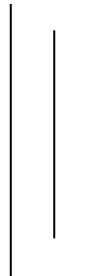


**PREPARATION AND QUALITY EVALUATION OF READY TO
USE THERAPEUTIC FOOD (RUTF) FOR CHILDREN BELOW
FIVE YEARS**



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2021

**Preparation and quality evaluation of Ready to Use Therapeutic Food
(RUTF) for children below five years**

*A dissertation submitted to the Department of Nutrition and Dietetics, Central
Campus of Technology, Tribhuvan University, in partial fulfillment of the
requirements for the degree of B.Sc. Nutrition and Dietetics*

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Approval letter

This *dissertation* entitled *Preparation and quality evaluation of Ready to Use Therapeutic Food (RUTF) for children below five years* presented by **Priya Pradhan** has been accepted as the partial fulfillment of the requirements for the **Bachelor degree in Nutrition and Dietetics**

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Regards,

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Abstract

Severe acute malnutrition has been the major cause of morbidity and mortality of people in the world. RUTF is an energy-dense, mineral- and vitamin-enriched food that requires no preparation and is specifically designed to treat severe acute malnutrition (SAM). RUTF is soft and can be consumed easily by children from the age of 6 months. According to the formulae, eleven products of RUTF (Ready to Use Therapeutic Food) were prepared using cereals, legumes, nuts, oilseeds, oil, sugar, SMP and vitamin and mineral premix.

On the basis of sensory evaluation, RUTF consisting 11 parts of wheat, 13 parts of maize, 11 parts of soybean, 1 part of bengal gram, 10 parts of pumpkin seed, 10 parts of peanut, 11 parts of milk powder, 17 parts of sunflower oil, 15 of parts sugar and 1 part of vitamin and mineral premix was found to be the best among the eleven products. The product was analyzed for proximate composition, mineral, vitamin, microbiological quality, organoleptic quality, rate of change in PV and AV, and cost calculation. The protein, fat, carbohydrate, crude fiber, total ash, vitamin C, iron and calcium of the product were found to be 13%, 35.76%, 45.44%, 2.7%, 1.80%, 55 mg/100gm, 13.2 mg/100gm and 535.6 mg/100gm respectively. The diet can supply 555 Kcal/100 gm. The energy contributed by the protein, fat and carbohydrate were found to be 9.35%, 57.9% and 32.71% of total Kcals respectively and contains all the essential amino acids required by the children under 5 years of age. The diet was found to be microbiologically safe. The total plate count and yeast and mold count in the product were 2.13×10^3 cfu/g and 42 cfu/g respectively. The cost of product was calculated as NRs. 28.10/100 gm. The rate of change in AV and PV under HDPE packaging when studied for four consecutive weeks were found within the acceptable limit.

Contents

Approval letter	iii
Acknowledgements	iv
Abstract.....	v
List of tables	ix
List of figures.....	x
List of abbreviations	xi
Introduction.....	1-5
1.1 General introduction.....	1
1.2 Statement of the problem	3
1.3 Objectives.....	4
1.3.1 General objectives.....	4
1.3.2 Specific objectives	5
1.4 Significance of the study	5
1.5 Limitations	5
Literature review	6-36
2.1 Food and nutrition	6
2.2 Components of nutrition	7
2.2.1 Macronutrients	7
2.2.2 Micronutrients.....	7
2.3 Nutritional status of children in Nepal	8
2.3.1 Recommended Dietary Allowances (RDAs) for vulnerable groups	10
2.4 Malnutrition	11
2.4.1 Causes of malnutrition	12
2.4.2 Categories of malnutrition	13
2.4.3 Diagnosis of acute malnutrition.....	16
2.4.4 Integrated management of acute malnutrition	16
2.5 Ready-to-use supplementary food (RUSF).....	22
2.6 Ready-to-use therapeutic food (RUTF)	23
2.7 Raw materials and their nutritive value	24
2.7.2 Cereals	24
2.7.3 Legumes.....	25
2.7.4 Oil seeds.....	27
2.7.5 Skim milk powder.....	28
2.7.6 Sunflower oil.....	28

2.7.7	Sucrose.....	29
2.7.8	Mineral salt	29
2.8	Technology of processing of RUTF.....	30
2.8.2	Soaking or steeping.....	30
2.8.3	Drying	31
2.8.4	Roasting	31
2.8.5	Milling and sieving	32
2.8.6	Blending.....	32
2.8.7	Packaging of RUTF	32
2.9	Packaging	32
2.9.2	Food packaging materials	33
2.9.3	Special feature required for RUTF packaging.....	33
2.9.4	HDPE.....	33
2.10	Shelf life	34
2.11	Rancidity of fat and oils	34
2.12	Use of RUTF	36
Materials and methods		37-44
3.1	Materials.....	37
3.1.1	Wheat (Triticum aestivum).....	37
3.1.2	Maize (Zea mays)	37
3.1.3	Soybean (Glycine max)	37
3.1.4	Bengal gram (Cicer arietinum)	37
3.1.5	Pumpkin seed (Cucurbita pepo).....	37
3.1.6	Peanut (Arachis hypogaea L).....	37
3.1.7	Milk powder.....	37
3.1.8	Sunflower oil.....	37
3.1.9	Sugar	38
3.1.10	Vitamin and mineral premix	38
3.1.11	Packaging material.....	38
3.2	Methods.....	38
3.2.1	Processing of raw materials	38
3.2.2	Formulation.....	40
3.2.3	Experimental design	41
3.2.4	Product preparation.....	42
3.2.5	Evaluation of prepared RUTF.....	43
3.3	Cost calculation.....	44

3.4	Data analysis	44
Results and discussion		45-53
4.1	Formulations of the products	45
4.2	Sensory analysis of the products	45
4.3	Analysis of optimized RUTF	49
4.4	Microbiological quality of product	50
4.5	Rate of change in AV and PV and shelf life of the RUTF.....	50
4.5.1	Changes in acid value	50
4.5.2	Changes in peroxide value.....	51
4.6	Cost of product.....	52
4.7	Comparison of the prepared product with CG sarbottam pitho	53
Conclusions and recommendations.....		54
5.1	Conclusions	54
5.2	Recommendations	54
Summary.....		55-56
References.....		57-62
Appendices.....		63-68

List of tables

Table no.	Title	Page no.
2.1	Recommended Dietary Allowances for children below 3 years	11
2.2	Diagnostic criteria for acute malnutrition in children aged 6-59 months	16
3.1	Nutrient requirement in RUTF	40
3.2	Amount of ingredients given by Design Expert	41
4.1	Analysis of RUTF	49
4.2	Microbiological assay of the products	50
B.1.1	Two-way ANOVA (no contrast) for color	64
B.1.2	Two-way ANOVA (no contrast) for flavor	64
B.1.3	Two-way ANOVA (no contrast) for texture	64
B.1.4	Two-way ANOVA (no contrast) for taste	65
B.1.5	Two-way ANOVA (no contrast) for overall acceptability	65
C.1	Chemical composition of selected ingredients	66
C.2	Cost calculation of the product (Formula B)	67
D.1	Composition of micronutrient premix (Fortivit-FBF-v-13)	68

List of figures

Figure no.	Title	Page no.
2.1	Prevalence of different forms of malnutrition	9
2.2	Trends of nutritional status of children under five years of age in Nepal	10
2.3	UNICEF conceptual framework	12
3.1	Flowchart for the processing of wheat	38
3.2	Outline for preparation of RUTF	42
4.1	Mean sensory score for color	46
4.2	Mean sensory score for flavor	46
4.3	Mean sensory score for texture	47
4.4	Mean sensory score for taste	48
4.5	Mean sensory score for overall acceptability	49
4.6	Change in acid value during storage at room temperature	51
4.7	Change in peroxide value during storage at room temperature	52

List of abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
ATP	Adenosine tri phosphate
AV	Acid value
BMI	Body mass index
CMAM	Community management of acute malnutrition
CSB	Corn soy blend
CTC	Community based therapeutic center
FDA	Food and drug administration
GRAS	Generally recognized as safe
IDA	Iron deficiency anemia
IDD	Iodine deficiency disorder
IMAM	Integrated management of malnutrition
LNS	Lipid nutrient supplement
MAM	Moderate acute malnutrition
MOHP	Ministry of health and population
MUAC	Mid upper arm circumference
MUFA	Monounsaturated fatty acid
NPU	Net protein utilization
PC	Polycarbonate
PE	Polyethylene
PEM	Protein energy malnutrition
PER	Protein efficiency ratio

PET	Polyethylene terephthalate
PS	Polystyrene
PUFA	Poly unsaturated fatty acid
PV	Peroxide value
PVC	Polyvinyl chloride
RDA	Recommended dietary allowances
RUSF	Ready to use supplementary foods
RUTF	Ready to use therapeutic foods
SAM	Severe acute malnutrition
SDG	Sustainable development goal
SMP	Skim milk powder
TSFC	Targeted supplementary feeding center
UN	United nations
UNOCHA	United nations office for the coordination of humanitarian affairs
WFP	World food program
WHO	World health organization
WVTR	Water vapor transmission rate

Part I

Introduction

1.1 General introduction

The World Health Organization (WHO) defines malnutrition as "the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions"(Onis, 2016). Malnutrition is an acute, sub-acute or chronic state of nutrition, in which varying degrees of over nutrition or under nutrition with or without inflammatory activity have led to a change in body composition and diminished function(Charles Mueller 1, 2011).

Malnutrition is a major factor in the massive morbidity and mortality of children throughout the world. Among the many groups vulnerable to malnutrition, children are the most important ones because (i) they are powerless, (ii) saving their life is more valuable than saving life of an older person, (iii) there are well developed means for assessing the nutritional status of children on an inexpensive and objective basis, and (iv) children's malnutrition is a massive problem all around the world (G. Kent, 1993). Malnutrition has more to do with the lack of proper care and nourishment of children rather than generally perceived factors that is caused by the lack of food, money and resources. Out of child mortality of some 100 thousand children under five years of age in Nepal 55% of the children die from malnutrition, vitamin A deficiency, Iodine Deficiency Disorder (IDD), Iron deficiency, Anemia and Zinc deficiency (UNICEF, 1998).

Under nutrition is a prevalent condition in many developing countries and a predisposing factor to various forms of diseases. In poor and deprived communities, children, the aged, pregnant women and the sick are particularly vulnerable to under nutrition. For the short term, it is frequently an emergency that requires immediate nutritional intervention with foods that are nutritionally potent enough to either prevent or correct the conditions of under nutrition. Such foods should also be able to provide enough energy to meet or supplement the daily requirements of the consumer (Krah, 2014).

Malnourished children are highly vulnerable to death and diseases. Treatment of such children is not always possible in hospital or Nutrition Rehabilitation Center in rural areas, so home based treatment is preferable and best in such cases. Home

treatment include food formulated from locally available food such as cereals (rice, barley, wheat, maize), pulses (gram, soya bean), milk powder, high quality vegetable oils, peanut, pumpkin seed etc. The treatment may also include commercially produced Ready to use therapeutic food. These foods are high energy dense foods fortified with vitamin and minerals which are very effective in the treatment of SAM. The supplement of both macro and micro nutrients make Ready to use therapeutic food more effective (Wagh, 2015).

Severe Acute Malnutrition (SAM) is defined as weight-for-height ratio of less than minus 3 standard deviations below the median reference population or weight-for-height ratio of below 70% or presence of nutritional edema (Muluken Berhanu Mena, 2018).

Ready to use therapeutic food is a mixture of different nutrients designed specially to address the problem of malnutrition. The main ingredients of the formulation may include milk powder, cereals (rice or wheat or barley or maize etc.), vegetable oil, sugar, peanut paste or pumpkin seed paste, vitamin and mineral mix. Ready-to-use foods (RUF) are energy-dense food with a low moisture content that can be eaten directly from the packaging. When used for nutritional rehabilitation of children with SAM, such products are referred to as ready-to-use therapeutic food (RUTF). RUTF was originally developed as a home-based alternative to F100. RUTF, in the form of a solid or semi-solid feed, has a similar nutrient profile to F100 (except for the presence of iron) (Steve Collins 1, 2006).

The term ready to use therapeutic food refers to several varieties of ready to eat foods, ranging from those prepared from locally available foods by village women in their own self-help groups for the malnourished children in their village, to those prepared according to specific formulas in factories to be shipped all over the world. The term now almost always refers to the latter, and specifically a peanut and milk powder based spread with specified amounts of micronutrients, providing energy equivalent to WHO requirement i.e. 520-550 Kcal/100gm (Wagh, 2015).

RUTF should meets the following requisite criteria ; (Kapil, 2009)

- a) Caloric dense, high in proteins, vitamins and minerals;
- b) Simple to deliver and administer;
- c) Easy to use;
- d) Fast acting;
- e) Affordable and acceptable cost;

- f) Should not require trained staff to administer (parents can deliver it to a child);
- g) Culturally acceptable;
- h) Packed in single-serve packets (each packet may contain fixed amount of calories 400- 500 calories);
- i) Acquires little preparation before use;
- j) Adequate shelf life and stability;
- k) Can be stored in varied climatic conditions and temperature;
- l) Resistant to bacterial contamination; and
- m) Does not cause addiction to child.

