsymbol{Energy\ content\ of\ Test\ meal} = \boldsymbol{EC + EP + EF}$$ .... Equation (7)

Where, EC =Energy from carbohydrate

EP =Energy from Protein

EF =Energy from Fat

3.3.8. Catechin and Caffeine analysis

Catechins and caffeine content of black tea can be analyzed by high pressure liquid chromatography (Shemadzu, Japan). The analytical conditions were as follows; the column will be shim-pack FC-ODS which has a thickness of (150mml × 4.6mml.D.). There was two mobile phase, mobile phase A was Sodium Phosphate buffer solution at a pH of 2.6 and mobile phase B was Acetonitrile gradient elution method. The flow rate will be 2ml/min and column temperature was adjusted to 40°c. The gradient program was adjusted as shown in the proceeding table.

Table 3.1, Gradient program of caffeine and catechin analysis using HPLC

Time in minute	B.Conc (%)
0	7
6	7
20	20
20.01	50
25	50
25.01	7
35	STOP

Detection was performed by UV –VIS spectroscopy at 270nm using 10 µL volume of injection.

3.4. Detailed flow diagram of berbere flour preparation

Berbere can be prepared differently and different spices are added during the process of preparation. The *berbere* consumed by the children obtained from local market. According to information obtained from the orphanage, there was a single supplier for *berbere* and other condiments for the last two years. Therefore data regarding the process of preparation as well the type and amount of ingredients added was obtained from the supplier.

The spiced added to prepare *berbere* which was consumed by the children; was red pepper, onion, garlic, basil (Ocinmum basilicum), false cardamom (Aframomum Korarima), Ethipian Caraway (Trachyspermum ammi), black seed and salt. The proportion of the red pepper with the spices was less standardized. Around one kilogram of onion, garlic, false cardamom and salt; and around 500g of basil, Ethiopian Caraway and black seed were added on seventeen kilo gram of red pepper. The process of preparation was shown in the following diagram.

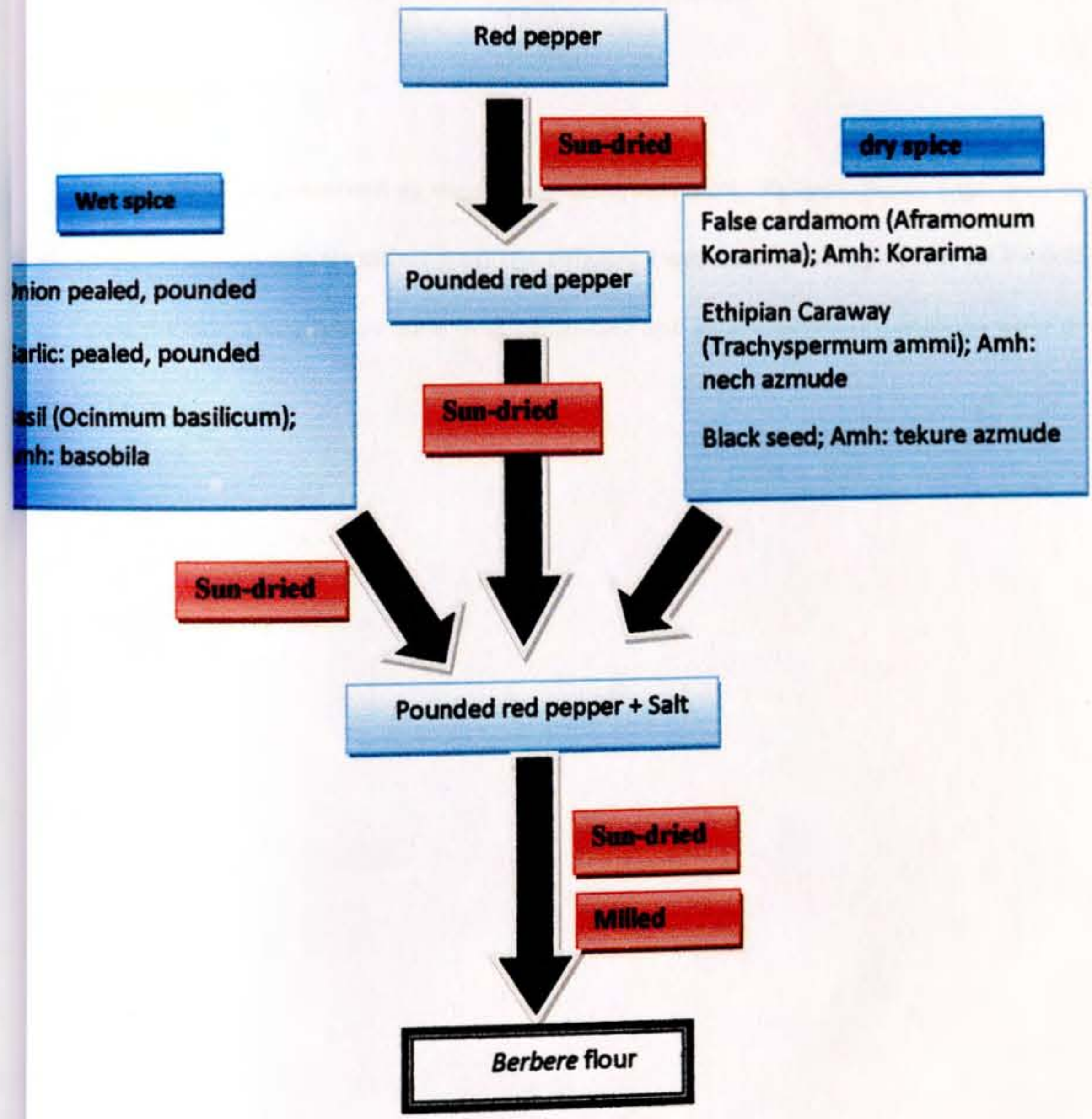

Figure3.2, detailed flow diagram of *berbere* flour preparation

3.4. Ethical approval

Ethical approval was obtained from the Human Ethics Committees of Addis Ababa University. The purpose and methods of the study was explained in detail to the administrator of the orphanage and written informed consent was also obtained.

