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Sensory Evaluation of Calcium, Iron and Zinc Fortified Whole Wheat Flour Chapati

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Abstract

Nutrition play important role in prevention of disease and promote good health if taken with clear understanding of importance of certain foods and assist in prevention of disease. Calcium is very important for bone and teeth health. Similarly zinc is needed for the brain modulation, gene expression DNA, RNA metabolism. Calcium decreases in young adult as they age. When calcium is not enough in the diet the body take calcium from the bone and lead to bone weakness. Zinc helps in wound healing and effect on learning and memory and regulates immune function. Zinc is used to prevent inflammation in lining of digestive tract. Iron is very important for healthy pregnancy, increased energy and better athletic performance. Iron treats anemia and used for brain function and helps in immune boasting, synthesis of neurotransmitters. So calcium carbonate and ferrous sulphate also the sulphate of zinc have already been used as iron zinc and calcium fortification. Two chapati will be prepared one fortified with 114mg Calcium carbonate with 48g of wheat flour and the other chapatti co-fortified with 1.5mg iron and 1.5mg zinc with 48g flours must be fortified manually. Then finally the proximate analysis and sensory evaluation will be performed on the products. In the end the study will be subjected to statistical design to interpret the result.

Abbreviations: AI: Adequate Intake; CDC: Centers for Disease Control; CHD: Coronary Heart Disease; DALY: Disability-Adjusted Life Year; DFE: Dietary Folate Equivalents; DRI: Dietary Recommended Intake; DRV: Dietary Reference Value; EAR: Estimated Average Requirement; EDTA: Ethylenediaminetetraacetic Acid; FAO: Food and Agriculture Organization; FFL: Feasible Fortification Level; FNB: Food and Nutrition Board; GAIN: Global Alliance for Improved Nutrition; GDP: Gross Domestic Product; GMP: Good Manufacturing Practice; HACCP: Hazard Analysis Critical Control Point; ICCIDD: International Council for Control of Iodine Deficiency Disorders; IDD: Iodine Deficiency Disorders; IIH: Iodine-Induced Hyperthroidism; ILO: International Labour Organization; INACG: International Nutritional Anemia Consultative Group; IOM: Institute of Medicine; IRLI: International Resource Laboratory for Iodine; IVACG: International Vitamin A Consultative Group; IZiNCG: International Zinc Nutrition Consultative Group; NGO: Nongovernmental organization; NRV: Nutrient Reference; VAD: Vitamin A Deficiency; WFP: World Food Programme; WHO: World Health Organization; TAZ: Total Absorbed Zinc; GBD: Global Burden of Disease; YLD: Year loss due to Disability; DW: Disability Weight

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Chapter 1

Introduction

Zinc is important for the health and zinc deficiency play important role in causing disease. Zinc play important role in prevention of diarrhoea and infection about 800,000 children has increase diarrhoea and infection result in death per year worldwide. Zinc deficient occurs in huge number of male and female and Nutritionists provide good food choice to overcome the deficient [1]. Many techniques have been taken to approximate the adequacy of zinc in diet in many place of the world. For example World Health Organization (WHO) in 1996 used technique to estimate minimum amount of zinc that is required to overcome the deficiency in individual. It is important to also notice bio-availability of zinc from typical diet consumed in many places of the world in order to obtain minimum zinc intake to overcome the deficiency. Then compare the estimated dietary intake of zinc suppose 210 survey conducted determine the mean intake of zinc and the minimal dietary of zinc that did not meet the minimal dietary intake by comparing percentage of normative level of zinc intake. This method provide conclusion of the percentage of zinc intake throughout the world but in area where consumption of plant based diet indicate zinc intake is from low to moderate bio-availability. For example 148 survey conducted mostly in western Europe and the U.S only one dietary survey indicate lower than the minimal intake of zinc among the 47 survey conducted in population with moderate zinc bio-availability and 40 indicate average. And 15 surveys conducted in population with low zinc bio-availability and no report shows mean greater then normative zinc intake. Zinc in developing country is the most important area for research of zinc intake. Although this analysis couldn't provide estimated regional zinc deficiency and prevalence in 2001, Brown published used available data on per capita food availability from 72 countries to approximate the prevalence of zinc and the poor intake of zinc worldwide Calcium is very important in bone health. Calcium is reported as important nutrient for the support and strengthens bone health. In patient of bone loss required critical care and continuous calcium intake. And vitamin D help in the absorption of calcium bone for the bone health. Survey conducted on South Asia population which shows 90% of women may not acquire enough calcium and 50% of women treated with bone loss do not have enough vitamin D) [2,3]. In country like Pakistan where there is abundant sunlight it is hard to believe that is so many Pakistanis that are vitamin D deficient .A huge percentage of adult population have low level of vitamin D having less vitamin D. There is commonly high scarcity of vitamin D in Pakistani parturient and their newborns. In country like Pakistan Endocrinologist and bone health commonly recommend vitamin D doses oral like In drop D or vitamin D shot [4-6].

Survey conducted shows less vitamin D levels (56.2%) compared to the means of males who had vitamin D (15.3%). Inadequacy has been record by 11.3% females with 9.6%and male1.6%. Decrease of vitamin D related with increased in cancer and CVD mortality and autoimmune disease like multiple sclerosis. Osteopenia indicate bone density that is low than normal density but not low to called osteoporosis. Osteopenia is without symptoms. Osteopenia show the beginning sign of osteoporosis but not everyone with osteopenia later develop osteoporosis [7,8].

The accurate way to examine osteopenia and osteoporosis is through BMD test (bone mass density) through DEXA (dual energy x-ray absorptiometry) scan. In osteopenia T-score show range of -1 and -25 and osteoporosis T-score shows -2.5 which indicate inadequate calcium [8]. Calcium is very important for many function of the body for example cell function and neuromuscular activity, blood coagulation and cardiac function but especially to the bone health as it help in structural design throughout the life even though body store 99% of calcium for the teeth and bone also calcium is present in plasma and because of plasma calcium is attain balance in our body and if plasma is low in our body then the bone help resorption adequate calcium is very necessary to attain balance and calcium in the intestine is absorbed with the help of vitamin D [9]. Along with calcium, Vitamin D is very important for bone heath the major function of vitamin D help in absorption of calcium in the intestine which maintain serum of calcium concentration. Vitamin D can be obtained from sunlight and from the diet and supplement. In case were the vitamin D is reduce calcium absorption from the intestine also reduce leading to increased osteoclast formation which increase the mobilization of calcium from the bone. If there is continuous deficient of vitamin D the body absorbed calcium from the bone leading to ricket in children and osteoporosis and osteomalacia [10,11].