1.2 Statement of the problem

The period between weaning and the age of five is nutritionally regarded as the most vulnerable period of the life cycle because that is when rapid growth, loss of passive immunity and the development of the immune system against infection occur. The first 2 years of a child's life are particularly important, as optimal nutrition during this period lowers morbidity and mortality, reduces the risk of chronic disease, and fosters better development (Steve Collins 1, 2006).

Nepal being a developing country, malnutrition has been its major problem. The trend of malnutrition is higher in the under five children. Acute malnutrition affects 10% of children aged below 5 years in Nepal, where 2% are severely malnourished and 8% are moderately malnourished (MOHP, 2016). So, management of acute malnutrition is the most. As per the WHO decision making criteria, wasting prevalence is at a critical level in Nepal, affecting an estimated 430,000 children under five years of age at any point in time (Steve Collins 1, 2006). So, integrated management of acute malnutrition (IMAM) program has been launched to reduce the burden of acute malnutrition in Nepal where the severely acute malnourished are treated using RUTF and RUSF are used in the management of moderate malnutrition (UNOCHA, 2015a).

Severe acute malnutrition remains a major killer of children under five years of age. Until recently, treatment has been restricted to facility-based approaches (i.e. hospital), greatly limiting its coverage and impact. New evidence suggests that large numbers of children with severe acute malnutrition can be treated in their home without being admitted to a hospital (W. WHO, UNICEF, UN, 2007). The community-based approach involves timely detection of severe acute malnutrition in the community and provision of treatment for those without medical complications with ready-to-use therapeutic

foods. If RUTF is properly combined with facility-based approach for those malnourished children with medical complications and implemented on a large scale, community-based management of severe acute malnutrition could prevent the deaths of hundreds of thousands of children (Nutrition, 2010).

Nepal being an underdeveloped country, the prevalence of SAM is high in the children. Not all can afford the inpatient care or the facilities in the country are not enough for all the effected children. So, in a case home based treatment with Ready to use nutritious food is one of the best ways to treat SAM in rural areas. The nutrient composition of Ready to use therapeutic food is similar to F-100, the standard milk-based formula which has been demonstrated effectively in the treatment of severe acute malnutrition. Ready to use nutritious food contains significant amounts of milk powder, which has traditionally been used successfully in treating malnourished children (Mark J. Manary, 2005b). Ready to use nutritious food have a similar nutrient composition to F100, which is the therapeutic diet used in hospital settings. But unlike F100, Ready to use nutritious food is not water-based, meaning that bacteria cannot grow in them. Therefore these foods can be used safely at home without refrigeration and even in areas where hygiene conditions are not optimal (W. WHO, UNICEF, UN, 2007).

Poverty and food insecurity seriously constrain accessibility of nutritious diets, including high protein quality, adequate micronutrient content and bioavailability, essential fatty acids, low anti-nutrient content, and high nutrient density. Ready to use therapeutic food can be prepared from locally available foods which are within the accessibility of all the people and equally contain the required quantity of nutrients as well. Thus RUTF could be the best in the management of outpatient care or home based treatment of SAM children (Wagh, 2015). Ready to use foods has been highly effective in the treatment of various forms of acute malnutrition, including kwashiorkor, nutritional marasmus, and several forms of wasting. This has been proved by a number of studies (Latham, 2011).

1.3 Objectives

1.3.1 General objectives

The main objective of the study was to prepare ready to use therapeutic food (RUTF) for the children below five years.

1.3.2 Specific objectives

- a. To prepare RUTF in accordance to the specification given by WFP.
- b. To evaluate microbiological quality of the prepared RUTF.
- c. To perform sensory and physiochemical analysis of the prepared RUTF.
- d. To study storage stability of the product at room temperature.

1.4 Significance of the study

RUTF has been effective in the treatment of SAM and the use of locally available foods in the preparation of RUTF has been highly recommended (WHO, 2012b). The study initiated by this dissertation work can be further extended in wide aspect (e.g. evaluation of nutritional quality, clinical trials etc.) in the future and if all the experimental result gives positive response, this diet can be prescribed for children with severe acute malnutrition and can be produced in the industrial scale. This formula will be beneficial especially to the malnourished children of refugee camp, slum dwellers, and flood victim. Any factory, government and non-government agencies, local agencies and others whose primary aim is to improve the nutritional status of people can produce the RUTF using this formula, and this work will provide the basis for the further work in this field.

1.5 Limitations

1. In-vitro analysis could not be done.
2. Analysis of amino acid and fatty acid composition of the product could not be performed.

Part II

Literature review

2.1 Food and nutrition

Food is anything edible in nature that is socially and culturally accepted. Beside something to eat, food is an integral part of society, region, or a country. In general, human eat everything which he considers safe from toxicological point of view. Food is a relative concept. A food which is considered edible in one culture or a community may not be edible in other culture or community. Food is also thought in terms of energy and nutrition (Hartog, 2006). Food supplies both the energy for all the body's functions and the building blocks for growth and maintenance. Even in fully grown adults there is a requirement for energy and to build and maintain body components that are being replaced. For example, the human stomach is constantly being lost and replaced. Also there is increasing evidence that diet play a major role in our defense against disease, including chronic disease such as cancer and heart disease. Mental processes and behavioral attitudes appear to be influenced by nutritional status and specific nutrients (Norman N. Potter, 2007).

Nutrition is the scientific study of food and how it nourishes our body and influences our health. It studies how foods are consumed, digested, metabolized, and nutrients are stored and how these nutrients affect our body. It also encompasses the factors influencing the quality and quantity of food, attempts made to maintain food safety and the issues related to global food supply (Netherlands, 2012). Nutrition is the intake of food, considered in relation to the body's dietary needs. Good nutrition an adequate, well balanced diet combined with regular physical activity is a cornerstone of good health. Poor nutrition can lead to reduced immunity, increased susceptibility to disease, impaired physical and mental development, and reduced productivity whereas better nutrition is related to improved infant, child and maternal health, stronger immune systems, safer pregnancy and childbirth, lower risk of non-communicable diseases (such as diabetes and cardiovascular disease), and longevity. Healthy children learn better. People with adequate nutrition are more productive and can create opportunities to gradually break the cycles of poverty and hunger. Good Nutrition is associated with human well-being. Right from the pregnancy to birth, during the infancy and in adulthood good nutrition plays a vital role in physical and mental well-being of human

being including brain functioning, immune system and physical activities, which ultimately leads to increase overall productivity of human being. Good nutrition flows throughout the life cycle and across the generations, so it plays a central role in the country's overall development ((IFPRI), 2014).

2.2 Components of nutrition

2.2.1 Macronutrients

Macronutrients are nutrients that provide calories or energy and are required in large amounts to maintain body functions and carry out the activities of daily life. There are three broad classes of macronutrient: proteins, carbohydrates and fats (Venn, 2020). Protein, fat and carbohydrates are macronutrients that make up the bulk of a diet and supply the body's energy. In resource-poor populations, carbohydrates (i.e. starches and sugars) are often a large part of the diet (80%) and the main source of energy. Fats supply energy and are important in cell formation. Proteins are required to build new tissue and are derived mostly from animal origin such as milk, meat and eggs. These animal byproducts contain essential amino acids that cannot be produced by the body but must be eaten. Protein from cereals and pulses alone do not provide the sufficient balanced essential amino acids. Therefore, to obtain the correct balance without requiring protein from animal sources, cereals and pulses must be combined when planning a meal (Becker, 2013).

2.2.2 Micronutrients

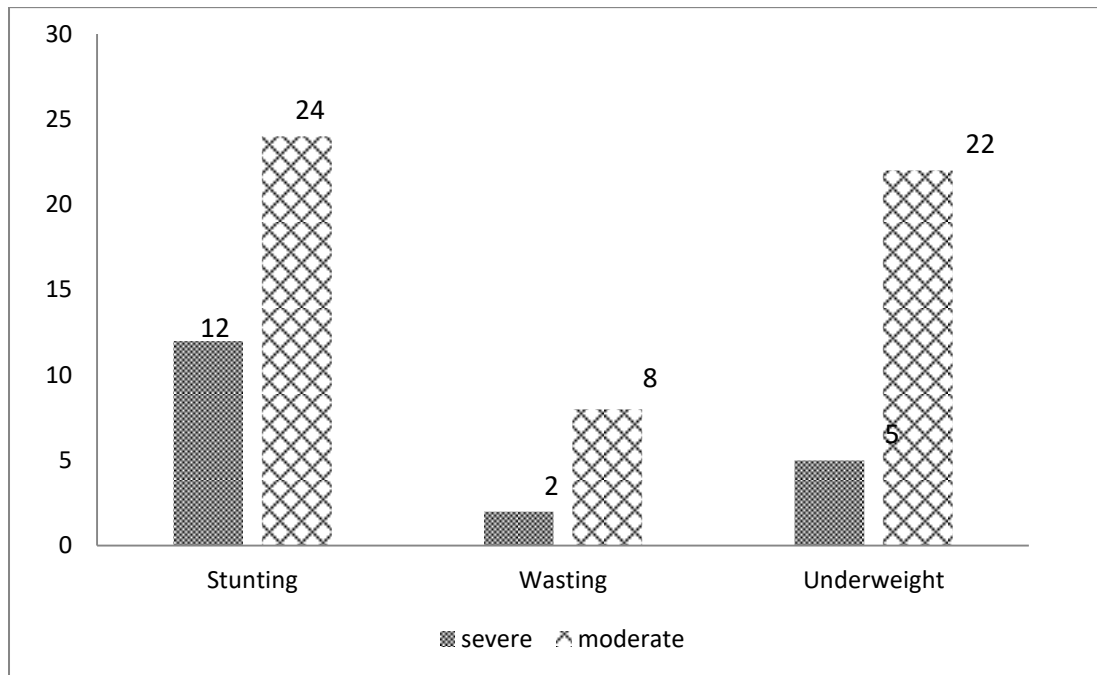
Micronutrients are one of the major groups of nutrients our body needs. They include vitamins and minerals. There are around forty different micronutrients that are essential for good health. Micronutrients are divided into two classes. Most micronutrients are classed as type I, which includes iodine, iron, vitamins A and C. Deficiencies in type I micronutrients do not affect growth (i.e. the individual can have normal growth with appropriate weight and still be deficient in micronutrients) and thus deficiency in type I micronutrients is not determined by anthropometric measurement. Deficiencies in type I micronutrients will cause major illness such as anemia, scurvy and impaired immunity. Type II micronutrients, including magnesium, sulphur, nitrogen, essential amino-acids, phosphorus, zinc, potassium, sodium and chloride, are essential for growth and tissue repair. Type II micronutrients are required only in small quantities, but the correct

balance is essential for good health. A deficiency in any of the type II micronutrients will lead to growth failure, measured by stunting and wasting (Shenkin, 2006).

2.3 Nutritional status of children in Nepal

Nutritional status is the condition of the health of the characteristics individuals as influenced by the utilization of the nutrients. It can be determined only by the correlation of information obtained through a careful medical and dietary history, a through physical examination and appropriate laboratory investigations (Corinne H. Robinson, 1990). Nutritional status is a requirement of health of a person convinced by the diet, the levels of nutrients containing in the body and normal metabolic integrity. Normal nutritional status is managed by balance food consumption and normal utilization of nutrients (international). The nutritional status of children under age five is an important measure of child's health. Children nutritional status is a reflection of their overall health. When children have access to an adequate food supply, are not exposed to repeated illness, and are well cared for, they reach their growth potential and are considered well nourished. Malnutrition is associated with more than half of all child deaths worldwide. Undernourished children are more likely to die from common childhood ailments and, for those who survive, have recurring sicknesses and faltering growth. Three quarters of the children who die from causes related to malnutrition are only mildly or moderately malnourished showing no outward sign of their vulnerability (CBS, 2015). One of the 17 goal of SDGs is to end all forms of malnutrition by 2030 and Government of Nepal has already set its target to achieve it (NPC, 2015).