3.5. Data analysis

All results were presented as mean ± standard deviation. Paired-samples t-test was used to compare within-subject differences. The difference was considered significant for $P < 0.05$. The experimental analysis was done in quadruplicate and all the laboratory analyses were done in triplicate.

Chapter 4 – *Results and Discussion*

4. Result and discussion

4.1: Anthropometric data

The mean age of study participants was 40 months and ranged from 24-54 months. The average weight and height of the children was 14 Kg and 93 cm, respectively (**Table 4.1**).

Table 4.1, the age (month), weight (Kg) and Height (cm) of the study participant

Age	Weight	Height
40±9.8	14.±1.8	93±6.7

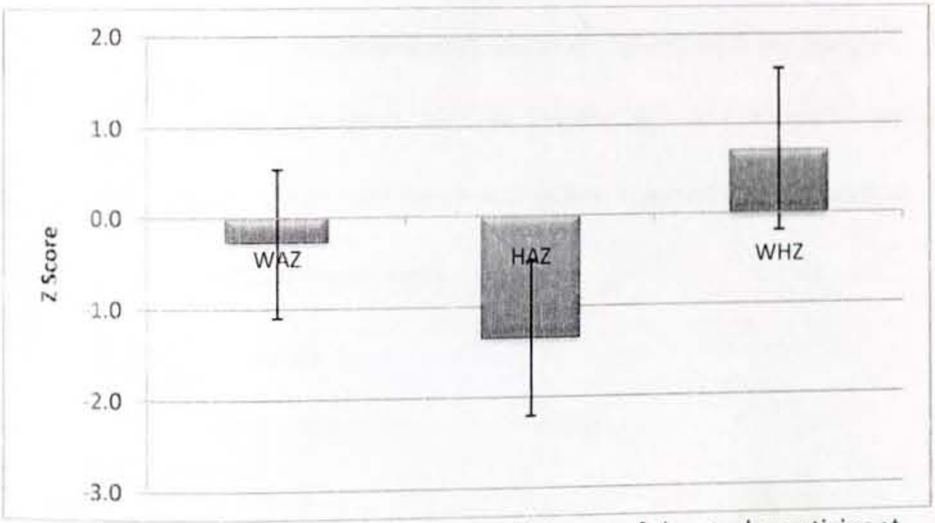

Figure 4.1, Nutritional Status of the study participant

The nutritional status of the children in the study was similar to previous findings (EDHS, 2011). More than half of the study participants were at the borderline of Z score for stunting, underweight, and wasting, suggesting that there is a high probability for these children to become malnourished in the near future. In addition, five of the study participants were excluded from the study due to malnutrition.

In this circumstance, determining the habitual intake of the children will be beneficial to understand the cause of poor nutritional status.

4. 2: Portion estimation of meals

The children's habitual consumption was investigated through meal observation and weighing of foods provided at meal time. The habitual food intake from major meals including breakfast, snack, and lunch was determined. The mean value of habitually intake and the standard deviation are presented in **Figure 4.2.**

On the first day, the children consumed bread with tea for breakfast, *chechebesa* (home prepared bread which was mixed with *berbere* and oil) with tea for snack, and Macaroni with *berbere*-spiced sauce for lunch. On the second day, the children had bread with tea for breakfast and snack while their lunch was *injera, a fermented pancake,* with lentil-based stew without *berbere* (*alecha meser wet*).

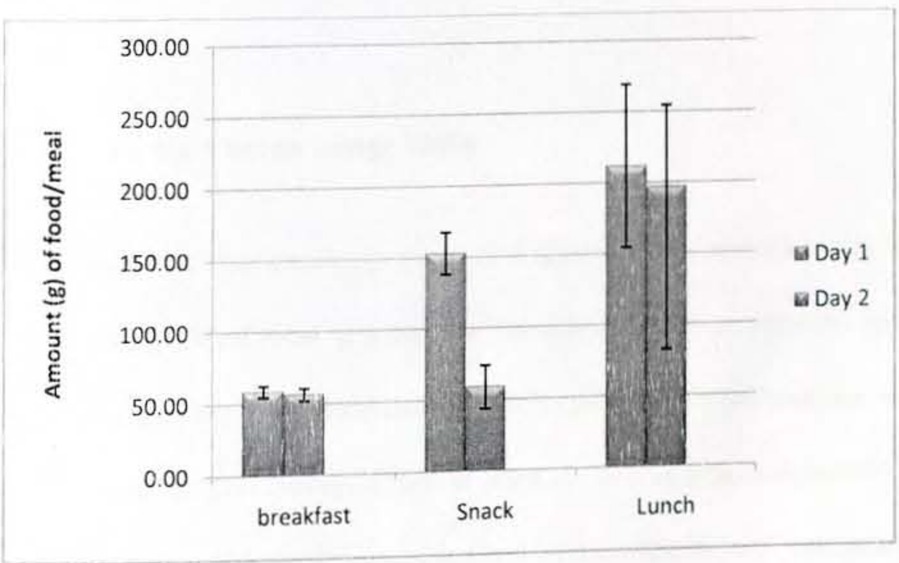

Figure 4.2, Two days habitual consumption of breakfast, snack and lunch in gram.