The optimal scientific tool for the diagnosis of osteoporosis is BMD test. BMD test help in the decision of calcium reach food intake and it also inform about the later bone health at the age of 65 and older for the women and at the age of 70 or older for the male despite of clinical possibility. In menopause often there is increase bone loss

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and most rapid loss of bone occur during first five years after the menopause [12]. Decrease in estrogen after the period of menopause cause bone resorption result in low calcium. To obtain good bone health required and delay osteoporosis it is important to maintain good lifestyle and exercise in adolescence and maintain healthy eating habit and maintain it through the life, significantly increased the duration of interval of bone loss as menopause [11,13,14].

The world health organization (WHO) overview the global prevalence of anemia. In 1985, WHO approximate that 30% of world is anemic. WHO approximate 37% of women were anemic in 1992 and in 2008 WHO reported that 24.8% of the world population was anemic which also mentioned 42% of pregnant women, 30% of non-pregnant women and 47%of preschool children [15-18].

Most worldwide anemic prevalence was approximate at 29% in pregnant women, and 43% was estimated of anemic in children and 29% in pregnant women, with decreased since 1995 in every group. The Global Burden of Disease (GBD) record that 2000 approximately anemic of 2% of all YLD and 1% disability regulate in long life and in the year 2004 shows nearly the same result [19,20].

These analyses give detail approximate of prevalence and the epidemiology of anemia and its effect on world health with key factor like age, sex, and differentiated between 1990 and 2010. With the help of data from 406 from Demographic and Health Survey also WHO database has data from both sub national and national survey. The 17 root the cause to anemia shared out by the data used in Global Burden of Disease, Injuries and Risk Factor 2010(GBD) study. The other authors approximate the effect of anemia on global health. To value the disease burden approximate it deserves to observe under the cover at their calculation. The disability weight (DW), is the first concept that shows the critical health loss that related with a clinical condition. The expert committee find out the critical health loss by DW and for 2010 GBD, The DWs were formed by idea from general public through comprehensive international study like Indonesia, Tanzania, Bangladesh, Peru and the US with internet survey. The contributor were asked of 2 hypothetical patients with differ health status and asked which were healthier. The internet survey were said to differentiate the value of different disease prevention and lifesaving, and 220 health condition were ranked , and DWs classified as mild, moderate, and severe anemia (from 0 for mildest and 1 indicate death) mild anemia is third anemia of all condition. And in moderate and severe anemia has DWs of 0.058 and 0.164 in 2010 shows mild, moderate, and severe anemia with large anonymous record than above years [21].

The YLD, shows the total number of year that is reduce than the ideal health state and can be calculated by multiplying the DW with the prevalence and find the approximate burden (9) The authors establish that anemia result for 8.8% of the world's YLD, but in 1990 to 2010, the occurrence of anemia worldwide decreased. Children of age less than 5 years still has increased occurrence of severe anemia compare to overall tendency and result of the records [19,22]. In country like South Asia has 37.5% of the world anemia and in sub-Saharan Africa showed 23.9% of the world anemia burden. The prime reason of anemia worldwide contributes to 3 syndromes the first is iron deficiency like anemia and hookworm and the second is hemoglobinpathy for example sickle cell anemia and malaria. The at most reason for anemia is iron deficiency and malaria especially in sub-Saharan dessert. African focal point the contradictory statement for anemia control in such areas, iron supplementation insofar is found to increased malaria risk. And the record also shows that there is increased chronic kidney disease over the two decade of the study and is the prime cause of anemia of 80+ age group [23].

Objectives

- To develop wheat flour chapati fortified with iron and zinc
- To develop wheat flour chapati fortified with calcium
- To do and compare the proximate analysis of the food products
- To compare the quality of food products through sensory evaluation

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Chapter 2

Review of Literature

Deficiency of micro-nutrients

Micro-nutrient play important role in our body and micro-nutrient deficiency also called "hidden hunger". Lack calcium cause weakness of bone and tooth and reduce blood clothing [24]. Half of the world population suffered from micro-nutrient malnutrition [24,25]. Iron deficiency causes anemia and decreases physical activity due to decrease in oxygen which is carried out by the haemologobin. Zinc deficiency lead to non-functioning immune system and cause stunted growth. Goitre is caused as result of decrease iodine along with iron deficiency. iron deficiency has direct role in production of thyroid hormone. Calculation of goitre reaction measured when given iodized salt and iron supplement [26,27].

Blood haemoglobin is increased with the increased in the quantity vitamin A in the society. Anemia is caused by the reduction of vitamin B2 and several other micro-nutrients. Vitamin C improve intake of non-haem iron from the diet. So conclusion fortification reduce iron and several other micro-nutrient while considering the interaction of element with each other that is present in the food and prevent several micronutrient deficient diseases [28,29]. The medical sign of deficiency of iron in Pakistan shows 29% occur in child below 5 years old and 48.7% occur in mother. The national nutritional survey on 2001-02 show zinc deficiency 37% in preschool children and 41.4% of mother [30]. Iron is most common micro-nutrient deficient. Rice is the main staple food for more than half of global population. This study show black rice is higher in iron content then in other rice type. The rice in husk and chaff iron is fairly high. The bio-availability of iron is very low in vegetable which almost 10%. Several methods were used to improve bioavailability of iron in rice such as biochemical and physical approach apart from genetic engineering and bleeding in rice grain [31].

Iron and zinc play important role in cognitive impairment and in growth and development anaemia and blindness and increased morbidity and mortality rate. Deficiency could be due to poor intake or poor absorption or utilization or increased loss. Most country that is developed has large mineral in fresh food but due to cultural and economics or religious have limited mineral. Staple diet is plant based and contains more fiber, phytate which inhibit absorption of non-haem iron and zinc. In dark green leafy vegetable contain b-carotene has poor bioavailability of iron and zinc. The rural diet beside the poor bioavailability of mineral has low fat content. An intervention plan required which very important to reduce deficiency of many minerals dietary changes and dietary strategies which fulfil mineral requirement and also dietary strategies involve selection pattern and food production practices. The result increased in availability and utilization of food that increased absorption of mineral to overcome mineral deficiency. The nutritional adequacy of Fe, Zn depends on the bioavailability and their amount. There are many strategies that are used in production of food to increase the absorption of mineral content in staple food of plant based especially in developing country. The deficiency of iron is very commonly known as micronutrient deficiency which result in 1.5 to 2 billion people of whom 500 million people have iron deficiency anemia most of them from developing country [32,33]. Iron absorption is measured when iron is absorbed into RBC at 1.4 day. And the absorption of zinc is measured from excretion of urine sample in 48 to 72 hours. WHO defined recommended Anemia as a haemoglobin as concentration of haemoglobin < 110 g/L in children aged 6 mo-6 y or < 120 g/L in children aged > 6 y. Iron deficiency was defined as a plasma ferritin concentration<12g/L [34].

A serious world issue is nutritional deficiency in developed country and lack of legislation and governmental control [35]. To overcome micronutrient deficiency many strategies is formed which include dietary modification supplementation and food fortification which could short or long term. The strategies have economically and technologically shown to enhance micronutrient deficiency to reduce mineral deficiency in population [36-37].