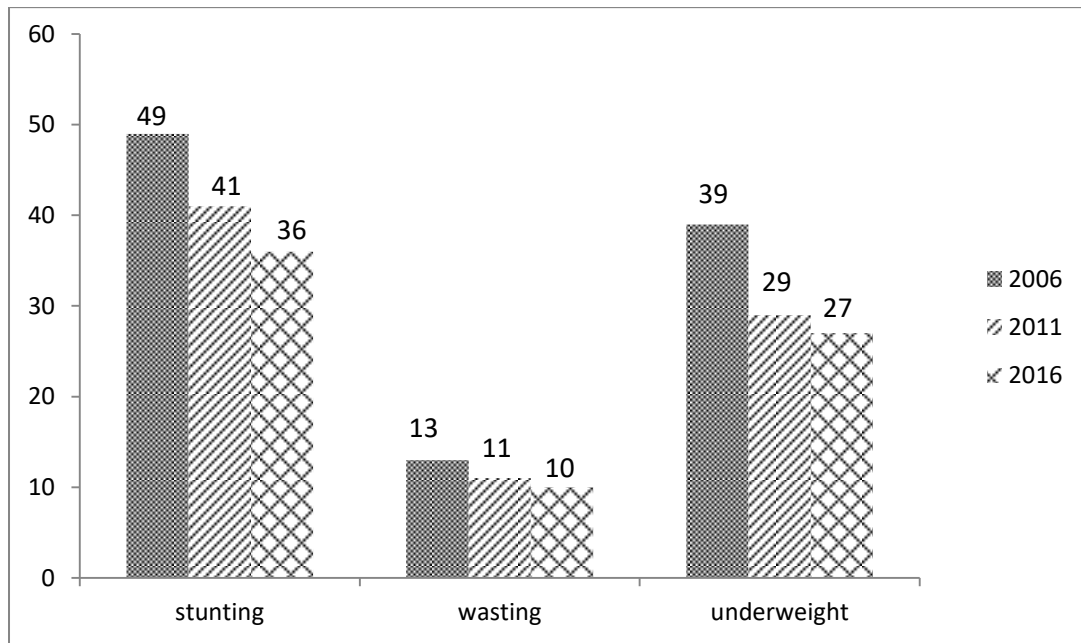
According to NDHS 2016, 36% of the children under five years of age are stunted, out of which 12% are severely stunted. Similarly, 10% of children are wasted, out of which 2% are severely wasted and 27% of the children under five years of age are underweight, out of which 5% are severely underweight (NDHS, 2016).



Source: (NDHS, 2016)

Fig 2.1 Prevalence of different forms of malnutrition in Nepal

The consequences of malnutrition are a significant concern for the government economy as well as society. The high mortality due to malnutrition leads to the loss of the economic potential of the child. It affects children in many ways, predisposing them to different infectious diseases, psychosocial mal development, and cognitive deficiencies (UNOCHA, 2015b).



Source: (ICMR, 2010)

Fig 2.2 Trends of nutritional status of children under five years of age in Nepal

2.3.1 Recommended Dietary Allowances (RDAs) for vulnerable groups

The recommended dietary allowance (RDA) is the average daily dietary intake level that suffices to meet the nutrient requirements of nearly all (97–98%) healthy persons of a specific sex, age, life stage, or physiological condition (such as pregnancy or lactation). The RDA is a nutrient intake goal for planning the diets of individuals (R.H. GLEW, 2006). Diets of various types can be devised to meet recognized nutritional needs. However, RDAs should be provided from a selection of foods that are acceptable and palatable to ensure consumption (Press, 1989). Child nutrition is vital for proper growth and development and as the sick and aged they are considered vulnerable because they have special nutritional requirement (RDAs) to achieve the required rate of growth for a period (Maria Hermoso 1, 2010).

Table 2.1 Recommended Dietary Allowances for children <3 years

Nutrients	0-6 months	6-12 months	1-3 years
Body wt. (kg)	5.4	8.4	12.9
Net Calories (Kcal/day)	92 kcal/kg	80 kcal/kg	1060
Proteins (g/day)	1.16 g/kg	1.69 g/kg	16.7
Visible fat (g/day)	-	19	27
Calcium (mg/day)	500	500	600
Iron (mg/day)	46µg/kg	05	09
Vitamin A (µg/day) Retinol	350	350	400
B- Carotene	-	2800	3200
Zinc (mg/day)	-	-	5
Magnesium (mg/day)	30	45	50
Thiamine (mg/day)	0.3	0.2	0.5
Riboflavin (mg/day)	0.4	0.6	0.8
Niacin (mg/day)	710µg/kg	650 µg/kg	8
Pyridoxine (mg/day)	0.1	0.4	0.9
Vit B12 (µg/day)	0.2	0.2	0.2-1.0
Ascorbic acid (mg/day)	25	25	40
Dietary folate (mg/day)	25	25	80

Source: (ICMR, 2010)

2.4 Malnutrition

Malnutrition refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients. Malnutrition has been defined in different ways some believe that it is a result of an imbalance in the intake of nutrient; whereas other say that it is the result of too little or even too much intake of certain nutrient. There are still other who say it is a clinical syndrome with typical symptoms and signs depending on the type of nutrient responsible for the disease. Nevertheless, both over nutrition and under nutrition are considered malnutrition. Malnutrition has been defined as a pathological state resulting from a relative or absolute deficiency or excess of one or more of the essential nutrients in the diet (Jelliffe, 1966).

2.4.1 Causes of malnutrition

The UNICEF conceptual framework, developed in the 1990s and shown below, summarizes the causes of malnutrition;

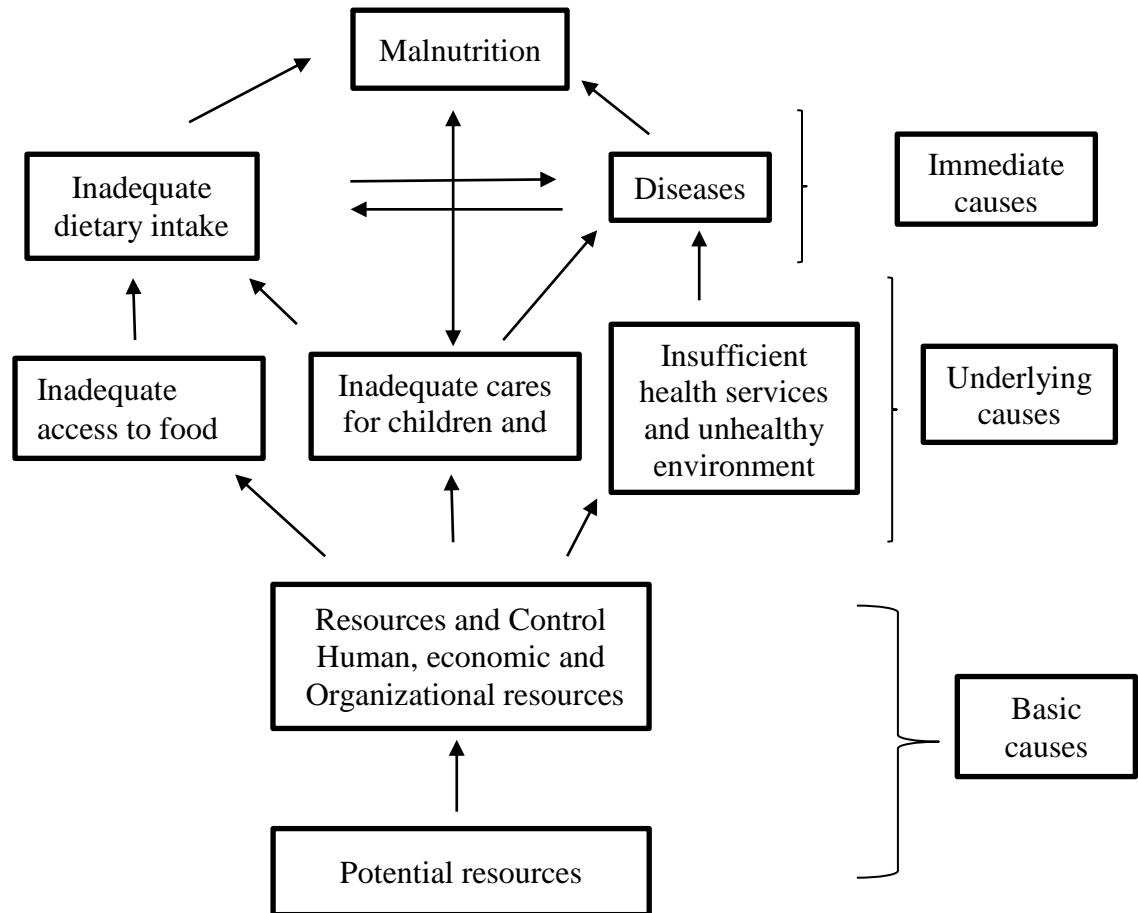


Fig 2.3 UNICEF conceptual framework

Source: (UNICEF, 1990)

2.4.1.1 Immediate causes of malnutrition

Lack of food intake and disease are immediate cause of malnutrition and create a vicious cycle in which disease and malnutrition exacerbate each other. It is known as the malnutrition infection complex. Thus, lack of food intake and disease must both be addressed to support recovery from malnutrition (UNICEF, 1990).

2.4.1.2 Underlying causes of malnutrition

Three major underlying causes of malnutrition (UNICEF, 1990) include:

- Food: Inadequate household food security (limited access or availability of food).

- Health: Limited access to adequate health services and/or inadequate environmental health conditions.
- Care: Inadequate social and care environment in the household and local community, especially with regard to women and children.

2.4.1.3 Basic causes of malnutrition

The basic causes of malnutrition in a community originate at the regional and national level, where strategies and policies that affect the allocation of resources (human, economic, political and cultural) influence what happens at community level. Geographical isolation and lack of access to markets due to poor infrastructure can have a huge negative impact on food security. When conducting an assessment to determine the causes of malnutrition in a community, it is important to research the actions at each level and how these actions, or inactions, influence malnutrition rates (UNICEF, 1990).

2.4.2 Categories of malnutrition

Types of malnutrition fall into two general categories, acute and chronic. Acute malnutrition, most often demonstrated by wasting, is frequently seen in temporary or cyclical settings like emergencies, seasonal depressions, and highly infectious-disease environments (Reinhardt, 2014).

2.4.2.1 Chronic malnutrition

This is a condition that develops when children do not eat the correct balance of nutrients in the first 1,000 days of life (from conception to the age of two), resulting in the irreversible stunting of their cognitive and physical development (WHO, 2017). Chronic malnutrition is defined as a form of growth failure that causes both physical and cognitive delays in growth and development. Stunting, also known as linear growth failure, and is defined as the inability to attain potential height for a particular age, and it is the most common measurement used to identify chronic malnutrition. Compared to children who have been given optimal opportunities to grow and develop, a chronically malnourished child will be challenged to attain the same height, will likely not develop the same cognitive ability, and will have higher risk of poor health outcomes throughout life. To treat a patient with chronic malnutrition requires a long-term focus that considers household food insecurity in the long run; home care practices (feeding and hygiene practices); and issues related to public health (Reinhardt, 2014).

2.4.2.2 Acute malnutrition

Acute malnutrition is a devastating epidemic. Acute malnutrition is a recent and severe weight loss as a result of acute food shortage and/or illness. It is the leading causes of morbidity and mortality of children aged 6–59 months as risk of death is nine times higher than that of children without it. The human body needs energy and nutrients to function. If food intake is inadequate, the body begins to break down body fat and muscle, the metabolism begins to slow down, thermal regulation is disrupted, the immune system is weakened, and kidney function is impaired (WHO, 2017). The acute malnutrition is further classified into severe acute malnutrition and moderate acute malnutrition on the basis of mid upper arm circumference (MUAC) and weight for height z-score (Ayana, 2015). Clinical forms of acute malnutrition can be defined by the characteristics of severe wasting, also called marasmus, and/or bilateral pitting edema, found in kwashiorkor as well as in a range of other clinical signs (Dibari, 2013).

2.4.2.2.1 Severe acute malnutrition (SAM)

Severe acute malnutrition (SAM) is defined as a weight-for-height measurement of 70% or less below the median, or three SD or more below the mean National Centre for Health Statistics references values, the presence of bilateral pitting edema of nutritional origin, or a mid-upper-arm circumference of less than 110mm in children age 1-5 years (Lancet, 2006). Severe acute malnutrition is a life threatening condition requiring urgent treatment. Until recently, the recommendation was to refer these children to hospital to receive therapeutic diets along with medical care. The situation changed recently with the advent of ready to use therapeutic foods (RUTF) which allows the management in the community of large numbers of children who are severely malnourished above the age of 6 months without medical complications (Steve Collins 1, 2006).

SAM affects nearly twenty million children under 5 years, causing up to 1 million deaths each year by increasing susceptibility to death from severe infection. The most susceptible age for malnutrition is 6 to 18 months, (when growth velocity and brain development are especially high); however, with many low-income settings introducing solids to children as young as two months, it is increasingly recognized that SAM may occur in infants aged < 6 months. SAM is defined by two distinct clinical entities:

- i. **Severe wasting** (marasmus; defined as middle upper arm circumference [MUAC]<115mm in children 6-59 months, or a weight-for-height/length <-3 Z

scores according to the 2006 WHO growth standards) in children aged 0 to 59 months;

- ii. **Nutritional edema** (kwashiorkor; defined as bilateral pitting edema).