The day-to-day variation in meal size was found to be minimal except for the snack. The snack provided on day one and day two was not the same food furthermore the meal size provided on the first day was almost three times that of the second day. This suggests that the observed variations may be due to differences in food preference and/or portion size. Indeed, previous studies in children have shown that increasing meal portion size and palatability increases food and energy intake (Corbit & Stellar, 1964; Rolls *et al.*, 2002).

The children in this study consumed significantly lower amount of food per meal which is similar to other Ethiopian children for whom consumption was lower than their age appropriate meal size (Baye *et al.*, 2013; Gibson *et al.*, 2009). Several factors that can hamper food intake in these children can be identified. This include consumption of components of tea (i.e. caffeine) (Hase *et al.*, 2001) and red pepper (Yoshioka *et al.*, 2004). Given that these children habitually consume tea and red-pepper spiced (*berbere*-spiced) foods, to what extent this affects their food intake is of interest.

4.3: Effect of drinking black tea on energy intake

The effect of drinking black tea on energy intake and appetite was investigated by observation and weighting of the breakfast meal to determine the role black tea on satiation and satiety by observing the intake of subsequent meal (snack) which was provided without tea. White bread was used as a test meal and on average 150ml of black tea or a very light tea where its caffeine and catechin content were almost zero was used as a control. The data of food intake in grams in the presence and absence of black tea is presented as follows **(Table 4.2)**.

Table 4.2, Mean test meal intake (g) and energy intake (kcal) in the presence and absence of black tea

	Test meal (Breakfast)		Test meal (Snack)	
	Mean	Standard deviation	Mean	Standard deviation
Food Intake (g)				
Control	75	22	67	18
Tea	83	25	73	17
Energy intake(Kcal)				
Control	192.5	57	172.5	46.1
Tea	213.4	64.6	187.3	43.7

The data are expressed as mean ± standard deviations.

There is no significant difference observed in energy intake. This is in contrast to previous findings in rats where Keemun black tea extract significantly reduced food intake (Du *et al.*, 2005). Likewise studies show that caffeine and catechin have a significant negative effect on energy intake and appetite (Roberts 2005; Hase *et al.*, 2001). Moreover Roberts (2005) suggested that the possible mechanism for weight loss from consumption of a herbal supplement containing black tea and caffeine by human subject in addition to thermogenesis might be reduction in energy intake. However in the present study only black tea was used to assess its effect on appetite and energy intake and the presence of additional caffeine might promote its effect on reduction of energy intake as observed in the previous studies.

There was a difference in caffeine and other tea bioactive ingredient content in black tea among varieties and processing method of tea leaves (Astill *et al.*, 2001). Indeed,Henning *et al.*, (2003) showed that the caffeine content in tea can vary from 25.3 ± 0.2 to 55.1 ± 0.1 mg/ 100ml as well catechin from 2.7 ± 0.3 to 16.2 ± 0.1 mg/ 100ml of tea by analyzing 9 varieties of black tea. Whether higher caffeine containing black tea or the ones used in this study leads to same findings needs further investigation.

Furthermore, the bioactive ingredient which is found in tea is not only caffeine and catechine but there are other ingredients like theophylline. On top of this, studies show that theophylline attenuates 5-HT-2C receptor dependent anorexia and anxiety. This suggests that tea may not produce adverse effect on appetite since it contains both caffeine and theophylline (Alam *et al.*, 2011).

Even though the energy intake of the children was below the age appropriate energy intake, according to the present finding the habitual consumption of black tea could not be the factor for lowering food and energy intake of the children. Therefore, the other food component which is routinely found in the children's menu containing a bioactive ingredient with the potential of reducing food intake was red pepper.

4.4: Effect of *berbere*-spiced food consumption on energy intake

The effect of *berbere*-spiced food on satiation was studied using low-fiber foods: pasta, macaroni and rice, with a sauce made with (treatment) or without (control) *berbere*. The study was planned in line with the habitual menu so the study was performed whenever the menu contained one of the above foods. The data of food intake in gram; and energy intake in Kcal with *berbere* spiced sauce and without are presented as follows (**Table 4.3**).

Table 4.3, Effect of *berbere* on food intake (g) and energy intake (Kcal)

	Test meal (lunch)	
	Mean	Standard deviation
Food Intake(g)		
Control	193	61
berbere	201	58
Energy intake (Kcal)		
Control	267.1	102.4
berbere	304.3	99.8

There is no significant difference observed in food/ energy intake (P> 0.05). This result was in line with (Smeets & Westerterp-Plantenga, 2009) finding on adults as the authors concluded lunch containing capsaicin had no effect on satiety, energy expenditure, and PYY, but increased GLP-1 and tended to decrease ghrelin; however other studies revealed that the addition of red pepper to a meal decreased the desire to eat as well protein and fat intakes at the next meal(Yoshioka *et al.*, 1999). Nevertheless the *berbere* in this study contain red pepper onion, garlic, basil (Ocinmum basilicum), false cardamom (Aframomum Korarima), Ethipian Caraway (Trachyspermum ammi), black seed and salt with the presence of this entire bioactive ingredient in *berbere* the expected effect of red pepper on energy intake might be limited by the other bioactive ingredients. Studies indicated that red pepper lowered subsequent energy intake in a dose-dependent manner (Yoshioka *et al.*, 2004). Moreover, the maximal tolerable dose of red pepper in the soup suppressed fat intake compared with the placebo (Yoshioka *et al.*, 2004) . The habitual dose of *berbere* given to the children as in this study is much lower than the previous studies which is done on adult, therefore might have underestimated its effect on energy intake.