Food Fortification

Food fortification is crucial strategies to overcome mineral deficiency which increase iron intake of population. Much country they used to fortified staple food like rice and maize etc. The primary goal of fortification of mineral is to overcome the deficiency and improve human healthy. Fe and I, Zn deficiency problem to the globe and affecting 30% of the world population. Anaemia and goitre are the risk factor in disease and death two billion of people suffer from it across the globe [38].

In country like Indonesia the wheat flour is fortified with 60mg of iron/kg. Several studies have shown in developing country zinc deficiency decrease mortality and morbidity

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from diarrhea and respiratory disease and assist in growth when using zinc supplementation. Wheat flour has less iron and zinc but iron is fortified in country like Indonesia has iron co-fortification with zinc. However zinc when co-fortified with iron which reduce iron absorption. Previous studies proved that iron has little effect on zinc absorption when zinc-iron ratios are 1:1 but an inhibitory effect on zinc absorption when zinc-iron ratios are 1:2 [39].

The fortification of wheat and maize flour enhance the rank of micronutrient and reduce micronutrient deficient which are found out to be problem for public health. The acceptable foods that are similar or other food nutrient have been examined before fortification. The fortification of wheat and maize flour can be attained by proper understanding of element that are required to be added and their interaction with other element in the food which influence public health and both national level and international level. So conclusion, the nutrient and the stable quantity depend upon the nutrient requirement of the people and the deficiency of the nutrient in the population. In most case deficiency of mineral and vitamin people prefer supplement but in the case of fortification of flour involve stable quality control and quality assurance. The Quality assurance and quality control is system which remove mistake while fortification which result in quality required of nutrient that are fulfilled. In developing countries fortification is very vital as there is deficiency of nutrient such as vitamin B12 vitamin A and iron, folic acid and zinc [40].

Food fortification is process of adding one or more nutrient in the food nutrient not important if it contains in the food or not the main goal is to prevent that deficiency due to micro- nutrient in specific population. Fortification is different from enrichment. Enrichment is the drinking back of nutrient that is washed out during the process. Fortification process usually occur on staple food as mean to provide more micro nutrient that lack in the food consume to buy particular population. So in 1 930 specific population was target such as iodine deficiency. In iodine deficiency iodine is fortified in Salt [41]. Food fortification involve biofortification, microbial biofortication, synthic biology industrial fortification and home fortification various type of fortification which is different depend on the type of procedure and technique used to fortified particular food. Biofortification include produce staple food with dense fortification with the help of brecing technique. Microbial biofortification includes probiotic bacteria that B-carotene within the food or it produces inside the material [42,43]. Commercial fortification and industrial fortification is a fortification of commercial product like rice, cooking oil, sauces etc. home fortification includes the fortification of food for vulnerable population with micro-mineral are the deficiency of vitamin and mineral.

Fortification of Different Food Item

Bread from wheat flour is staple food in country like Iran northern India as well in Middle East. In Iranian city bread is entirely made from flour of 80% to 90% extraction rate flat bread of bazari and sangak of different degree of refining. The local whole meal are mae into extraction rate of difference bread stepherd bread and tanok or lavosh during the preparation leaven is removed but when leaven is used fermentation is limited because short time allowed and due to resistance of whole meal to action of yeast. The nutrition proportion of iranian bread is healthy for large population the bread consumed in rural and urban contain substantial amount of fiber and phytate average of about 0.7% by weight in rural and half weight in urban bread because city bread used lower flour extraction [44].

Fiber concentration is less than phytate concentration. Fiber concentration is consistently high 50% of schoolboy energy intake is from bread and 75% or more in urban and rural families. The village diet is rich in Zn, Fe, and Ca, to complete the daily requirement. But because of poor intestinal absorption zinc and iron deficiency is fulfilled with supplementation. Calcium supplementation is not well documented. Bread is fortified with large amount of calcium and zinc but the body store of minerals is depleted which result in mineral absorption [45]. To determine variety level of zinc fortification on TAZ among the young children are difficult by the fact that specific intake conjecture equations are not yet accessible for children. The model of adult gives satisfying for data obtained from children of age 2 that eat low phytate diet [46]. Zinc compounds are widespread consider as safe (GRAS) for human utilization, and are acceptable to use in fortification of food. Zinc oxide is the inexpensive chemical form of GRAS zinc compounds, although concerns have been increase about its bioavailability because it is does not dissolve at neutral pH .However, three separate studies shows there is no different in the intake of zinc oxide and zinc sulphate [47]. There is keen role of sodium ethylenediaminetetraacetate (NaEDTA) increase zinc intake, primarily because NaEDTA is familiar to enhance iron intake. Recent study shows iron intake from the diet supplement contains iron and calcium is 42% and from the diet supplement contains iron and zinc is 53%. The study shows that diet which contain iron and calcium reduce iron compare to diet supplement contain iron and zinc as calcium interact with iron and reduce iron bioavailability in the body which can lead to iron deficiency. Zinc and iron in diet improve iron requirement in the body. Sequence of iron is negative which shows zinc compound on iron [48,49].

Fortification of iron was done in U.S in infant formula, the goal was to reduce the prevalence or prevent anemia and other disease which is the main cause for iron deficiency later

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in children below 5 years old. The fortification of iron, vitamin A and B vitamin on whole wheat flour to make chappati for children and adults is a way of reducing iron deficiency. This procedure was performed by Venezuela since 1993. The result shows remarkable decrease of iron deficiency when compared before and after the process of fortification [50-52]. Milk fortification with iron and vitamin C shows quick reduction of iron deficiency in children below 5 years old. The fortification of soy sauce with iron was performed in china and shows productive result so the procedure was performed on 10,000 Chinese women and children with huge chance of anemia. This research carried out through double blind control placebo for 2 years and after 6 month it was found out decrease in anemia for all age group [53-55].

Iron supplement and iron fortification on wheat flour is productive and has more advantage in reduction of anemia and this both the procedure can be used when perform efficacy in iron deficiency individual. Fortification process has broad advantage as it upgrade not only one micronutrient deficient in individual but also multiple micronutrients deficient in individual. Serum retinol is increased when taken iron supplement [56]. Micronutrient malnutrition is restorable by food fortification .food fortification is good procedure for prevention and cure of micronutrient deficiency. The several fortification of food prevent the shortage of many micronutrient in the most reasonable way rather using only one micronutrient that is deficiency in our body. Cereal flour fortified commonly with zinc, calcium and iron. Many investigator report iron interfere intake of both calcium and zinc as calcium, iron and zinc interact during fortification which decrease their bioavailability in the body [30,57].