Children with SAM are classified according to the absence or presence of medical complications:

- i. **Uncomplicated SAM:** children who are clinically well without signs of infection or other indication for hospital admission, with a retained appetite ('passed an appetite test'). Retained appetite is regarded to indicate the absence of severe metabolic disturbance. These patients are deemed to be most appropriately managed as outpatients, with ready-to-use therapeutic foods.
- ii. **Complicated SAM:** children who have clinical features of infection, metabolic disturbance, severe edema, hypothermia, vomiting, severe dehydration, severe anemia or a lack of appetite, requiring inpatient treatment initially with low protein milk-based feeds. Children are discharged to continue nutritional management as an outpatient is recommended when complications have resolved (Berkley, 2016).

2.4.2.2.1.1 Marasmus

Marasmus is a form of severe under nutrition, referred to alternatively as non-edematous malnutrition. It is due to consumption of diet deficient in both protein and calorie. It is most commonly seen in weaned infant of about 6-18 months (WHO, 2017). The Well come classification associated marasmus with "severe wasting of fat and muscle, which the body breaks down to make energy leaving 'skin and bones'. A child with marasmus is extremely thin with a wizened 'old man' appearance (Dibari, 2013).

2.4.2.2.1.2 Kwashiorkor

Kwashiorkor is a form of severe under nutrition, also referred to as edematous malnutrition. This type of under nutrition is common in children between the age of 6 months and 3 years, a period of life when complementary feeding plays an important role in child's growth and development (WHO, 2017). Kwashiorkor is associated with growth failure (when compared with healthy children) and characterized by edema, loss of appetite, thin, sparse or discolored hair and skin with discolored patches that may crack and peel. Kwashiorkor is associated with a pitting edema affecting both sides of the body (bilateral). Edema is defined as "swelling from excessive accumulation of watery fluid in cells, or tissues" (Dibari, 2013).

2.4.2.2.1.3 Marasmic Kwashiorkor

Marasmic kwashiorkor is caused by acute or chronic protein deficiency and chronic energy deficit and is characterized by edema, wasting, stunting, and mild hepatomegaly. Marasmic kwashiorkor is a form of severe under nutrition diagnosed by the presence of risk of short-term death and is assessed by the circumference of the left middle upper arm (MUAC), whereas nutritional indices referring to z-scores are more indicated to define the anthropometry of children presenting MAM or SAM. In addition, the presence of bilateral pitting edema is considered diagnostic for SAM (Dibari, 2013).

2.4.3 Diagnosis of acute malnutrition

Acute malnutrition is determined by a child's weight and height, by calculating weight for height as "z-score", and presence of edema. All patients with bi-lateral edema are considered to have severe acute malnutrition. MUAC is often the screening tool used to determine malnutrition for children in the community under five years old. A very low MUAC (<11.5cm for children under five years) is considered a high mortality risk and is a criterion for admission with severe acute malnutrition (WHO, 2013).

Table 2.2 Diagnostic criteria for acute malnutrition in children aged 6-59 months

	Measure	Cut-off
Severe acute malnutrition	Weight for height	<-3 SD
	MUAC	<115 mm
	Bilateral pitting edema	Grades 1, 2, 3
Moderate acute malnutrition	Weight for height	<-2 SD and \geq -3 SD
	MUAC	<125 mm and \geq 115mm

Source : (WHO, 2013)

2.4.4 Integrated management of acute malnutrition

Integrated Management of Acute Malnutrition (IMAM) is a new approach in the management of severe acute malnutrition for effective care and rehabilitation. It also addresses management of moderate acute malnutrition (UNOCHA, 2015a).

2.4.4.2 Principles of IMAM

IMAM is a strategy to address acute malnutrition. IMAM focuses on the integration of effective management of acute malnutrition into the ongoing routine health services at all levels of the health facilities whilst still striving for maximum coverage. It also aims to integrate the management of acute malnutrition across the sectors to ensure that treatment is linked to support for continued rehabilitation of cases and to wider malnutrition prevention programs and services focused on the critical 1000-day windows (UNOCHA, 2015a).

IMAM is based on the same principles as the initial CMAM program (UNOCHA, 2015a). These are as follows:

Maximum coverage and access – IMAM is designed to achieve the greatest possible coverage by making services accessible and acceptable to the highest possible proportion of a population in need.

Timeliness – IMAM prioritizes early case-finding and mobilization so that most of the cases of acute malnutrition can be treated before complications develop.

Appropriate care – Provision of simple, effective outpatient care for those who can be treated at home and clinical care for those who need inpatient treatment. Less intensive care is provided for those suffering from MAM.

Care for as long as it is needed - By improving access to treatment and integrating the service into the existing structures and health system, IMAM ensures that children can stay in the program until they have recovered.

2.4.4.3 Management of severe acute malnutrition

The severely malnourished child with an adequate appetite is best managed at home with ready-to-use therapeutic food. The treatment of severely malnourished includes feeding the children with a RUTF until they have gained adequate weight. In some settings, it may be possible to construct an appropriate therapeutic diet using locally available nutrient-dense foods with added micronutrient supplements. However, this approach requires very careful monitoring because nutrient adequacy is hard to achieve. In addition to the provision of RUTF, children need to receive a short course of basic oral medication to treat infections. Follow-up, including the provision of the next supply of RUTF, should be done weekly or every two weeks by a skilled health worker in a nearby clinic or in the community (UNICEF, 2007a).

Management of severe acute malnutrition (SAM) in children comprises two potential phases: stabilization and rehabilitation. During the initial stabilization phase, children receive treatment for dehydration, electrolyte imbalances, intercurrent infections and other complications. In the rehabilitation phase (applicable to children presenting with uncomplicated SAM or those with complicated SAM after complications have been resolved), catch-up growth is the main focus and the recommended energy and protein requirements are much higher. In-hospital rehabilitation of children with SAM is not always desirable or practical - especially in rural settings - and home-based care can offer a better solution. Ready-to-use therapeutic food (RUTF) is a widely used option for home-based rehabilitation (Lombard *et al.*, 2019)

The severely malnourished child with immediately life threatening complications should be stabilized in an inpatient facility. Treatment of severe malnutrition in the facility involves feeds of small amounts of liquid food every two hours. The recommended daily energy intake of 100kcal/kg/day is provided by a milk based formula called F-75. Once the child with complicated severe malnutrition has regained appetite and is no longer unstable, usually after a few days of treatment in a health facility, he or she is best managed as the child with uncomplicated severe malnutrition. At this point the child's diet is advanced to a high energy, high protein ready to use therapeutic food (M. J. a. S. Manary, H. L. , 2008).

RUTFs have been used at the rehabilitation stage of treating SAM and have been proven successful in the implementation of community based therapeutic care (CTC) (Steve Collins 1, 2006). This concept was developed as an alternative to the in-patient therapeutic care system which has been relatively ineffective in curtailing the effect of SAM (Isabelle Defourny, 2009) due to cost the availability of facilities and their effect on the number of people reached by these interventions (Karakochuk, 2012).

Children with severe acute malnutrition need safe, palatable foods with a high-energy content and adequate amount of vitamins and minerals. RUTF are soft or crushable foods that can be consumed easily by children from the age of six months without adding water. When there are no medical complications, a malnourished child with appetite, if aged six months or more, can be given a standard dose of RUTF adjusted to their weight. Guided by appetite, children may consume the food at home, with minimal supervision, directly from a container, at any time of the day or night. Because RUTF

do not contain water, children should also be offered safe drinking water to drink (UNICEF, 2007a).

Ready to use high energy foods to be provided to the caregiver of a malnourished child has been a paradigm shift in the management of malnutrition. Home-based treatment has been recommended during the rehabilitation phase of treatment for malnutrition in areas where follow up is possible (Ashworth, 2006).

Ready-to-use therapeutic food (RUTF) is a high-energy, micronutrient enhanced paste used to treat children under age 5 who are affected by severe acute malnutrition (UNICEF, 2013b). Ready-to-use therapeutic food (RUTF) provides a scientifically based combination of easily accessible macronutrients, plus essential minerals and vitamins. It is very energy dense and does not need to be mixed with water. This certainly makes it an appropriate food for treatment of severe acute malnutrition. RUTF is highly effective in the treatment of various forms of severe acute malnutrition, including kwashiorkor, nutritional marasmus, and several forms of severe wasting (Latham, 2011).

The use of RUTF has transformed the treatment of severe acute malnutrition, in part, because it allows those children without medical complications to be cured right in their own homes and communities. This approach is referred to as the community-based management of severe acute malnutrition. RUTF is essential for the community-based management of children who are suffering from uncomplicated severe acute malnutrition and who retain an appetite. First, it provides all the nutrients required for recovery. Second, it has a good shelf life, and does not spoil easily even after opening. Third, since RUTF is not water based, the risk of bacterial growth is very limited, and consequently it is safe to use without refrigeration at household level. Fourth, it is liked by children, safe and easy to use without close medical supervision. Finally, it can be used in combination with breastfeeding and other best practices for infant and young child feeding (UNICEF, 2013a).

2.4.4.4 Management of moderate acute malnutrition

Moderate acute malnutrition is treated by adding a nutrient rich supplemental food that provides the daily recommended dietary allowance of all micronutrients in addition to the child's habitual diet. The treatment of MAM in children below the age of 5 years requires the consumption of nutritionally adequate foods, including exclusive breastfeeding before 6 months of age (UNICEF, 2007a). In addition, clean drinking

water, good sanitation practices, and preventive (vitamin A supplementation, immunization) and curative services need to be available to children to optimize growth, development, and survival (WHO/UNICEF, 2003).

The WHO recommends that the dietary management of MAM should be based normally on the optimal use of locally available, nutrient-dense foods, using a number of approaches to ensure adequate intakes of nutrients, including dietary diversification and fortification of certain staple foods (WHO, 2012b). Unfortunately, available and affordable foods often fail to meet nutrient needs, particularly when families cannot afford frequent consumption of animal-source foods (Arimond, 2013) or in situations of food shortage and emergencies. In such conditions, specially formulated supplementary foods are usually required to contribute to an adequate intake of required nutrients (Osendarp, 2015). The development of specially formulated ready-to use therapeutic foods (RUTFs) has enabled treatment of SAM in the community and made a difference to child survival which has resulted in a growing interest in using these types of products for the management of MAM as well, and RUSFs have been developed and are increasingly being used for the management of MAM (Arimond, 2013).

Besides highly recommending the use of super cereal plus as the best option in the case of Nepal in treating of MAM children, there are a number of choices for using the specialized nutritious foods as lipid-based nutrient supplement (LNS), large quantity (92-100g) such as Plumpy Sup (peanut-based), eeZeeRUSF (peanut based), Acha Mum (chickpea-based) etc. which can be effectively used as RUSF for treating MAM children aged 6-59 months and malnourished pregnant and lactating women with less than 6 months infants. The RUSF can be eaten directly from the sachet without prior cooking, mixing or dilution. Each nutrient supplement has the same nutritional value to control and monitor dietary intake (UNOCHA, 2015a).

Principles of nutritional management of children with moderate acute malnutrition

According to (WHO, 2012b) the principles of nutritional management of children with moderate acute malnutrition are as follows:

1. Every child needs to receive nutrition of a sufficient quality and quantity to enable normal growth and development as defined by the WHO growth and development standards.

2. Management of moderate acute malnutrition in children 6–59 months of age should include essential nutrition actions such as breastfeeding promotion and support, education and nutrition counseling for families, and other activities that identify and prevent the underlying causes of malnutrition, including nutrition insecurity. Interventions to improve food security include the provision of conditional or non-conditional cash transfers and support to agriculture, such as crop diversification
3. Children 6–59 months of age with moderate acute malnutrition need to receive nutrient dense foods to meet their extra needs for weight and height gain and functional recovery.
4. Nutrient-dense foods enable children to consume and maximize the absorption of nutrients in order to fulfill their requirements of energy and all essential nutrients. Animal-source foods are more likely to meet the amino acid and other nutrient needs of recovering children. Plant-source foods, in particular legumes or a combination of cereals and legumes, also have high-quality proteins, although they also contain some anti-nutrients such as phytates, tannins or inhibitors of digestive enzymes, which may limit the absorption of some micronutrients, particularly minerals.
5. The amounts of anti-nutrient compounds and naturally occurring toxins, cyanogen, alkaloids or other potentially poisonous or deleterious ingredients can be minimized by using appropriate food processing methods, such as soaking, germination, malting and fermentation.
6. Supplementary foods, particularly when they represent the main source of energy, need to provide nutrients at levels that do not cause adverse effects in moderately malnourished children when consumed for several months.
7. Determination of the amount of supplementary food that needs to be given to a moderately malnourished child requires consideration of the availability and nutrient content of the child's habitual diet, including whether the child is being breastfed, the likelihood of sharing of the supplementary food within and beyond the household, and access to other foods.
8. The formulation of supplementary foods should be safe and effective, particularly where moderately malnourished children use this food as their only source of energy.