Even though a review made by Westerterp-Plantenga *et al.*,(2006) suggested that tea, caffeine & spices have the potential to produce significant effects on metabolic targets such as satiety, two of the habitual consumed food group that is drinking black tea and consumption of *berbere* spiced foods had insignificant role in reducing the appetite and energy intake of preschool children in this study. Since the children are habitually provided with portion sizes is much lower than their age appropriate meal portion size (Baye *et al.*, 2013; Gibson *et al.*, 2009), this could result in lower food and energy intake.

4.5. Effects of increasing portion size on energy intake

The role of increasing portion size on energy intake and appetite was investigated by providing additional 100% of the habitual intake of breakfast. The data of food intake in gram and energy intake in Kcal is presented in **(Table 4.4.)**

Table 4.4: Mean food intake in gram and energy intake in Calorie of under five children during the habitual portion and larger portion size of breakfast

	Break fast	
	Mean	Standard deviation
Food Intake (g)		
habitual portion	57	2
Larger portion size	78*	19
Energy intake (Kcal)		
habitual portion	146	5
Larger portion size	201*	49

* Significant difference in mean between habitual and larger portion size, P <.001

Serving a larger portion size relative to a habitual one had a significant effect on food and energy intake and this was observed (P< 0.001). This is in line with previous reports that showed that amount of food that is presented during a single meal directly affects energy intake (Rolls *et al.*, 2002).

In addition large portion size promoted high daily intake in Hispanic and African American children (Fisher *et al.*, 2007). Even though the finding of the present study is in line with other studies; Fisher *et al.* (2003) stated that large entrée portions may constitute an "obesigenic" environmental which can influence preschool-aged children to have excessive intake at meals. In the present investigation, the children from age 2 to 5 years were served similar kind of portion size and the provided as well as the larger portion size was probably lower than the age appropriate meal portion size. Since the children have a high probability for being malnourished providing a larger portion size could increase their food and energy intake.

4.6. Implication of increasing portion size to other nutrients

Increasing portion size of the test meals not only increased the energy intake of the children, but also provided additional nutrients to the children. **Table 4.5** shows the difference in nutrient intake from breakfast when the habitual or the larger portion size was served.

Table 4.5 The difference of nutrient content of the food when the habitual portion size and larger portion size served

	Mean food intake (g)	Protein content (g)	Carbohydrate content(g)	Fat content (g)	Energy content (Kcal)
Habitual portion size	57	5.2	30.5	0.4	146
Larger portion size	78*	7.2*	41.7*	0.6*	201*

*Significant difference in mean between habitual and larger portion size in nutrient content, P <.001

increasing the portion size, thus also improves the macronutrient intake of the children significantly at p value < 0.001. Serving larger portion size meal could thus be one strategy to increase nutrients intake. Furthermore a study show that increasing the intake of micronutrient like iron improves children appetite therefore increasing portion size might have additional value of increasing the appetite of children at the same time as the micronutrient intake increases (Stoltzfus *et al.*, 2004).

4.7. Biochemical analysis of test meals and tea

4.7.1. Proximate composition of test meal

The proximate composition of the test meals (i.e. bread, rice and pasta; which is prepared with *berbere* sauce, macaroni and pasta; which is prepared without *berbere* sauce) was determined and it is presented in (**Table 4.6**)

Table 4.6, Proximate composition of test meals

Food type/ Test meal	Moisture *g/100g*	Ash *g/100g*	Protein *g/100g*	Fat *g/100g*	Fiber *g/100g*	Carbohydrate *g/100g*
Bread	31.4± 0.9	0.7 ± 0.1	9.2 ± 1.2	1.8 ± 0.0	3.4 ± 0.4	53.7 ± 2.4
Rice with *berbere*	61.8 ±0.7	11.3±1.1	5.5 ± 0.1	9.8 ± 0.8	0.9 ± 0.1	10.6 ± 1.7
Pasta with *berbere*	63.3 ± 2.2	7.5 ± 0.2	8.4 ± 0.8	7.3 ± 0.8	1.4± 0.8	12.1 ± 0.8
Macaroni without *berbere*	63.5 ± 2.6	8.3 ± 0.1	8.2 ± 0.2	8.0 ± 1.8	1.3 ± 1.3	10.6 ± 2.9
Pasta without *berbere*	61.4 ± 0.4	9.7 ± 0.4	8.3 ± 0.2	6.3 ±0 .8	1.4 ± 0.3	13 ± 0.6

The nutritional compositions of the test meals were not dense in energy and were not also rich in macronutrient; however, this data could not represent the day to day intake of the children. The study was done in line with the orphanage's food menu; yet it was done whenever there was low protein and fiber containing foods. Different investigation showed that presence of high protein and fiber in the meal could affect food and energy intake of individuals (Slavin and Green 2007; Weigle *et al.*, 2005).

The moisture content of the test meals was high. Since these meals were prepared for preschool children it should be easily chewable in order to attain this, the prepared meals will not be allowed to absorb all the water which was added during the preparation process.

4.7.1. Analysis of tea

The caffeine and catechin content of black tea was analyzed using HPLC and the mean concentration of caffeine and catechin in the cup of tea which contains 150ml of black tea was determined. The calibration curve of caffeine and catechin Standard was presented in **Figure 4.3** & 4.4 respectively.