Wheat is vital food grain that contains major calories, protein and mineral for Pakistanis. About 70% of the total or more is used as wheat flour to form chapati or bread little or there is no use of fermentation. Calcium fortification assists those individual that suffer from intolerance of lactose and individual with form of traditional dairy. Several study shows that every wheat flour tortillas standard are strengthen with calcium citrate, calcium carbonate then they are differentiate with the non-fortified tortillas. The moisture content is similar for both tortillas .using Hedonic scale people were ask to check tortillas on the basis of taste, smell, texture, etc. People preferred more control tortillas over the commercial tortillas. Despite of the difference consume were equally purchasing both the tortillas. The study shows achievable form of calcium is through fortification [58]. Zinc oxide is the most globally used for fortification in food. Zinc compound less advantage over zinc oxide. Zinc oxide is less expensive and stable form of zinc compound and the food remain same in which zinc oxide is added. The sulphate of zinc is the second most global used for zinc fortification. To compare these two zinc compound, zinc sulphate and zinc oxide their absorption. The study is conducted in around 10 of the women were taking corn tortillas strengthen with zinc. The fragment intake of oxide of zinc that had been strengthened in corn tortillas was found alike to those sulphates of zinc.

Dietary For various nutrient are not fulfilled from the food they consumed. Nutritional scientist produce dietary standard, and guidelines, food pyramid, RDA, RDI during the period of last decade nutritionist are more concern on safety, security, malnutrition. The nutrient is divided on to micro nutrition and macronutrient. It is important to know the amount of iron added in the food like rice, maize, white rice should be 25 and 80 ppm again relay on the critical need of the population. The good source of iron relay on the increase bio available and the cheap [59,60]. Feso4 is not suitable for product that are stored for long period because it cause oxidation rancidity and ultimately because reduction in shelf life. Feso4 has disadvantage when used in food as it change the color the as well the flavor over time and reduce acceptance in the market calcium, phytates, and polyphenol influence bioavailability of non-heme iron. A good result of fortification program relay on micronutrient stability and food added. Fortificant stability depends on heat, moisture, air, light and acid packing, distribution or storage bread and several serial. The form iron to used relay on its stability, functional, bioavalibity. The form of iron that high function stability are always less absorbable and those with high bioavailability has the highest capability to damage [61,62].

Fortification of Wheat Flour in Different Countries

The fortification of wheat flour with micronutrients begin first in Canada and U.S in the year of 1940s by 1950s latin America like chile also start the fortification of wheat flour by forwarding legislation that promote food fortification. A large population was iron deficient that promote the fortification of iron. Fortification of wheat flour with iron and B vitamin in countries like North Africa and Middle East and central Asia countries start wheat flour fortification like Azerbaijan, Kyrgyz and Mongolia. Asian countries like Pakistan, Thailand, and china also agreed for the fortification of wheat flour as it decreased the occurrence of anemia or other disease due to iron deficient. Jordan has also start the fortification of the wheat flour because the consumption of wheat flour is more in Asian countries as wheat flour can be used in bread, chapatti, and in baking purpose to make biscuit or bun. In Thailand and Guatemala Study report that fortification of wheat flour in village areas is more important has there is increased vulnerable population. in country like Chile has less food fortification of less than 1%.The ministry of health in Chile asked to add folic acid to the wheat flour of 2.2mg/kg to reduce the issue of neutral tube defect with the help of

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fortification of wheat flour that reduce 40% in neural tube defect. Later it was observe that fortification of wheat flour with folic acid upgrade folate status in especially pregnant women. So fortification of whole wheat is in improve mineral deficiency worldwide 18% in 2004 to 27% in 2007. The number of who can purchase wheat flour has increased 540 million and during pregnant consuming fortified wheat flour who later give birth to newborn has increased by 1,4 million. The countries that made compulsory to fortify while wheat has also increased 33 in 2004 to 54 in 2007 [63].

Even though of the achievement more than third population of the world have no availability of fortified wheat flour fortification of wheat flour are more required for the vulnerable group like that women of child bearing age. Single fortification nutrient is less productive than multiple fortifications multiple fortification is by adding two or more micronutrient in co-fortification way [64].

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Chapter 3

Materials and Methods

The research study was carried out to determine the proximate profile of prepared garlic and ginger Chapatis as well as to develop fortified chapatis with Zinc sulphate and ferrous sulphate and Calcium carbonate.

Collection of Raw Materials

Wheat flour was bought from the market and all the three chemicals ferrous sulphate, zinc sulphate and calcium carbonate procured from The University of Agriculture Faisalabad.

Analysis of Raw Material

The analysis of wheat flour chapatis and fortified wheat flour chapatis was done to determined their various content i.e, moisture, crude fat, crude protein, crude fiber, ash content and nitrogen free extract the analysis work was performed in the Research Laboratory of The University of Faisalabad.

Preparation of Composite Flour

Various ingredients of wheat flour chapatis are fortified chapatis are shown in Table 1 Every chapati made is different from others according to treatments as it is fortified with different salts whereas the control chapati is not fortified. The second chapati is co-fortified with zinc sulphate and ferrous sulphate and the third chapati fortified with calcium carbonate. Different treatment is shown in Table 2.

Ingredient	Quantity
Garlic	666mg
Ginger	1333mg
Ferrous sulphate (feso**4**)	1.5mg
Zinc sulphate (znso4)	1.5mg
Calcium carbonate(caco3)	114mg
Whole wheat flour	48g

Table 1: Ingredient/Quantity.

Treatment	Description	Quality of sample
To	Control (chapatti not fortified)	0
T1	Chapati with zinc sulphate and ferrous sulphate	1.5mg
		1.5mg
T2	Chapati with calcium carbonate	114mg

Table 2: Different Treatment Used to Prepared Composite Flour.

Proximate Analysis of Composite Flours

Proximate Analysis: Chapati samples will be evaluated for moisture, ash, crude fat, crude protein, and nitrogen free extract according to their standard procedure described by the American Association of Cereal Chemists (AACC, 2000).

Moisture Content: The moisture content in chapati was determined by drying the sample in the oven at 105±5 till the weight of the chapati sample becomes constant according to the method described in AACC (2000).

Moisture%= Weight of fresh sample (g) - Weight of dried sample (g) x 100 /Weight of fresh sample (g)

Ash content: The ash content of chapati was assessed according to the method defined in AACC (2000). Ash estimation was conducted by direct incineration of sample obtained in a crucible. The crucible was heated on the oxidizing flame till it produces no fumes. Then kindled in a muffle kiln at 550 C till grayish white residue was obtained % ash was calculate according to formula:

Ash % = Weight of ash (g) x 100 /Weight of sample (g)

Crude fat content: Crude fat determination was conducted using soxhlet kit according to AACC (2000). 48 of chapati sample were taken. It was taken in separate thimbles and placed in an extraction tube of soxhlet kit after wrapping in

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the filter paper. The modification of temperature of heater was so that incessant drops of ethanol fell on the sample in the extraction tube. The reminders were shifted into a dry weighted china dish. Then this china dish was placed into a hot air oven for evaporation for either 4-5 hrs. Then this china dish was taken out and placed in desiccation to cool it and then again weighted. The crude fat content was determined by taking 48g chapati using ethanol as solvent in a soxhlet apparatus according to the procedure given in AACC (2000).