9. The mineral components should be authorized by a regulatory body. The Codex Alimentarius includes a list of approved additives and fortificants for foods for infants and young children. In areas where coeliac disease is common, attention should be given to avoiding early introduction of wheat products. Additionally, because of the impaired digestive capacity of malnourished children, water-soluble salts should be used where possible.
10. Hygiene standards should comply with the Codex Alimentarius for infant and young children's food. These are being revised and will be discussed and agreed at the 34th session of the Codex Committee on Nutrition and Foods for Especially Dietary Uses in July 2012. It is advisable to give instructions for the safe and hygienic preparation of meals, e.g. those containing fortified blended food.

2.5 Ready-to-use supplementary food (RUSF)

Supplementary feeding is the provision of nutritious rations to targeted individuals that supplement the energy and nutrients missing from the diet of those with higher nutritional needs (WHO, 2012a). Food supplementation or dietary supplementation is a practice in which people are provided with a product to complement their daily dietary intake with the intention to improve the nutritional value of their diet. Food supplementation is normally done in the form of food aids in areas of food shortages where nutritious foods such as corn soy blend (CSB) are distributed to people (Nackers, 2010). In children with SAM this may come as RUTF whereas in those with MAM it has been described as RUSF (Isabelle Defourny, 2009).

RUSF is a food supplement that is intended to be eaten during two to three months, as part of a nutritional program, to treat moderate acute malnutrition for children 6 months and older. Product is intended to be eaten directly from the package with no necessary dilution, mixing or cooking. One package contains one daily dose of 100g. It is a fortified lipid-based paste/spread that is stabilized and individually packaged in robust sachets that are packed in sturdy cartons. RUSF is generally made with heat treated oil seeds/pulses/cereals, sugar, milk powder, vegetable oils, vitamins and minerals (WFP, 2016).

Ready-to-Use Supplementary Foods (RUSF) have been developed to provide a nutrient-dense alternative to more conventional supplements. There have been a number of studies on the efficacy of RUSF in enhancing the rate of recovery from MAM and SAM or even preventing them (Defourny, 2009) found that adding child-targeted RUSF

supplementation to a general food distribution resulted in increased hemoglobin status and linear growth. In a similar study (Karakochuk, 2012), realized that the treatment of moderate acute malnutrition with RUSF resulted in higher recovery rates in children, despite the higher amount of energy content in CSB. RUTF supplementation has been found to improve the rate of weight gain and improvement of appetite, a shorter period of convalescence as well as a higher rate of recovery from cough in children after an episode of acute uncomplicated *Plasmodium falciparum* malaria (Van Der Kam, 2012).

2.6 Ready-to-use therapeutic food (RUTF)

Therapeutic foods are prepared foods that contain calories, vitamins and minerals. They do not require cooking and have a long shelf life. Therapeutic foods are foods designed for specific, usually nutritional, therapeutic purposes as a form of dietary supplement. The primary examples of therapeutic foods are used for emergency feeding of malnourished children or to supplement the diets of persons with special nutrition requirements, such as the elderly. RUTF is an energy-dense, mineral- and vitamin-enriched food that requires no preparation and is specifically designed to treat severe acute malnutrition (SAM). RUTF has a similar nutrient composition to F-100 therapeutic milk, which is used to treat SAM in hospital settings. RUTF is soft and can be consumed easily by children from the age of 6 months. Because RUTF is not water-based, bacteria cannot grow in it and it can be used safely at home without refrigeration and in areas where hygiene conditions are not optimal (USAID, 2017). RUTF is a mixture of nutrients designed and primarily addressed to the therapy of the severe acute malnutrition without complications. The main ingredients of the formulation are powdered milk, peanuts butter, vegetal oil, sugar, and a mix of vitamins, salts, and minerals. The effectiveness of ready to use therapeutic food within the person's own home for the treatment of severe acute malnutrition in children under five years of age has been found not to be different than standard care. The formulation of RUTF is a need of low income countries and developing countries to combat malnutrition of children (Wagh, 2015).

The first RUTF Plumpy nutR by Nutriset is basically made up of peanuts, sugar, oil and milk but is quite expensive because it has to be imported. Also in the case of local production, milk powder which is imported is used as protein source, not making it any less expensive. Researchers have therefore been concentrating on the use of locally

available raw material for a formulation that will provide nutrient composition very similar to the proven Plumpy nutR (Briend, 2010).

RUTFs are energy dense, micronutrient enhanced pastes used in therapeutic feeding. These soft foods are a homogenous mix of lipid rich foods, with a nutritional profile similar to the WHO recommended therapeutic milk formula used for inpatient therapeutic feeding programs. Typical primary ingredients of RUTF include peanuts, oil, sugar, milk powder and vitamin and mineral supplements (UNICEF, 2013a).

Ingredients: 92g of each sachet Plumpy' Sup contains peanuts, sugar, whey, vegetable oil, milk, soy protein, cocoa, vitamins and minerals with 500 kcal, 13g protein (10%) and 31g fat (55%). 92g of each sachet RUTF contains peanut, sugar, milk, solids, vegetable oil, vitamins and minerals with 500 kcal, 13g protein (11%) and 31g fat (56%). Similarly, 100g of each Acha Mum sachet contains chickpeas, vegetable oil, milk powder, sugar, vitamins, minerals and soya lecithin with 520 kcal, 13g protein (10%) and 29g fat (50%) It does not contain any ingredient of animal origin, except for those derived from milk (UNOCHA, 2015a).

Ration: Each admitted individual will be provided 1 sachet of RUTF per day for a period of 60 to 90 days as therapeutic food. However, all caregivers, mothers and children will be encouraged to utilize nutritious food available at household level. The RUTF will be provided as a fortnightly ration with a special provision for one month in case of geographical difficulty for each individual. Each beneficiary is required to come for a follow up visit at the end of each fortnight to the targeted supplementary feeding center (TSFC) (UNOCHA, 2015a).

2.7 Raw materials and their nutritive value

2.7.2 Cereals

A cereal is any of the edible components of the grain of cultivated grass, composed of the endosperm, germ and bran. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop and are therefore staple crops (Centre", 2016). Cereals are the most important staple foods for mankind worldwide. The major cereals are wheat, corn, rice, barley, sorghum, millet, oats, and rye. Botanically, cereals are grasses and belong to the monocot family Poaceae. Cereals are a staple food in most countries and are considered important sources of nutrients. They are a rich source of macronutrients (carbohydrates, fats, oils, and protein) and

micronutrients (vitamins, minerals) as well as bioactive phytochemicals (polyphenols, flavonoids, anthocyanin, carotenoids, etc.) (MacEvilly, 2003)

2.7.2.2 Wheat

Wheat (*Triticum* spp.) is the most important and strategic food crop for ensuring food security at the global level. It is a cereal grain (botanically, a type of fruit called a caryopsis) originally from the Levant region of the near east but now cultivated worldwide. Globally, wheat is the leading source of vegetal protein in human food, having higher protein content than the other major cereals maize and rice. Wheat is the most important source of carbohydrate in a majority of countries. 100 grams of wheat provides 341 calories and is an excellent source of multiple essential nutrients, such as protein, dietary fiber, manganese, phosphorus and niacin. Several B vitamins and other dietary minerals are in significant content. Wheat is 12.2% water, 69.4% carbohydrates, 1.7% fat and 12.1% protein (DFTQC, 2017).

Wheat protein is easily digested by nearly 99% of the human population as is its starch. With a small amount of animal or legume protein added, a wheat-based meal is highly nutritious (Veraverbeke WS, 2002).

2.7.2.3 Maize

Maize (*Zea mays*) is one of the main source of cereals for food, forage and processed industrial products. Maize is one of the most important and widely distributed cereals crops of the world. The chief proteins of maize are glutelin and prolamine (zein). The zein fraction was shown to be very low in lysine content and lacking in tryptophan. Maize protein contains excess of Leucine and leucine interferes in the conversion of tryptophan to niacin and hence aggravates the pellargragenic action of maize. Whole maize is good source of thiamine, pyridoxine, pantothenic acid, fair sources of riboflavin but poor sources of niacin (Doebley, 2004).

2.7.3 Legumes

A legume is a plant or fruit/seed in the family Fabaceae (or Leguminosae). Legumes are grown agriculturally, primarily for their grain seed called pulse. Legumes are good foods for young children because they have a high protein content and are usually in expensive, or grown at own field. Although the protein in most legumes is only of moderate quality, it is supplementing other food proteins notably the cereals (Whyte, 1953).

Legumes are a significant source of protein, dietary fiber, carbohydrates and dietary minerals. Food legumes are comparatively rich in lysine and threonine and therefore a combination of cereal protein and legume protein comes very close to providing an ideal source of methionine and cysteine and legumes is in the large part offset by the higher proportions of these amino acids present in most cereals. The nutritional complementary of cereals and legumes is of the great importance, particularly for the people of the less developed world. Many of the legumes contain toxic factors such as trypsin inhibitor, haematoglulins etc. which can be removed by heat treatment before consumption (Prasad, 2016).

2.7.3.2 Soybean

It is the member of the family Leguminosae. Soybean (*Glycine max* L. Merrill) is the world's most important seed legume, which contributes to 25 % of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding (Dinesh Agrawal, 2013). It is well reputed pulse in hilly region of Nepal where it is cultivated mostly in a mixed crop and to less extent as pure crop under unplanned condition. Soybean is an important source of high quality, inexpensive protein and oil. At 38% soybean has the highest protein content of all food crops and is second only to peanut in terms of oil content (18%) among food legumes. Compared to other protein-rich foods such as meat, fish, and eggs, soybean is by far the cheapest. It also has a superior amino-acid profile compared to other sources of plant protein (WHO, 1998). It contains trypsin and growth inhibitor and hemagglutinin which can be inactivated by autoclaving soybean at pressure of 14lb for 30 min. or roasting in 100-110° C for some time (Khokhar, 2003). The PER of the heat processed soybean protein ranges from 1.9-2.2 and is increased markedly to 2.8-3.0 by supplementation with methionine. The chief protein of soybean is a globulin known as glycinin; other proteins present in small amount are phaseolin and legumelin. It is rich in lysine and threonine. The deficiency in sulphur containing amino acid can be overcome by either the addition of 0.15% of methionine result in better protein energy ratio than casein or by blending with other protein to provide a good balance of amino acids (Islam, 2007).

2.7.3.3 Bengal gram

Bengal gram (*Cicer arietinum*) is a leading nonoil seed legume, produced mostly in India and Pakistan. It contains about 10 mg iron, 5% fat, 17% proteins and very high potassium, phosphorous and calcium. It is, however difficult to assimilate most of the

calcium contained in this seed. It is rich in B-complex vitamins with a reasonable fiber and vitamin A and vitamin C contents. Its protein is deficient in two amino acids amply found in cereals but is rich in lysine which is deficient in cereals. Due to high content of sulphur and chlorine these grams have a high cleaning effect. The undesirable constituents can be minimized by soaking the legume in water for about 5-6 hours and then by boiling for 15 min (Dewan, 1994).

2.7.4 Oil seeds

2.7.4.2 Pumpkin seed

Pumpkin seeds have long been valued as a source of the mineral zinc and the World Health Organization recommends their consumption as a good way of obtaining this nutrient (foods). Pumpkin seeds are consumed directly as snack food in many cultures throughout the world. The seeds are nutrient-rich, with especially high content of protein, dietary fiber and numerous micronutrients. The pumpkin seeds are good source of protein and minerals such as iron, potassium, sodium, calcium and phosphorous (Giarni, 2003). The protein of pumpkin seed is high in phenylalanine, leucine and valine whereas markedly deficient in the sulphur-containing amino acids (methionine and cysteine). Pumpkin seeds have a high content of dietary fiber, in addition to being a protein source and presenting a high percentage of polyunsaturated oils therefore, they can be used in the preparation of new food products. It is also a good source of minerals required in human nutrition. Pumpkin seed contains some anti-nutritional factors like phytates but it can be reduced by boiling, cooking or roasting. The use of pumpkin seed flour in the product can enable product diversification and contribute to aggregate technological and nutritional quality and to reduce environmental impact (Elinge C. M. 1, 2012).

2.7.4.3 Peanut

Peanut or groundnut (*Arachis hypogea* L.), a member of the legume family, is an important food and oil crop. It is currently grown on approximately 42 million acres worldwide. It is the third major oilseed of the world after soybean and cotton. Peanuts contain easily digested monounsaturated fats. They are relatively high in calories, they are also rich in zinc and protein- both supportive for the immune system and long bone growth in reversing stunted height, while protein also contributes to muscle development. Peanuts are also a natural source of vitamin E, an antioxidant that helps to convert food into energy. It contain about 22- 28% protein and 42- 50% oil. Peanut are

good source of thiamine, niacin, pantothenic acid and phosphorous and fair sources of riboflavin, iron and calcium. The chief proteins in peanut are globulins known as arachin and conarachin. The limiting amino acid in peanuts proteins are lysine, threonine and methionine. The PER of protein ranges from 1.6-1.8 (Swaminathan, 2004).