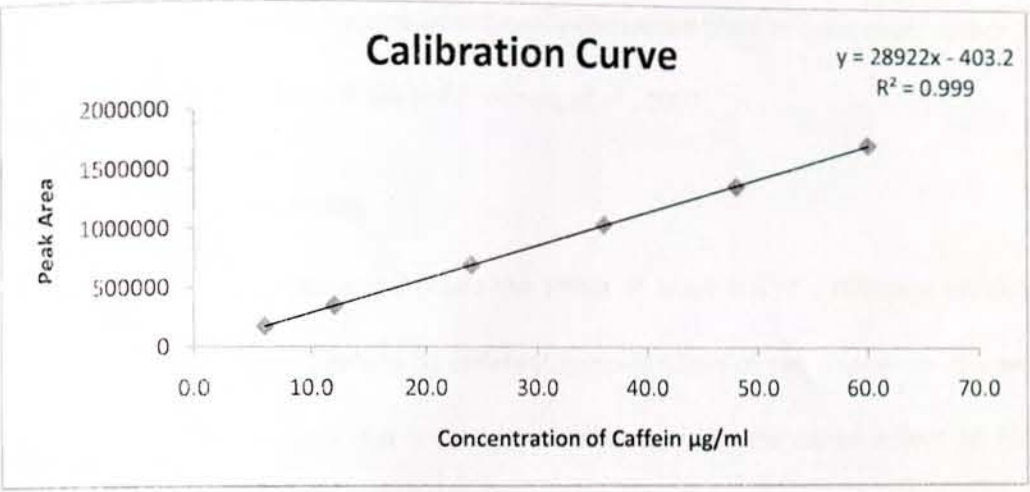

Figure 4.3, Calibration curve of caffeine standard.

According to the response of the sample concentration of caffeine in ml of black tea was range from 34.0 μg/ml to 52.6μmg/ml. Therefore the concentration of caffeine consumed by the children was 7mg/150ml of tea.

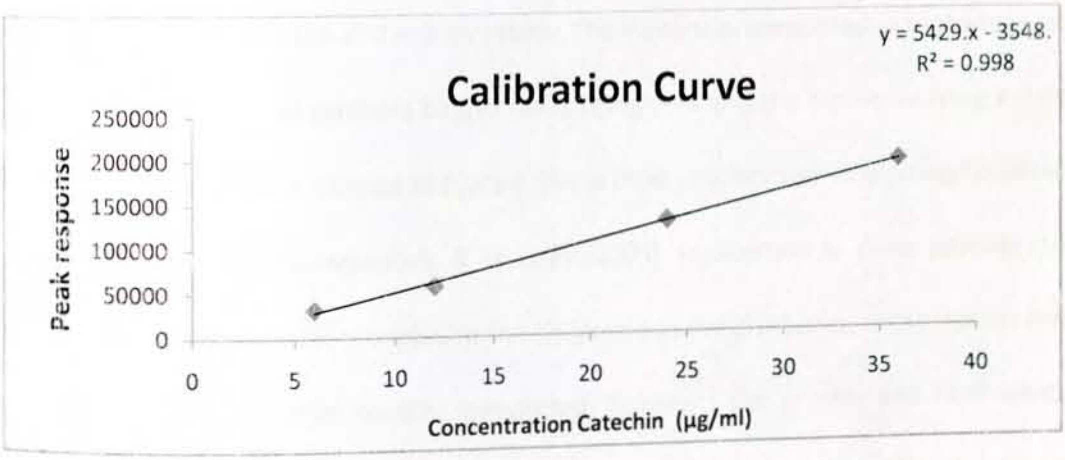

Figure 4.4, Calibration curve of catechin standard.

According to the response the concentration of catechin in black tea ranges from 0.9 μg/ ml to 3.38 μg/ ml. Therefore the concentration of catechin in black tea consumed by the children was 0.4 mg / 150 ml of black tea.

The caffeine and catechin content of habitually consumed black tea was much lower than other type of black tea like *Lipton* black tea (Henning *et al.*, 2003).

4.8. Limitation of the study

Even though it is an experiential study the effect of black tea at a different concentration of caffeine and catechin or *berbere* at different concentration of red pepper on the energy/food intake of the children was not investigated to determine the actual effect of black tea or *berbere*. In addition the proportion of composition of the spices in the *berbere* was defined in less standardized way.

In spite of these limitations in this study, within subject crossover study design was used to minimize error and to prevent the effect of physiological factors which may have positive or negative effect on appetite and energy intake. The study was conducted in orphanage where all the children are treated similarly by the same caregivers and the former feeding environment was maintained. Indeed, studies indicated that a child environment is a powerful determinant of their energy intake (Mrdjenovic & Levitsky, 2005). In addition to observational studies of food intake, the test meals provided to the children was weighed prior consumption and all the leftover also weighed after feeding completed. Nutrients like protein and fiber which affect food and energy intake was minimized in line with the orphanage meal menu. For instance foods containing meat which may contain high protein and *Injera* (fermented pan cake) which may have a higher concentration of fiber were not used as a test meal. Studies declared that high protein and high fiber in the meal affect food and energy intake (Slavin and Green, 2007). Furthermore the nutritional status of the children was determined and children with Z score for underweight and wasting >-2 and for stunting >-3 were participated in the experiment. In this study the role of habitually consumed concentration of black tea drinking, consumption of *berbere* and effect of portion size on food and energy intake was investigated.