Crude fat % = Weight of sample (g) - Weight of fat free sample (g) x 100 /Weight of sample (g)

Crude Fiber: The chapati sample after fat extraction was tested for crude fiber content by following the technique stated in AACC (2000). 48gof chapati in a 500ml beaker was marked. The sample was then heated with 25ml and 1. 25 percent H_2SO_4 solution for 30 minutes. The content was filtered and gave 2-3 washing with distilled water. The washed residue was transferred to 500ml beaker and again made the volume up to 200ml using distilled water and was heated with 2.5ml 1.25% NaOH solution for 30 minutes. The content was again boiled for 30 minutes. The content was filtered and 2-3 washing with hot water were given until it was alkali free. The residue was carefully transferred to a tarred crucible and dried in an oven at 100 C for 3-4hrs until constant was obtained. The content was heated on flame until the smoke ceased. The content was placed in muffle furnace at 500C for 4hrs until a grayish ash was obtained, then cooled in desiccators and weighted. The difference in the weight was calculated as crude fiber by using following formula:

Crude fiber % = Weight of insoluble matter (g) - Weight of ash (g) x 100/ Weight of sample (g)

Crude Protein: The percentage of nitrogen in the sample was determined by using kjeldhal method as described in AACC (2000). The sample (2g) was digested in the digestion tube with aid of 30ml conc. H_2SO_4 in the presence of 5g digestion mixture ($CuSO_4$:$FeSO_4$; K_2SO_4 in the ratio of 9:1:90) for 56hrs or till the digested material attained light greenish or transparent color. The material was diluted and distillation was done by taking 10ml of diluted material and 10ml of NaOH. The ammonia released was collection in 4% boric acid having methyl indicator. The solution was then titrated against 0.1 NH_2SO_4. Crude protein was calculated by using the following formula:

Nitrogen (%) = Vol. of 0.1 NH_2SO_4 x Vol. of dilution x 0.0014 x 100/ Wt. of sample (g) x Vol. of aliquot sample (ml)
Crude protein = Nitrogen (%) x 6.25

Nitrogen free extract: The NFE was calculated by using the formula according to Uraku, et al.

NFE (%) = 100 — (% moisture + % crude fiber + % crude protein + %ash + %crude fat)

Preparation of Chapatis

Chapatis were prepared without selected interval from the entire sample by using the method described by Haridas, et al. Control chapatis prepared by mixing 48g of flour with the measured quantity of water to develop the dough for chapatis and 666mg of garlic and 1333mg of ginger were add for each chapati for good taste and aroma and co- fortified chapati prepared by adding 48g of wheat flour and 666mg of garlic and 1333mg of ginger and 1.5mg of zinc sulphate and 1.5mg of ferrous sulphate. And single fortified chapatti prepared by adding 48g of wheat flour and 666mg of garlic and 1333mg of ginger and 114mg of calcium sulphate.

Sensory Evaluation

Sensory evaluation of chapati (T0, T1, T2) was performed by a trained panel for taste 9-point Hedonic scale. The quality characteristic of chapati like taste, color, texture, flavor, aroma and overall acceptability according to their sensory response were recorded. The evaluation process was conducted in booths separate for each panelist under clear white light in the Nutrition and Sensory Laboratory of The University of Faisalabad. The panelist was served the sample in plate accordingly with random codes on the day of evaluation. The panelist was utterly explained about the nature and essence of the experiment. During the process of evaluation the panelist were also served mineral water to neutralize the taste and to rinse for rationalized assessment. The panelist were requested and guided to give the product quality a rating by scoring for the parameters chosen for them Meilgaard, et al. [65].

Statistical analysis: The data were designed by employing completely randomized design also called CRD. The level of significance (ANOVA) was determined by CRD by applying the outlined principle and methodology by Steel, et al. [66].

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Chapter 4

Results and Discussion

Moisture content

Moisture content is the quantity of water in the food. Moisture content is crucial parameter for preservation and storage for the shipping of food product. The moisture content of food is very important as it give information of the type of packaging of food. The analysis of variance shows that all the chapati has same moisture content. The Table 1 also revealed that there is highly non-significant between the treatments of the composite flour. The finding also shows the moisture content of To 32.33, T1 32.33, T2 32.33. T1 is co- fortified of zinc sulphate (1.5mg) and ferrous sulphate (1.5mg). T2 is calcium carbonate of (114mg) and to control chapati is non-fortified. Pomeranz explained that moisture is the most important factor influencing the rate of deterioration of stored grain and their products and variation in moisture content can be due to storage, otherwise there might be not much variation in moisture content [67].

Ash Content

Ash content is the amount of inorganic particle in the food after the water and the organic matter is removed by heating the food in the present of organic agent. Ash content tells us about the total quantity of mineral within the food. And the analysis of variance shows that all chapati have different Ash content as the ingredient of all chapati is different from each other.

Nutritional Composition (%)	T_0	T_1	T_2
Moistire	32.3±0.057	32.3±0.057	32.3±0.057
Crude Fat	0.36±0.057	0.36±0.057	0.36±0.057
Crude Fiber	0.36±0.057	0.36±0.057	0.36±0.057
Protein	8.74±0.005	8.74±0.0057	8.75±0.005
Ash	0.6±0	1±0	1±0
Nfe	57.45±0.05	57.45±0.05	57.45±0.05

Table 3: Mean Values for Proximate Analysis.

Source of Variation	Sum of Square	Degree of Freedom	Mean Square	F-value	P-Value
Between Groups	0	2	0	0	1
Within Groups	0.02	6	0.003333		
Total	0.02	8			

Table 4: Analysis of Variance for Moisture Content.
*p > 0.05

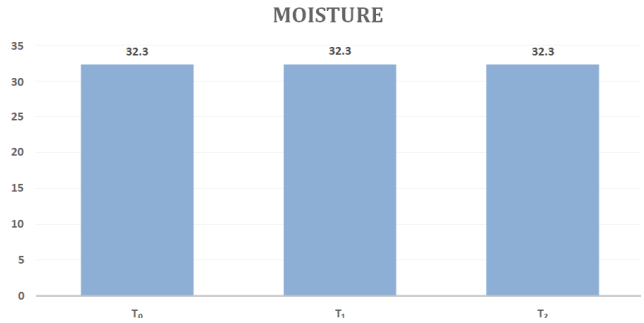

Figure 1: Estimation of Moisture Content.

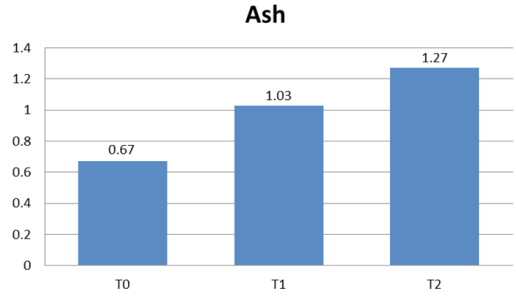

Figure 2: Estimation of Ash Content.