2.7.5 Skim milk powder

Skim Milk Powder (SMP) is the product resulting from the partial removal of fat and water from pasteurized milk. SMP contain about 35% protein and only traces of fat (0.5-1.0%). It is good sources of calcium and B-vitamins. SMP can be used as a supplement to the diets of children and adults. Milk possesses of protein of very high digestibility. Adding skim milk in complementary food improves weight gain, linear growth, and recovery from malnutrition and milk proteins also improves flavor, which is important for acceptability in vulnerable groups. Adding whey or skimmed milk powder to RUTF improves the protein quality, allowing a reduction in total amount of protein, which could have potential metabolic advantages. It also allows for a reduced content of cereals and legumes, thereby a reduction of potential anti nutrients (Hoppe, 2008).

2.7.6 Sunflower oil

Vegetable oils that are used in food are comprised of complex mixtures of triacylglycerol. Vegetable oils are triglyceride extracted from various parts of plants such as seeds, fruits, or plant seedlings. Under the chemical terms, they are a combination of triglycerides of higher saturated and unsaturated fatty acids. Oils are very concentrated source of energy. They improve the palatability and consistency of a food without increasing its bulk. The amounts eaten each day vary widely according to cooking patterns, availability and cost. It is availability and cost. It is difficult for a small child to eat enough food to cover his energy requirements unless his diet contains some fat or oil or fat rich food. Fats and oil are necessary for the absorption of fat soluble vitamins and sources of essential fatty acids. Their high-energy value helps to reduce the bulk of foods (Thomas, 2000).

Sunflower oil is pressed from the seeds of the sunflower. Sunflower oil is the non-volatile oil compressed from the seeds of sunflower (*Helianthus annuus*). Sunflower oil is commonly used in food for frying. Sunflower oil is a monounsaturated (MUFA)/polyunsaturated (PUFA) mixture of mostly oleic acid (omega9)-linoleic acid

(omega-6) group of oils. The oil contains appreciable quantities of vitamin E, sterols, squalene, and other aliphatic hydrocarbons (Swaminathan, 2004).

2.7.7 Sucrose

Sucrose, commonly known as “table sugar” or “cane sugar”, is a carbohydrate formed from the combination of glucose and fructose. Sucrose is a common, naturally occurring carbohydrate found in many plants and plant parts. The molecule is a disaccharide combination of the monosaccharides glucose and fructose with the formula $C_{12}H_{22}O_{11}$ which is easily hydrolyzed by enzyme sucrose present in intestinal juice. Sucrose is often extracted and refined from either cane or beet sugar for human consumption. It plays a central role as an additive in food production and food consumption all over the world and provides energy as well as palatability (Fennema, 1996).

2.7.8 Mineral salt

Mineral salt are inorganic salts and include phosphate, calcium, chloride, sodium and potassium. Mineral salts are essential to the body and its metabolic function. They take part, inter alia, to the fluids balance, development of enzymes and hormones, the composition of bones and teeth, transmission of nerve impulses and muscle contraction (WHO/FAO, 2006).

Calcium is essential for construction and maintenance of bone and normal function of nerves and muscles. Phosphorus is an important constituent of adenosine triphosphate (ATP) and nucleic acid and is also essential for acid-base balance, bone and tooth formation. Red blood cells cannot function properly without iron in hemoglobin, the oxygen-carrying pigment of red blood cells. Iron is also an important component of the cytochromes that function in cellular respiration. Magnesium, copper, selenium, zinc, iron, manganese and molybdenum are important co-factors found in the structure of certain enzymes and are indispensable in numerous biochemical pathways. Iodine is used by thyroid gland to help regulate metabolism and development of both skeleton and brain, among other things. Lack of iodine may severely affect child’s brain function and IQ. Similarly, sodium, potassium and chlorine are important in the maintenance of osmotic balance between cells and the interstitial fluid (WHO/FAO, 2006). (Beigler, 1976) listed the following minerals salts for elemental diet:

- Calcium gluconate
- Calcium citrate

- Calcium chloride.2H₂O
- Ferrous gluconate
- Ferrous ammonium sulphate.6H₂O
- Cupric acetate.2H₂O
- Manganous acetate.4H₂O
- Magnesium oxide
- Ferrous sulphate
- Potassium chloride
- Potassium Iodide
- Sodium chloride
- Zinc benzoate
- Zinc acetate.2H₂O

2.8 Technology of processing of RUTF

The common household processing methods such as cleaning, soaking, drying and cooking have been used to improve nutritional quality of the cereals and legumes. Processing of food such as soaking and roasting leads to a reduction in phytic acid and tannin and increases of the minerals solubility in foods and also improves the bioavailability of minerals in cereals and legumes(U. S. Ndidi, 2014a). Processing techniques reduce the levels of anti-nutritional organic factors, which including phytates, phenols, tannins and enzyme inhibitors by releasing exogenous and endogenous enzymes such as phytase enzyme formed during processing (Tarek, 2002).

2.8.2 Soaking or steeping

Soaking or steeping is the process of softening a hard food by immersing it in liquid, preferably water, for hours. Soaking is ideally done to enhance the nutritional value as well as the flavor of the soaked food. However, sometimes foods are soaked simply to transform them into a soft form that tends to blend smoothly. The essence of soaking is to turn the soaked food into a more palatable form which is convenient for digestion. Soaking is primarily used for increasing the absorption capacity of the body for nutrients from the ingested food (Foutch, 2009). Non-corticated grains are soaked in water for a short time lead themselves to easy husk removal. Soaking process increases hydration coefficient, seed weight, total protein, ash, fat, fiber, while non-protein nitrogen, total carbohydrates, starch, stachyose, raffinose, reducing sugars, and minerals

of cereals and legumes. Soaking the seeds in water and processing effectively removed the anti-nutrients. All anti-nutritional factors such as phytic acid, tannin, trypsin inhibitor and hemagglutinin activity were decreased during soaking in 0.5% sodium bicarbonate (el-Adawy, 2000).

Time period for steeping depends on temperature and degree of aeration of the steep water. A temperature of 10-12°C is recommended with steeping times of 40-60 hours. A temperature of 20-25°C is recommended with steeping times of 16-20 hours for legumes (N. L. Kent, 1994).

2.8.3 Drying

Drying removes moisture from food, and moisture is necessary for the bacterial growth that eventually causes spoilage. Successful dehydration depends upon a slow, steady heat supply to ensure that food is dried from the inside to the outside (Hendley, 2016). Drying involves the use of heat to vaporize the water present in the food, and also the removal of the water vapor from the food surface. The main objectives of drying include preserving foods and increasing their shelf life by reducing the water content and water activity (Guiné, 2018). In drying process, the removal of moisture at low temperature allows the maximal survival of enzyme and the least development of aroma and color (Hough, 1982).

2.8.4 Roasting

Roasting is a way to uniformly heat and cook food. Roasting is a dry heat method of cooking, where hot air from an oven, open flame, or another heat source completely surrounds the food, cooking it evenly on all sides (Gavin, 2018). Roasting can enhance flavor through caramelization and Maillard browning on the surface of the food. Dry roasting is a process by which heat is applied to dry foodstuffs without the use of oil or water as a carrier. Unlike other dry heat methods, dry roasting is used with foods such as nuts and seeds. Dry roasted foods are stirred as they are roasted to ensure even heating (Gahlawat, 1994).

Roasting reduces the moisture content, thereby concentrating the food value. Roasting also enhance acceptability by imparting a nutty flavor to the food. Most of the anti-nutritional factors or toxic effects of legumes (trypsin inhibitor, hemagglutinin, goitrogenic agents, cyanogenic glucosides, alkaloids, etc.) are partially or fully eliminated by roasting (U. S. Ndidi, Ndidi, C. U., Aimola, I. A., Bassa, O. Y., Mankilik, M. and Adamu, Z. , 2014b). Similarly, on roasting, in vitro protein and starch

digestibility of weaning foods increased by 15-21% and 16-19%, respectively. Roasting also improved in vitro iron availability by 12-19% (Gahlawat, 1994b).

2.8.5 Milling and sieving

The milling process removes material by performing many separate, small cuts. In the process of milling food grains, the main objective is to remove the coarse fibrous bran or the seed coat (Deosthale, Y.G., 1979). The outer bran in coarse grains is fibrous, bitter, astringent, or colored. Milling of the coarse grains is therefore desirable to confer adequate consumer acceptability to them. It is obvious that over milling or very high refining must be avoided, since it removes the aleuronic layers and germ rich in protein, vitamins, and minerals (Viraktamath, 1971).

Sieving is a traditional and a very easy method of separation. Sieving is defined as a method in which two or more components of different sizes are separated from a mixture on the basis of the difference in their sizes (Allen, 1990).

2.8.6 Blending

It is the homogenous mixing of the entire ingredient. It is the process of combining two or more ingredients together so that they lose their individual characteristics and become smooth and uniform. The main objective of blending is to combine or mix so that the constitute parts are indistinguishable from one another resulting into the lipid based paste product (Amagloh, 2012).

2.8.7 Packaging of RUTF

RUTF requires special packaging to extend its storage life as it is lipid based paste product. Following moisture pick-up certain physical, chemical and biological change will take place and quality degradation will begin. Packaging of RUTF shall be in food-grade sachets, hermetically sealed and strong enough to prevent leakage and protect the product throughout its shelf life. Sachet material shall not represent a hazard for infants and young children when sachets is opened and put in contact with the mouth (WFP, 2016).

2.9 Packaging

Packaging is the science, art, and technology of enclosing or protecting products for distribution, storage, sale, and use. Packaging also refers to the process of design, evaluation, and production of packages. Packaging means covering the product itself so that it is protected from damage, leakage, dust, pollution, contamination etc (S, 2002).

Packaging can be described as a coordinated system of preparing goods for transport, warehousing, logistics, sale, and end use. Packaging contains, protects, preserves, transports, informs, and sells (Soroka, 2002). Packaging is an essential part of processing and distributing foods. Whereas preservation is the major role of packaging, there are several functions for packaging, each of which must be understood by the food manufacturer (Coles, 2003).

2.9.2 Food packaging materials

Food packaging is packaging for food. A package provides protection, tampering resistance, and special physical, chemical, or biological needs. It may bear a nutrition facts label and other information about food being offered for sale (Paine, 1992).

2.9.3 Special feature required for RUTF packaging

RUTF are lipid based paste products. The fat content is generally high so, it is very susceptible to oxidative rancidity. Thus, RUTF should be packaged in food-grade sachets, hermetically sealed and robust enough to prevent leakage and protect the product throughout its shelf life. Sachet material shall not represent a hazard for infants and young children when sachets is opened and put in contact with the mouth (WFP, 2016).

2.9.4 HDPE

HDPE (High-Density Polyethylene) packaging is commonly used in many forms including bags, bottles, carboys and containers. HDPE is a commodity plastic derived from fossil fuels and is the most widely used types of plastic (Tansey, 2018b). High-density polyethylene (HDPE) is the third-largest commodity plastic material in the world, after polyvinyl chloride and polypropylene in terms of volume. It is a thermoplastic material composed of carbon and hydrogen atoms joined together forming high-molecular weight products. HDPE is a plastic with many applications in packaging due to its unique blend of properties. It is a hydrocarbon polymer created in a catalytic process that results in a final product containing a number of beneficial properties, including a high tensile strength, that complement many packaging needs. HDPE is produced at lower temperatures and atmospheric pressure as a liquid phase process. It softens at 120-130 °C and so it can be used for hot filling, steam sterilizing or cook in the bag applications. Due to its greater rigidity, it can be used in thinner gauges thereby saving money. It has excellent retention of essential oils such as aromas. In general, the

polyethenes are soft and flexible in film form with good impact resistance. However, they can be hard to open. They are very resistant to water and water vapor; the higher the density the greater the resistance (Tansey, 2018a). High-density polyethylene or HDPE is a commonly used petroleum thermoplastic and the most used of the three polyethylene for a wide range of applications. According to (McCartney, 2019) main advantages of HDPE are:

- a) It has low strength and hardness, but is very ductile and has good impact strength; it will stretch rather than break.
- b) It is water resistant and durable, so it is longer lasting when exposed to the elements compared to other polymers.
- c) It is a good electric insulator.
- d) It is heat sealable.
- e) It has good heat resistant properties.

2.10 Shelf life

Shelf life is the length of time that a commodity may be stored without becoming unfit for use, consumption, or sale. Shelf life is the recommended maximum time for which products or fresh (harvested) produce can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected (or specified) conditions of distribution, storage and display. Shelf life depends on the degradation mechanism of the specific product. Most can be influenced by several factors: exposure to light, heat and moisture, transmission of gases, mechanical stress, and contamination by things such as microorganisms. Product quality is often mathematically modeled around a parameter (concentration of a chemical compound) a microbiological index or moisture content (Azanha, 2005).