Chapter 5- *Conclusion and recommendations*

5. Conclusion and recommendations

The habitual concentration of black tea and *berbere*-spiced food consumption does not have a significant role on appetite and energy intake of the preschool children at the 7.0 mg/150ml of caffeine and 0.40mg/150ml of catechin concentration while increasing portion size have a significant effect on appetite and energy intake. Furthermore increasing portion size significantly increases nutritional content of the children intake in addition to energy intake. Therefore the role of habitual consumed black tea and *berbere* in relation to hindering intake could be considered as insignificant; however, black tea phenols have significant effect on iron bioavailability therefore tea should be provided two hours before or after meal.

In orphanage or other institution where large numbers of children live together availability of age appropriate portion size meal as well providing nutrient which fulfill daily recommended energy intake is difficult in developing counties due to financial constraint as well as lack of awareness. Different mechanism could be developed to prevent malnutrition; among this training of the caregivers regarding child feeding practice, providing ready to eat foods so that the burden of food preparation especially for the morning section could be minimized and the sustainability of those nutritious foods could be assured. However, in this case the cost of the ready to eat food should be affordable for such kind of institutions that is there should be mechanisms which balance the cost and availability of the nutrient dense food in the orphanage. In addition age appropriate portion size meal should be provided to battle malnutrition especially for children with high probability of being malnutrition. However researches should be carried to determine the age appropriate portion size in relation with energy and nutrient density. Furthermore considering the preference of the children regarding food groups could be another strategy to improve food and energy intake. Therefore in an orphanage age appropriate portion size of in relation with energy density should be determined and the children should be served according to their age with appropriate portion size. Since a single type of meal was used and this meal was almost always provided for breakfast, the effect of black tea on food and energy intake may not be observed therefore the effect should be assessed in a setting where habituation of a single food did not developed.

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Annexes:

Annex one, Informed Consent in Amharic

FOOD SCIENCE AND NUTRITION CENTER
COLLEGE OF NATURAL SCIENCES
ADDIS ABABA UNIVERSITY

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ጉዳዩ፡ የስምምነት ቅፅ

ውድ የህፃናት ማሳደጊያው ማእከል አስተዳደር

እኔ በአዲስ አበባ ዩኒቨርሲቲ የምግብ ሳይንስና ኒውትሪሽን ማእከል ትምህርታዊ ጥናት የማካሂድ ሲሆን ከአምስት አመት በታች በሆኑ ህፃናት የምግብ ፍላጎት ዙሪያ በህፃናት ማሳደጊያችሁ ውስጥ ጥናት ለመስራት ፍላጎት አለኝ። የጥናቱ አላማ ሻይ መጠጣትና በበርበሬ የተሰራ ምግብ መመገብ ከአምስት አመት በታች በሆኑ ህፃናት የምግብ ፍላጎት ላይ የሚያመጣውን ተፅኖ በተመለከተ ጥናት ማድረግ ነው። የህፃናት ማሳደጊያችሁ ይህንን ጥናት ለማካሄድ የተመረጠ ሲሆን ጥናቱን እንድናካሄድ ፍቃደኛ ከሆናችሁ የለት ተለት አመጋገባቸውን በማያዛባ መልኩ ህፃናቱ ከሻይ ጋርና ያለ ሻይ እንዲሁም በበርበሬ የተሰራ ምግብና ያለበርበሬ የተሰራ ምግብ ሲመገቡ የሚመገቡትን ምግብ ከመለካታችን በፊትና በልተው ከጨረሱ በኋላ የሚተርፈውን ምግብ በመመዘን ባመጋገባቸው ላይ ያለውን ለውጥ የምናጠና ይሆናል። ከዚህም በተጨማሪ የህፃናቱን ክብደትና ቁመት የምንለካ ይሆናል። በዚህ ጥናት ላይ መሳተፍም ሆነ አለመሳተፍ የህፃናት ማሳደጊያው ምርጫ ሲሆን በጥናቱ ላይ የምትሳተፉ ከሆነ ለጥናቱ የሚሰበሰቡ መረጃዎች በሚስጥር እንደሚያዙ እናረጋግጣለን።

ከዚህ በተጨማሪ በጥናቱ ላይ ለመሳተፍ እንደማትገደዱ መሆኑን እናሳውቃለን። ለመሳተፍ ፍቃደኛ ከሆናችሁ ጥናቱን በማእከሉ ለማካሄድ ዝግጁ መሆናችንን እየገለጽን ጥናቱ በህፃናቱም ላይ ሆነ በአሳዳጊዎቻቸው ላይ የሚያመጣው ምንም አይነት አሉታዊ ተፅኖ አለመኖሩን ልናረጋግጥ እንወዳለን።

ጥናቱን በተመለከተ ያላችሁን ማንኛውም አይነት ጥያቄ ሆነ አስተያየት የምንቀበል መሆኑን እየገለጽን በጥናቱ ላይ ለመሳተፍ ፍቃደኛ ከሆናችሁ አብረን ለመስራት ደስተኛ መሆናችንን እንገልፃለን።

የህፃናት ማሳደጊያው ማእከል አስተዳደር ስም

ፊርማ

The name and the stamp of the orphanage was hidden for the sake of confidentiality

Annex two, Informed Consent in English

FOOD SCIENCE AND NUTRITION CENTER
COLLEGE OF NATURAL SCIENCES
ADDIS ABABA UNIVERSITY

Date Oct 16/2013

Subject: **Informed consent form**

Dear Madame

We belong to the Food Science and Nutrition Center of Addis Ababa University. We need to study the appetite and energy intake of under five children. Our goal is to evaluate the role of drinking black tea and consumption of "*berbere*" spiced foods on the per school children appetite and energy intake. Your orphanage has been selected as a site of study. If you are willing to take part of the study; the habitually consumed meal that is containing tea or "*berbere*" will be weighed, the left over after the children completed consuming their meal will also be measured. Weight and the height will also be recorded. We are therefore here to ask for your consent to take part of the study. You are totally free to accept and refuse to participate in the study. If you decide to refuse, for any reason, there will not be any repercussions. If you accept, we guarantee you that confidentiality of all information collected will be assured. This study will not have any consequence neither to the children nor to your surroundings.