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Source of Variation	Sum of Square	Degree of Freedomn	Mean Square	F-value	P-Value
Between Groups	0.32	2	0.16	65535	0
Within Groups	0	6	0		
Total	0.32	8			

Table 5: Analysis of Variance for Ash Content. (*p < 0.05)

Crude Fat

Scientific term Fat is referred as lipid, dissolve in non-polar solvent. There is different method used to find the quantity of fats in particular food. One of the method is crude fat in which the material are dissolve in ether or hexane then the solvent is evaporated the remaining material is the crude fat. the crude fat of To 0.36, T1 0.36, T2 0.36 the result shows non-significant due to no addition of oil in the chapatis.T1 is co-fortified with zinc sulphate (1.5mg) and ferrous sulphate (1.5mg). T2 is calcium carbonate of (114mg) and to is control chapati non-fortified. Zeleny ascribed the decrease in fat content of flour due to no addition of oil or during storage due to the development of oxidative rancidity [69].

Crude Protein

Crude protein is the quantity of protein in particular food. And crude protein relay on the nitrogen collection in the food protein. The crude protein is used to calculate the quantity of carbohydrates that is presence in the food. The analysis of variance shows that there might be non-significant difference of TO 8.74, T 1 8.74, T2 8.74 .T1 is co-fortified with zinc sulphate (1.5mg) and ferrous sulphate (1.5mg) .T2 is calcium carbonate of (114mg) and To is control chapati non-fortified . The decrease in protein content of fortified flour samples was agreed with the findings of Fifield and Robertson (Tables 6 & 7, Figures 3 & 4).

Source of Variation	Sum of Square	Degree of Freedom	Mean Square	F-value	P-Value
Between Groups	0	2	0	0	
Within Groups	0.02	6	0.003333		
Total	0.02	8			

Table 6: Analysis Of Variance for Crude Fat Content. (***p > 0.05**)

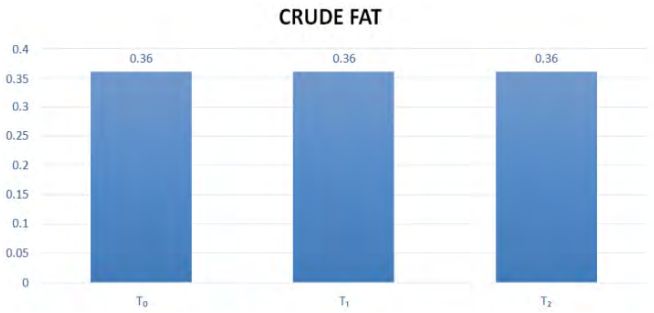

Figure 3: Estimation of Crude Fat Content.

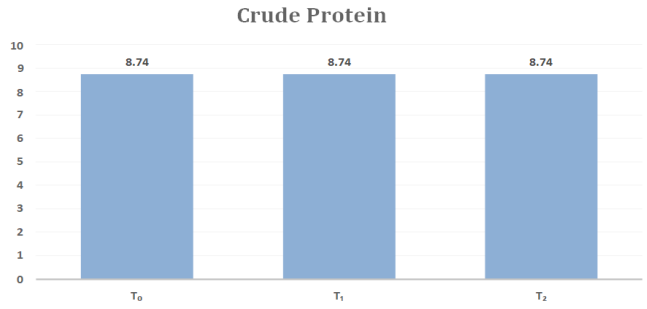

Figure 4: Estimation of Crude Protein Content.

Source of Variation	Sum of Square	Degree of Freedom	Mean Square	F-value	P-Value
Between Groups	0	2	0	0	1
Within Groups	0.0002	6	3.33E-05		
Total	0.0002	8			

Table 7: Analysis of Variance for Crude Protein Content.(*p > 0.05)

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rude Fiber

Crude Fiber is the amount of indigestible lignin,, cellulose, and the other fiber that are within the food. The finding of crude fiber of food and the feed for animal is obligatory worldwide. The analysis of variance of different treatment shows that all the three chapatis have different crude fiber .And the comparison of the treatment of different chapati T0 0.36, T1 0.36, T2 0.36. T1 is co-fortified with zinc sulphate (1.5mg) and ferrous sulphate (1.5mg). T2 is calcium carbonate of (114mg) and to is control chapati non-fortified .The more flour added the more is the crude fiber in the chapati. All the treatment has similar crude fiber due same quantity of wheat flour added in the chapatis [70].

Nitrogen free extract

Nitrogen- free extracts or NFE is important for the analysis and the calculation of nitrogen not only nitrogen but also help in calculation and analysis of sugar and starch content in food and animal feed. The nitrogen free extract is same for all the treatment of chapatis .The finding shows that T0 57.45, T1 57.45, T2 57.45 .T1 is co-fortified with zinc sulphate (1.5mg) and ferrous sulphate (1.5mg). T2 is calcium carbonate of (114mg) and to is control chapati non-fortified (Tables 8 & 9, Figures 5 & 6).

Source of Variation	Sum of Square	Degree of Freedom	Mean Square	F-value	P-Value
Between Groups	0	2	0	0	1
Within Groups	0.02	6	0.003333		
Total	0.02	8			

Table 8: Analysis of Variance for Crude Fiber Content.

c*p > 0.05

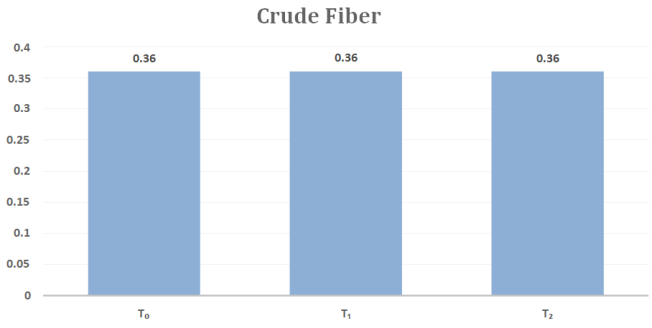

Figure 5: Estimation of Crude Fiber Content.

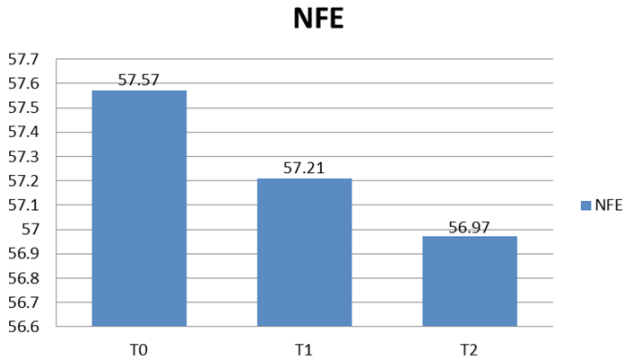

Figure 6: Estimation of NFE.