2.11 Rancidity of fat and oils

Oxidation of fats, generally known as rancidity, is caused by a biochemical reaction between fats and oxygen. In this process the long-chain fatty acids are degraded and short-chain compounds are formed. Oxidation primarily occurs with unsaturated fats by a free radical-mediated process. These chemical processes can generate highly reactive molecules in rancid foods and oils, which are responsible for producing unpleasant and noxious odors and flavors. These chemical processes may also destroy nutrients in food (Misra, 2014). Lipid oxidation is one of the major reasons that foods deteriorate and is caused by the reaction of fats and oils with molecular oxygen leading to off-flavors that

are generally called rancidity. Exposure to light, pro-oxidants and elevated temperature will accelerate the reaction. Rancidity is associated with characteristic off-flavor and odour of the oil. There are two major causes of rancidity. One occurs when oil reacts with oxygen and is called oxidative rancidity. The other cause of rancidity is by a combination of enzymes and moisture. Enzymes such as lipases liberates fatty acids from the triglyceride to form di and/or monoglycerides and free fatty acids and such liberation of free fatty acids is called hydrolysis (Hamilton, 1994). According to Hamilton (1994) hydrolysis is also caused by chemical action that is prompted by factors such as heat or presence of water. Rancidity caused by hydrolysis is called hydrolytic rancidity. Oxidation is concerned mainly with the unsaturated fatty acids. Oxidative rancidity is of special interest as it leads to the development of unfavorable off-flavors that can be detected early on in the development of rancidity, more so than in the case of hydrolytic rancidity. Fats and oils play an important role in the flavor, aroma, texture, and nutritional quality of foods. Fats and oils may be added during manufacturing or they may be inherent to the product or ingredient. The product may be pure oil or it may be part of a complex mixture with proteins, carbohydrates, minerals, and vitamins. The product may contain almost no fat or it may contain a considerable amount. Regardless of the source of fat, the amount of fat, or the product composition, predicting and monitoring fat and oil quality is an important component of developing and manufacturing high quality products (Asif, 2011).

As soon as a food is manufactured, it begins to undergo a variety of chemical and physical changes. Oxidation of lipids is one common and frequently undesirable chemical change that may impact flavor, aroma, nutritional quality, and, in some cases, even the texture of a product. The chemicals produced from oxidation of lipids are responsible for rancid flavors and aromas. Vitamins and other nutrients may be partially or entirely destroyed by highly reactive intermediates in the lipid oxidation process. Oxidized fats can interact with protein and carbohydrates causing changes in texture. Of course, not all lipid oxidation is undesirable. Enzymes, for example, promote oxidation of lipid membranes during ripening of fruit. For most products, though, predicting and understanding oxidation of lipids is necessary to minimize objectionable flavors and aromas arising from fat rancidity (Asif, 2011).

2.12 Use of RUTF

Ready to use therapeutic food (RUTF) are energy dense, micronutrient enhanced pastes used in therapeutic feeding. These soft foods are a homogeneous mix of lipid rich foods, with a nutritional profile similar to the World Health Organization recommended therapeutic milk formula used for inpatient therapeutic feeding programs. The standard dose of RUTF is adjusted according to the weight of the child under treatment. It can be consumed in the home at any time under minimal supervision until the child had gained adequate weight. Because RUTF do not contain water, children should also be offered safe drinking water to consume at will (UNICEF, 2013c).

Part III

Materials and methods

3.1 Materials

3.1.1 Wheat (*Triticum aestivum*)

Wheat was collected from Dharan market. Scientific name of wheat is *Triticum aestivum* and it is locally known as '*gahu*'.

3.1.2 Maize (*Zea mays*)

Maize was collected from Dharan. Scientific name of maize is *Zea mays* and it is locally known as '*makai*'.

3.1.3 Soybean (*Glycine max*)

White variety of soybean was collected from Dharan market. Soybean's scientific name is *Glycine max* and is locally known as '*Nepali bhatmas*'.

3.1.4 Bengal gram (*Cicer arietinum*)

Bengal gram was collected from Dharan market. Scientific name of bengal gram is *Cicer arietinum* and it is locally known as '*rato chana*'.

3.1.5 Pumpkin seed (*Cucurbita pepo*)

Pumpkin seed was collected locally from Bhojpur. Scientific name of pumpkin is *Cucurbita pepo* and its seed is locally known as '*farsi ko biya*'.

3.1.6 Peanut (*Arachis hypogaea* L)

Peanut was collected from Dharan market. Scientific name of peanut is (*Arachis hypogaea* L) and is locally known as '*badam*'.

3.1.7 Milk powder

Milk powder manufactured by Nestle, India, available in Dharan market was used.

3.1.8 Sunflower oil

Sunflower oil named '*Cello*' manufactured by Bagmati oil industries Pvt. Ltd. Biratnagar, Nepal was used.

3.1.9 Sugar

White crystalline sugar available in Dharan market was used.

3.1.10 Vitamin and mineral premix

Mixture of fat soluble and water soluble vitamin and mineral premix was obtained from Nutrifood industries Pvt. Ltd., Duhabi, Nepal. The composition of micronutrient premix is in appendix D.

3.1.11 Packaging material

HDPE packaging (20 μ) available in Dharan market is used as packaging material for the packaging of the product.

3.2 Methods

3.2.1 Processing of raw materials

3.2.1.1 Wheat

It was cleaned and soaked in water for 12 hours and then drained and was dried in cabinet drier at 50°C for 7 hours and 70°C for 5 hour until moisture was sufficiently reduced to about 11%. The dried wheat was then roasted and ground into flour and sieved(300 μ) and packed in air tight plastic bags (Tehseen yaseen, 2014). Fig 3.1 shows the flow diagram for the processing of wheat.

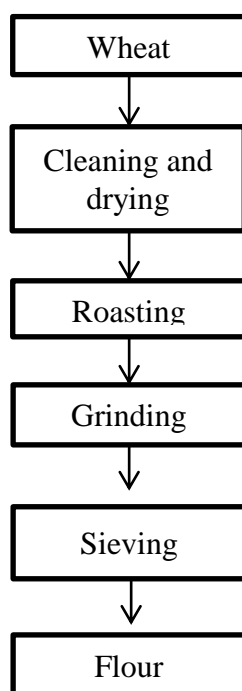


Fig 3.1 Flowchart for the processing of wheat

3.2.1.2 Maize

Maize was cleaned to remove impurities such as stones, weed seeds, other grains, broken kernels etc. The cleaned maize was steeped into water at room temperature (25-27 °C) for 12 hours. Then it was dried at 50°C unless moisture reduces about 20-25% and then it was again dried in cabinet drier to reduce the moisture to 10-12%. It was then roasted and ground in a mixture into flour. The flour was sieved and put into air tight plastic bags (Collins, 2010). The flow diagram for the processing of maize is same as wheat.

3.2.1.3 Soybean

It was cleaned and soaked for 8 hours with 1% w/v sodium bicarbonate at room temperature and then drained and was then dried in cabinet drier at 50°C for 6 hours and 70°C for 9 hour until moisture was sufficiently reduced to about 10%. It was roasted; dehusked, splitted cotyledons were ground in a grinder into flour. The flour was sieved and stored into airtight plastic bags (Pulami, 2013). The flow diagram for the processing of soybean is same as wheat.

3.2.1.4 Bengal gram

It was cleaned and soaked for 24 hours with 1% w/v sodium bicarbonate at room temperature and then drained and was then dried in cabinet drier at 50°C for 6 hours and 70°C for 8 hour until moisture was sufficiently reduced to about 11%. It was roasted; dehusked, splitted cotyledons were ground into flour. The flour was sieved and stored into airtight plastic bags (Pulami, 2013). The flow diagram for the processing of bengal gram is same as wheat.

3.2.1.5 Pumpkin seed

Pumpkin seed was cleaned sorted and soaked in hot water for 10-20 minutes. Then it was dried in cabinet drier at 50°C for 5 hours and 70°C for 7 hour. It was then roasted and grinded in grinder and finally the flour was sieved and stored in the airtight plastic bags (Ikujenlola A. Victor1, 2013). The flow diagram for the processing of pumpkin seed is same as wheat.

3.2.1.6 Peanut

Peanut was sorted and cleaned and dried in cabinet drier at 50°C for 6 hours and 70°C for 7 hour. It was grinded in grinder to form paste and was stored in airtight plastic bags (Mark J. Manary, 2005a). The flow diagram for the processing of peanut is same as wheat.

3.2.1.7 Sugar

White sugar crystals were ground in grinder and then packed in airtight plastic bags (Pulami, 2013).

3.2.2 Formulation

3.2.2.1 Basis of formulation

The preparation of diet was done on the basis of specification of basic nutrient composition given by (WFP, 2016). Table 3.1 shows the basic nutrient requirement in RUTF:

Table 3.1 Nutrient requirement in RUTF

Nutrients	Nutritional values per 100 g finished product
Energy (Kcal)	510-560
Protein (g)	11-16
Fat (g)	26-36
ω -3 fatty acids (g)	0.30-1.8
ω -6 fatty acids (g)	2.6-6.1
Retinol (Vit A) (μ g)	550-1150
Thiamin (Vit B1) (mg)	1
Riboflavin (Vit B2) (mg)	2.1
Niacin (Vit B3) (mg)	13
Pantothenic acid (Vit B5) (mg)	4
Pyridoxine (Vit B6) (mg)	1.8
Biotin (Vit B7) (μ g)	60
Folic acid (Vit B9) (μ g)	330
Cobalamin (Vit B12) (μ g)	2.7
Ascorbic Acid (Vit C) (mg)	60
Calcium (Ca) (mg)	535-750
Copper (Cu) (mg)	1.4-1.9
Iodine (I) (μ g)	100-140
Iron (Fe) (mg)	10-14
Magnesium (Mg)	150-225
Phosphorus (P) (mg)	450-750
Potassium (K) (mg)	900-1400

Sodium (Na) (mg)	270
Zinc (Zn) (mg)	11-14

Source : (WFP, 2016)

3.2.2.2 Calculation of amounts of ingredients

For the formulation of RUTF, the amounts of ingredients were calculated on dry weight basis. Legumes were taken as the source of protein and the cereals as the staple source. Pumpkin seed and sesame were chosen as a source of energy and to maintain the level of essential fatty acids. Mineral salts for mineral source, sugar as sweetening agent, oil as a source of energy and to maintain the level of essential fatty acids. Amount of vitamin and mineral premix was added to achieve vitamins and mineral level in RUTF.

3.2.3 Experimental design

Using the Design expert version 10, eleven formulations were prepared as sample A to K by varying the amount of Bengal gram and peanut.

Table 3.2 Amount of ingredients given by Design Expert

Ingredients	A	B	C	D	E	F	G	H	I	J	K
Wheat(g)	11	11	11	11	11	11	11	11	11	11	11
Maize(g)	13	13	13	13	13	13	13	13	13	13	13
Bengal gram(g)	1	1	6	5.9	5.6	1	3.6	3.5	1	2.9	3.5
Soybean(g)	11	11	11	11	11	11	11	11	11	11	11
Peanut(g)	17.7	10	10.3	20	16.5	13.4	10	12.5	20	15	20
Pumpkin seed(g)	10	10	10	10	10	10	10	10	10	10	10
Milk powder(g)	11	11	11	11	11	11	11	11	11	11	11
Sugar(g)	11.6	14.9	11	5	6.8	11.5	10.3	10.8	9.9	7.9	5.4
Oil(g)	12.6	17	15.6	12	14	17	19	17	13	17	14
Premix(g)	1	1	1	1	1	1	1	1	1	1	1
Total(g)	100	100	100	100	100	100	100	100	100	100	100

3.2.4 Product preparation

The calculated amounts of ingredients for eleven different formula (A, B, C, D, E, F, G, H, I, J and K) were calculated on dry basis. Flow chart diagram of different ingredients used for the preparation of RUTF is shown in Fig 3.2.

3.2.4.1 Grinding and milling

All the roasted cereals and legumes were ground using the grinder available in laboratory of Central Campus of Technology, Dharan.

3.2.4.2 Sieving of the ground powder product

All the ground flour was sieved by using 300 μ sieve available in the laboratory.

3.2.4.3 Mixing

The calculated amounts of ingredients were weighed according to the formulation and mixed together homogeneously.

3.2.4.4 Packaging

After completion of proper mixing, the product was packed immediately in airtight plastic containers, and then it was repacked in experimental packaging material HDPE. The package was kept at room temperature for shelf life evaluation.