Don't hesitate to ask us any questions regarding the objective or the process of the investigation. If you are willing to proceed through the study, we are very glad to work in your orphanage.

Name of the administrator of the orphanage:

Signature:

The name and the stamp of the orphanage was hidden for the sake of confidentiality

Annex three, anthropometric data recording format

NO	ID of the child	**Anthropometric data**	
…………	……………………………	Height (cm) 1…………………	2…………………
		Weight (Kg) 1………………	2…………………
		Age (Months) 1………………	2…………………
…………	……………………………	Height (cm) 1…………………	2…………………
		Weight (Kg) 1………………	2…………………
		Age (Months) 1………………	2…………………
…………	……………………………	Height (cm) 1…………………	2…………………
		Weight (Kg) 1………………	2…………………
		Age (Months) 1………………	2…………………
…………	……………………………	Height (cm) 1…………………	2…………………
		Weight (Kg) 1………………	2…………………
		Age (Months) 1………………	2…………………
…………	……………………………	Height (cm) 1…………………	2…………………
		Weight (Kg) 1………………	2…………………
		Age (Months) 1………………	2…………………
…………	……………………………	Height (cm) 1…………………	2…………………
		Weight (Kg) 1………………	2…………………
		Age (Months) 1………………	2…………………

Annex four, Sample chromatogram

5/20/2014 23:25:20 1 / 1

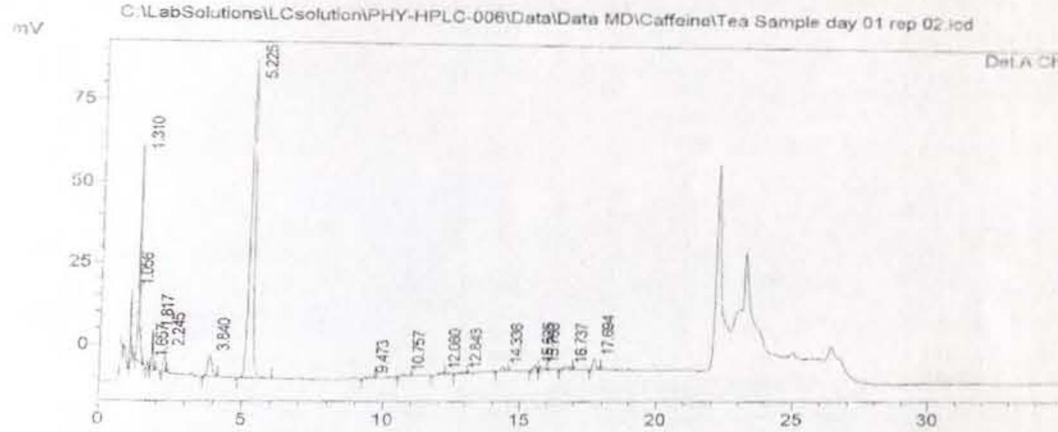
Treatment /Tea chromatogram

Analysed By:- Mahlet Dejene

C:\LabSolutions\LCsolution\PHY-HPLC-006\Data\Data MD\Caffeine\Tea Sample day 01 rep 02.lcd

Acquired by	: Admin
Sample Name	: Tea Sample
Sample ID	: Tea Sample day 01
Tray#	: 1
Vail #	: 11
Injection Volume	: 10 uL
Data File Name	: Tea Sample day 01 rep 02.lcd
Method File Name	: Catechins.lcm
Batch File Name	: Tea sample.lcb
Report File Name	: Default.lcr
Data Acquired	: 5/13/2014 11:21:00 PM
Data Processed	: 5/14/2014 4:29:39 AM

C:\LabSolutions\LCsolution\PHY-HPLC-006\Data\Data MD\Caffeine\Tea Sample day 01 rep 02.lcd



1 Det.A Ch1/270nm

PeakTable

Detector A Ch1 270nm

Peak#	Ret. Time	Area	Height	Area %	Theoretical Plate#	Tailing Factor	Resolution
1	1.056	47528	22518	3.245	5459	1.968	0.000
2	1.310	206445	66070	14.095	4350	2.145	3.722
3	1.657	5852	2131	0.400	5595	1.129	4.133
4	1.817	39481	11568	2.696	4826	1.240	1.659
5	2.245	28824	6858	1.968	5200	1.041	3.728
6	3.840	66784	6442	4.560	2762	1.146	7.557
7	5.225	983105	96944	67.121	6044	1.103	4.237
8	9.473	5000	361	0.341	10186	0.985	13.187
9	10.757	5658	377	0.386	11430	0.889	3.101
10	12.080	4723	490	0.322	33260	0.818	3.963
11	12.843	4486	425	0.306	30735	1.016	2.735
12	14.336	8527	1098	0.582	70491	1.094	5.866
13	15.525	12414	1238	0.848	44442	0.000	4.659
14	15.708	10837	965	0.740	17034	0.000	0.469
15	16.737	8907	818	0.608	58220	0.784	2.714
16	17.694	26100	3384	1.782	107453	1.006	3.879
Total		1464670	221685	100.000			

C:\LabSolutions\LCsolution\PHY-HPLC-006\Data\Data MD\Caffeine\Tea Sample day 01 rep 02.lcd