Source of Variation	Sum of Square	Degree of Freedom	Mean Square	F-value	P-Value
Between Groups	0	2	0	0	1
Within Groups	0.015	6	0.0025		
Total	0.015	8			

Table 9: Analysis of Variance for NFE Content.

*p > 0.05

Sensory Evaluation of Chapatis

Flavor: Flavor is the impression of sensory of particular food. It is control by the chemical sense of taste and smell. The "trigeminal senses", that recognizes the chemical of irritant located in the mouth and throat. The chemical sense of smell identifies the taste of the food which in other words identifies the flavor of the food. The taste of the food is limitless. The taste of food is limited to salt, sour, bitter or sweets, umami, and basic taste [71]. The word flavor is usually term as the combined chemical sense of taste and smell. In food industry the flavor of food are natural identical that means the chemical is similar to natural flavor of food but are produce commercially instead of taking extract from

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nature food .The reason for using natural identical flavor is high cost or unavailability of natural flavor when making food in food industry. In treatment T0 that is control chapati without fortified has score 7 which is like moderately and in the treatment T1 has 8 score which means like very much similarly in the treatment T2 has score 8 which means like very much. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University.

Color: Color is typically human visual perception color consist yellow, green, and white, black. In food, color is important parameter of sensory evaluation. Different food has different color. In food industry food color is used when the natural food color fade away during processing of food. The result of different treatment of chapatis shows that all the treatment T0, T1, T2 has similar score of color 8 which is like very much without using food color. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University. (Figures 7 & 8).

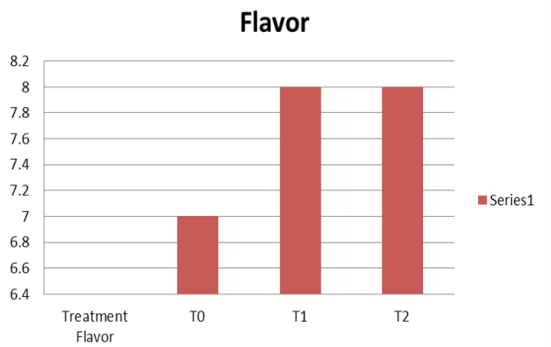

Figure 7: Comparing Flavor of Different Chapatis.

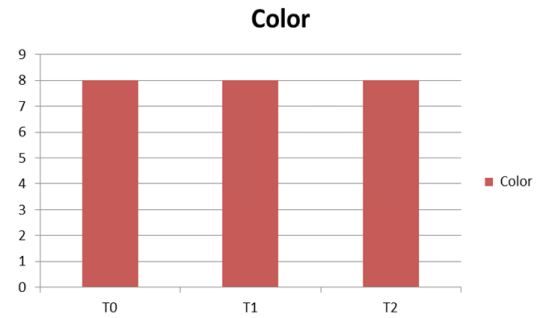

Figure 8: Comparing Color of Different Chapatis.

Taste: Taste receptors in cell located on the taste bud in the mouth or oral cavity specifically in the tongue. Other than the taste bud in the tongue human have the upper of the tongue and the epiglottis. The gustatory cortex is the part of the brain that is responsible mainly for the taste perception.in the structure of the tongue there is thousands of mini bumps called as papillae which can be seen in naked eye. And within each of the papillae have hundreds of taste buds and is present in the upper, lower and the side of the tongue. There is overall five type of taste which is sweetness, sourness, saltiness, bitterness, and umami. The treatment of all the chapatis when sensory evaluated according to Hedonic scale shows that treatment T0 that is the control chapati have scored of 8 which means like very much], and treatment T1 have scored 8 which means like very much, and T2 shows 7score which is like moderately. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University.

Appearance: The first important ascribe of any food is it appearance. The appearance of food depends on the color of the food. Every food has its natural appearance the define particular food. The alter during the ripening of fruit or the change in color cause loss in its appearance which make the fruit an acceptable to consume. However, the color of food is not the only definition for appearance. Different treatment of chapatis have the same appearance and according to hedonic scale all the chapatis of T0, T1, T3 scored 8, 8 ,8 which means like very much foe all the treatment of chapatis. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University. (Anonymous, 2002. The American Journal of Clinical Nutrition) (Figures 9 & 10).

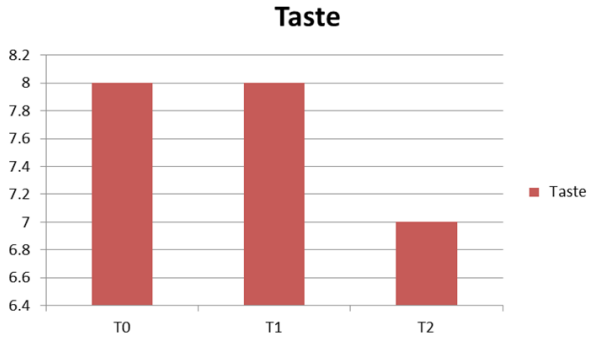

Figure 9: Comparing Taste of Different Chapatis.

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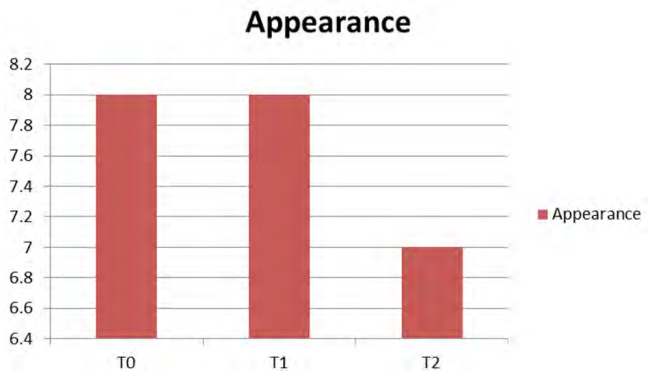

Figure 10: Comparing Appearance of Different Chapatis.

Figure 11: Comparing Acceptability of Different Chapatis.

Aroma: Aroma is of odor that is produce from the particular food. Different food has different aroma that define particular food. Aroma is the chemical of one or more volatile compound. Aroma generally move from low concentration that human understand the type of aroma through their sense of smell. Ascent can be pleasant or unpleasant smell but aroma, fragrance and scent are only referred to pleasant smell they are mostly used in cosmetic industry and food. By using hedonic scale for different treatment of chapatis shows that of T0 scored 7 which means like moderately and T1 scored 8 which means like very much and T2 shows 7 which like moderately. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University (Anonymous, 2002. The American Journal of Clinical Nutrition).