5/24/2014 23:02:04 1 / 2

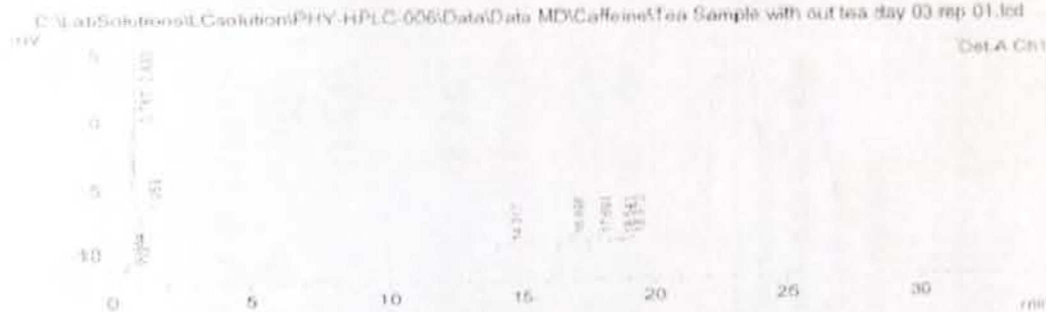
Control /VLT chromatogram

Analysed By:-Mahlet D.(Caffine)

C:\LabSolutions\LCsolution\PHY-HPLC-006\Data\Data MD\Caffeine\Tea Sample with out tea day 03 rep 01.lcd

Acquired by	: Admin
Sample Name	: Tea Sample
Sample ID	: Tea Sample with out tea day 03
Tray#	: 1
Vial #	: 10
Injection Volume	: 10 uL
Data File Name	: Tea Sample with out tea day 03 rep 01.lcd
Method File Name	: Catechins.lcm
Batch File Name	: Tea sample.lcb
Report File Name	: Default.lcr
Data Acquired	: 5/13/2014 8:59:12 PM
Data Processed	: 5/24/2014 10:57:14 PM

C:\LabSolutions\LCsolution\PHY-HPLC-006\Data\Data MD\Caffeine\Tea Sample with out tea day 03 rep 01.lcd



1 Det.A Ch1/270nm

PeakTable

Detector A Ch1 270nm

Peak#	Ret. Time	Area	Height	Area %	Theoretical Plates	Tailing Factor	Resolution
1	0.514	196	63	0.445	353	1.479	0.000
2	0.781	2835	1332	6.436	8601	0.809	4.416
3	0.833	24054	9241	54.602	2143	2.489	0.977
4	1.059	8949	3436	20.315	4665	2.303	3.382
5	14.317	1204	141	2.734	34401	1.109	86.211
6	16.698	2717	266	6.167	56046	0.818	9.025
7	17.693	1117	215	2.532	130744	0.936	4.165
8	18.541	1182	136	2.682	95678	0.970	3.893
9	18.977	1799	212	4.083	90674	0.881	1.793
Total		44053	17043	100.000			

C:\LabSolutions\LCsolution\PHY-HPLC-006\Data\Data MD\Caffeine\Tea Sample with out tea day 03 rep 01.lcd

Annex five, the Orphanage children Food menu

ምናሌ ከ 2 - 4 ዓመት ያሉ ህፃናት የምግብ ፕሮግራም

ሰዓት	ሰኞ	ማክሰኞ	ረቡዕ	ሀሙስ	አርብ	ቅዳሜ	እሁድ
1:00 ቁርስ	ዳቦ በሻይ	ቅንጬ	እንቁላል በዳቦ	ዳቦ በሻይ	ገንፎ በቅቤ	ቅንጬ	ዳቦ በሻይ
4:00 መክሰስ	ጨጨብሳ በሻይ	ዳቦ በሻይ	ዳቦ በማርማላት ሻይ	ገንፎ በቅቤ ሻይ	የገብስ ቆሎ በማርማላት	ጨጨብሳ በሻይ	ዳቦ በሻይ
6:00 ምሳ	ፓስታ በዳቦ	ሲጋወጥ በእንጀራ	ሩዝ በአትክልት	ሲጋወጥ በእንጀራ	መኮረኒ በዳቦ	ምስር ወጥ በእንጀራ	መኮረኒ በስጋ
9:00 መክሰስ	ዳቦ በወተት	ብስኩት በሻይ	ዳ[illegible] በወ[illegible]	The stamp of the orphanage was hidden for the sake of confidentiality	ብስኩት በሻይ	ዳቦ በሻይ	ብስኩት በሻይ
2:00 እራት	ሽሮ ወጥ በአትክልት	መኮረኒ በዳቦ	ም[illegible] በእንጀራ	[illegible]	ድንች ምስር በእንጀራ	ሩዝ በዳቦ	ሲጋ ወጥ በእንጀራ

Annex six, food intake recording format

6.1 Treatment/control meal intake recording format to observe the effect of tea

NO	ID of the child	Break fast			Snack		
		Type of food	Amount (g)	Cup of Tea / Hot water (ml)	Type of food	Amount (g)	Cup of Hot water (ml)
......	....	....	....	....	....	....	....
......	....	....	....	....	....	....	....
......	....	....	....	....	....	....	....
......	....	....	....	....	....	....	....
......	....	....	....	....	....	....	....

6.2 Treatment/ Control meal intake recording format to observe the effect of berbere spiced food consumption

NO	ID of the children	Lunch	
		Type of food	Amount (g)
....	....	....	....
....	....	....	....
....	....	....	....
....	....	....	....
....	....	....	....
....	....	....	....