Overall Acceptability: Food acceptability depend on many factors that varies from individual to individual. Acceptability is intuition but varies from person to person based on sensory properties of food, individual culture, physiological status, previous exposure and expectation or the environment in which the food is consumed. Acceptability of food is multiplex depend on individual behavioral model/ food choice. The finding of different treatment of the chapatis T0, T1, T2, were scored based on hedonic scale 8, 8, 7 which like very much, like very much, like moderately. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University (Anonymous, 2002. The American Journal of Clinical Nutrition) (Figures 11 & 12).

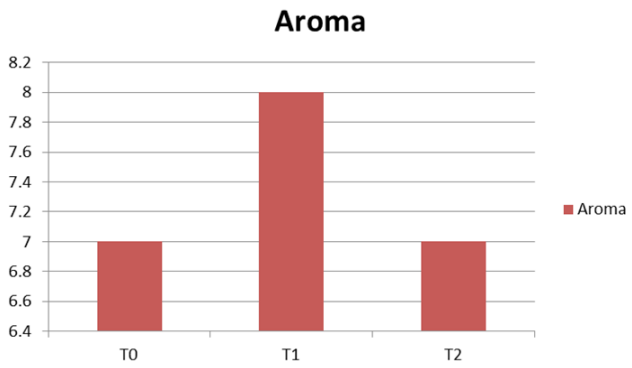

Figure 12: Comparing Aroma of Different Chapatis.

Texture: Texture is the properties of food that is sense by touch with either mouth or the hand. Different food has different texture that is soft, hard, mushy, and crunchy. Texture also defines as the acceptability of the food in the market. Texture play crucial role in sensory property and it is important characteristic than taste, appearance, and smell. Texture makes the food interesting and mouth-fell by the interaction of the surface in the mouth and the food. Texture also defined as the process of the brain during the mastication of the food. By using hedonic scale for sensory evaluation different treatment of chapatis show texture of treatment T0 7, T1 7, T2 8 which means like moderately, like moderately, like very much. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University (Anonymous, 2002. The American Journal of Clinical Nutrition).

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Chew-ability: Chew-ability is defined as the degree or the quality of the food that is easily teared or cut during the mastication. Chew-ability of food is either soft or easy to chew or hard that is difficult to chew or slightly chew. Different food has different degree of chew-ability like depend on the texture of the food. By using hedonic scale the treatment of each chapatis show chew-ability T0 7, T1 8 T2 7 which is like moderately, like very much, like moderately. There were 4 panelists who gave score for each treatment of chapatis. Panelists that are allergic to the ingredient of the food were removed from his study. This study of sensory evaluation and hedonic scale was approved by the Institutional Review Board at Georgia Southern University (Anonymous, 2002. The American Journal of Clinical Nutrition) (Figures 13 & 14).

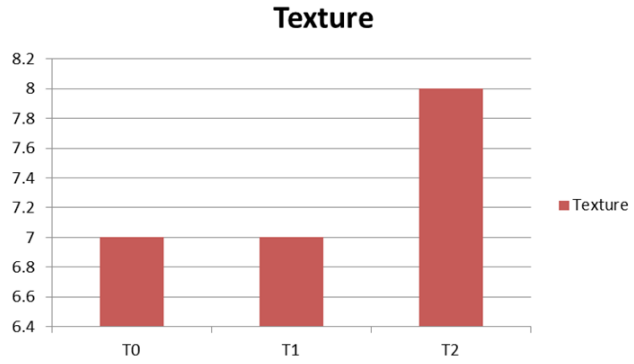

Figure 13: Comparing the Texture of Different Chapatis.

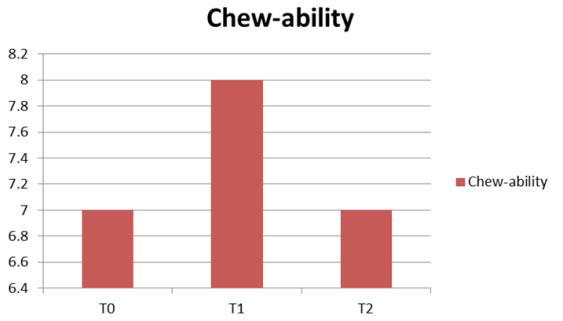

Figure 14: Comparing Chew-Ability of Different Chapatis.

Summary

Food Fortification is process in which the extra minerals or vitamins are added in food to improve the food nutritional quality of food and improve the deficiency of micro-nutrient for individual with micro-nutrient deficiency. When it comes to fortification of food it is best to choose staple food that is consumed in large amount in any country. Wheat flour is the staple food of country like Pakistan, India. Wheat is consumed in large quantity 94% across the worldwide in form cereal, maize rice, pasta etc. Fortification of wheat flour is productive, cheap and easy method to overcome the deficiency of minerals. A large amount of population is deficiency in minerals and vitamins. One of example is anemia most of women suffer from anemia. Anemia is condition in which the amount of red blood cell is less to the physiological needs of the body. When there is imbalance between the production and the damage of erythrocytes. The deficiency of iron occurs when there is poor intake or problem related to absorption, utilization or excessive iron loss from the body.

The World Health Organization commands the addition of zinc in the form of zinc sulphate in whole wheat flour and maize flour especially to infant formula and readymade cereals. Certain country made compulsory the addition of zinc like in Indonesia and In 2012 According to the Food fortification Initiative at least 20 countries were had obligatory for zinc fortification in wheat flour .and in country like Mexico addition of zinc to the wheat and maize is voluntary. Adequate calcium is very important for the bone growth to obtain peak bone mass which later decrease the risk for disease like osteoporosis, colon and cancer .Osteoporosis is very Common in woman worldwide particular to Asian woman it has been approximated that 50% of the hip fracture will occur in Asian women. In country like Pakistan where the wheat flour is the staple food it is good to fortified the whole wheat. Good achievable fortifications of calcium consist of proximate composition, Mineral profile and the sensory evaluation of the whole wheat chapati.

Conclusion

- Food fortification is very innovative to improve the nutritional quality of vulnerable group like young children and woman. The word MNP is term as micro-nutrient powder that are added in the food either semi-solid or solid food.
- Food fortification is easy and assists the body to meet the micro-nutrient that is deficiency. Micro-nutrient is adding in the food of the children in the form of powder as children don't like to take in the form of drugs.
- Food fortification is best in place were plant based meal is common and tea consumption is excess (high fiber and phytate) and consumption of animal source is very less.
- It is very important to know the quantity to add by measuring the micro-nutrient otherwise it can cause toxicity.

Recommendation

- Wheat flour fortified is very important for therapeutic and is significant for developing country with measuring

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of the quantity of micro-nutrient.

- It is very important to know GRAS the general recognize as safe of micro-nutrient that are added in the food for fortification.
- For fortification to be success it is good to make wise selection of food for fortification like staple food consumed in large amount by particular group of population.
- Food fortification is best for people consume a large amount of plant based meal and less animal source and more tea.
- Food fortification is cheap and easy way to overcome micro-nutrient deficiency especially in the developing country.

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