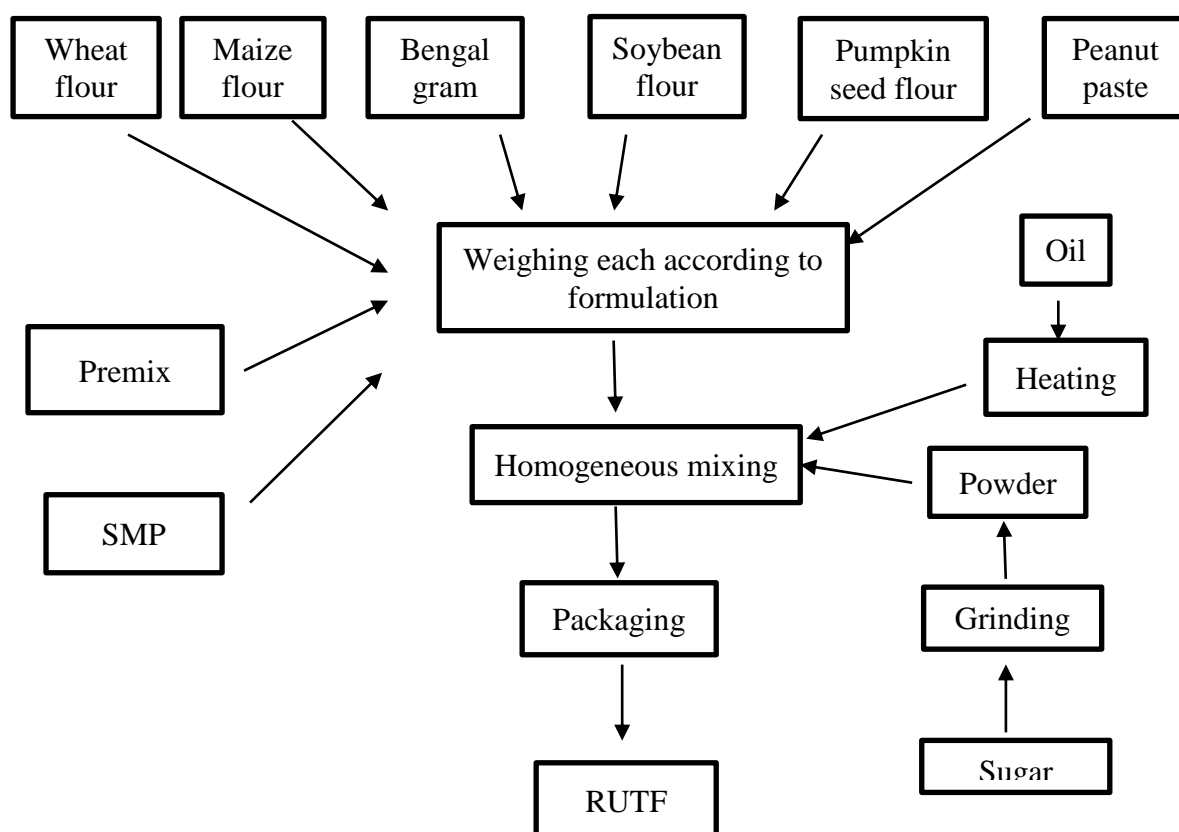


Fig 3.2 Outline for preparation of RUTF

3.2.5 Evaluation of prepared RUTF

3.2.5.1 Sensory evaluation

Sensory evaluation was performed by 9-point hedonic scoring (9 = like extremely, 1 = dislike extremely) for color, flavor, taste, texture and overall acceptance. The evaluation was carried out by 10 panelists comprising of teachers and students of Central Campus of Technology including 4 females and 6 males. Sensory evaluation was carried out in individual booth with adequate light and free from obnoxious odors. Each panelist was provided with 11 samples coded random numbers and evaluation card (Appendix A). They were provided with portable water for rinsing between samples. Verbal communication among the panelist was prohibited. They were asked to evaluate the samples individually using a score card.

3.2.5.2 Physicochemical analysis of product

3.2.5.2.1 Moisture content

Moisture content was determined by using hot air oven as per Rangana, 1986.

3.2.5.2.2 Crude fat

The fat content was determined by Soxhlet method as per Rangana, 1986.

3.2.5.2.3 Crude protein

The crude protein was determined by using Kjeldahl's method as per Rangana, 1986.

3.2.5.2.4 Crude fiber

Crude fiber was determined as per Rangana, 1986.

3.2.5.2.5 Total ash

Total ash content was determined by ashing in electric muffle furnace (ambassador, working temperature 900°C, UK) as per Rangana, 1986.

3.2.5.2.6 Total carbohydrate

Total carbohydrate was determined by difference method Rangana, 1986.

3.2.5.2.7 Calcium

Calcium content was determined by volumetric method as per KC and Rai, 2007.

3.2.5.2.8 Vitamin C

Vitamin C content was determined as per KC and Rai, 2007.

3.2.5.2.9 Iron

Iron content was determined as per Rangana, 1986.

3.2.5.3 Determination of energy value

One of the methods specified by FDA was employed. This uses the general factors of 4, 4, and 9 calories per gram of protein, total carbohydrate, and total fat, respectively, to calculate the calorie content of food (**Francisca I Bassey, 2013**).

Total energy = energy from carbohydrate + energy from protein + energy from fat

3.2.5.4 Evaluation of microbiological quality

It is done to assess bacterial, fungal and yeast load under laboratory condition. For analysis, 10 g of each sample is aseptically weighed and diluted to (1:10), i.e., 10 g in 90 ml sterilized distilled water and mixed well. Pour plate method and spread plate method can be used for yeast and fungus AOAC, 2005.

3.2.5.4.1 Total plate count (TPC)

TPC was carried out using the method of AOAC, 2005.

3.2.5.4.2 Yeast and mold

Yeast and mold was determined according to the method of AOAC, 2005.

3.2.5.5 Rate of change in AV and PV

For lipid stability, free acid value and peroxide value (PV) of the extracted oil were determined over the storage period of the product. Following chemical analysis was carried out in each interval of week.

3.2.5.5.1 Acid value

Acid value was determined according to Rangana, 1986.

3.2.5.5.2 Peroxide value

Peroxide value was determined according to Rangana, 1986.

3.3 Cost calculation

The costs of the best RUTF from eleven different formulations were calculated including a profit of 10% (Appendix C).

3.4 Data analysis

Data on sensory analysis were tabulated for comparison and were graphically represented using Microsoft excel-2010. Data were statistically processed by Genstat for analysis of variance (ANOVA). Means of the data were separated whether they are significant or not by using LSD (least square difference) method at 5% level of significance.

Part IV

Results and discussion

4.1 Formulations of the products

The amounts of ingredients were calculated on dry weight basis, for the formulation of RUTF. Cereals were taken as staple source of food; legumes were taken as source of plant protein and milk powder as a source of animal protein. Similarly, pumpkin seed, sesame and sunflower oil as source of energy and to maintain essential fatty acids, vitamin and mineral premix as a source of minerals and vitamins, sugar as a sweetening agent.

From the calculation of nutrient composition from food composition table by DFTQC, eleven products were developed on the basis of WHO/UN specification of RUTF.

4.2 Sensory analysis of the products

The prepared eleven RUTF formulae were subjected to sensory evaluation. The samples were provided to 10 semi trained panelists. The semi trained panelists evaluated for various parameters of RUTF namely color, flavor, taste, texture and overall acceptability. The panelists were requested to provide scores in the score sheets as per their perception. Data were analyzed statistically and best product was found out.

Vertical error bars represent \pm standard deviation of scores given by 10 panelists. The ANOVA at 95% level of confidence ($p < 0.05$) showed that the formula A, B, C, D, E, F, G, H, I, J and K were significantly different from each other in sensory attributes.

4.2.1 Color

The average sensory score for color was 7.8, 8.2, 7.7, 7.8, 7.8, 8.1, 8.2, 8, 7.9, 7.7 and 7.7 for A, B, C, D, E, F, G, H, I, J and K respectively. The analysis of variance showed that in case of color, there were no significant differences between the samples.

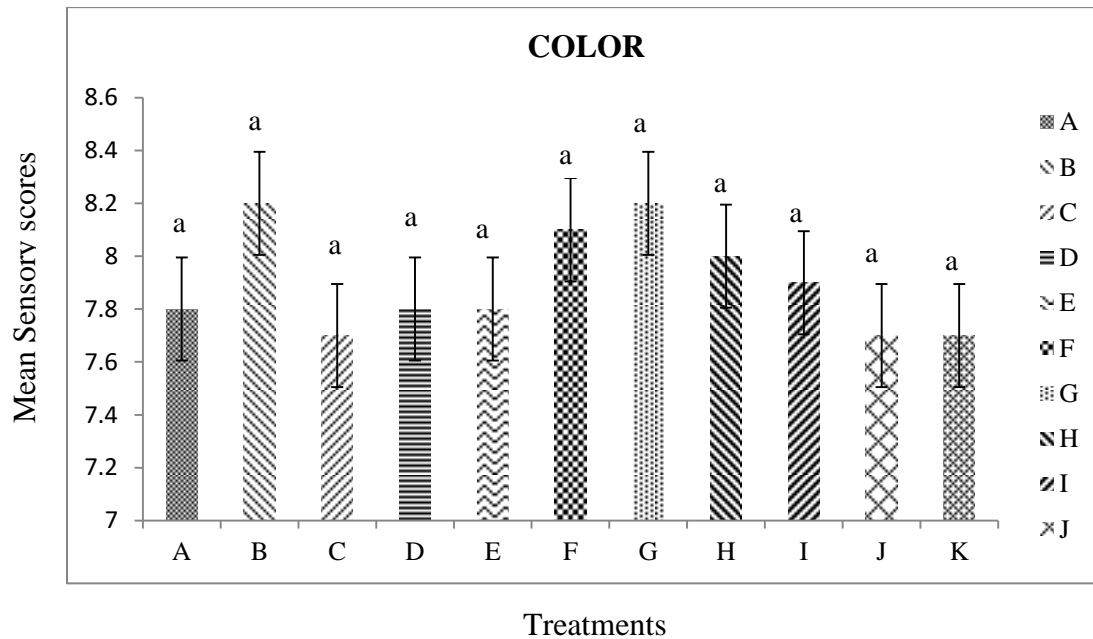


Fig 4.1 Mean sensory score for color

Figure 4.1 represents the mean sensory scores for color of RUTF. Value on top of the bar shows that the samples were not significantly different from each other at 5% level of significance.

4.2.2 Flavor

The average sensory score for flavor was 7.4, 7.7, 7.6, 7, 6.8, 7.3, 7.7, 7.3, 7, 6.9 and 6.8 for A, B, C, D, E, F, G, H, I, J and K respectively. In case of flavor, there were no significant differences between the samples.

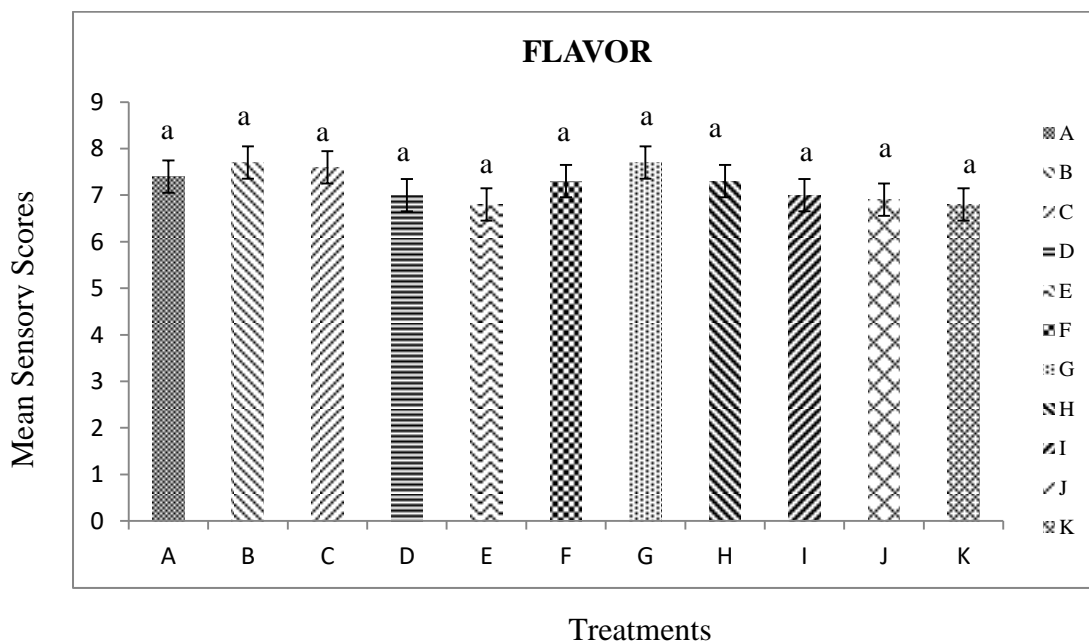


Fig 4.2 Mean sensory score for flavor

Figure 4.2 represents the mean sensory scores for flavor of RUTF. Value on top of the bar shows that the samples were not significantly different from each other at 5% level of significance.

4.2.3 Texture

The average sensory score for texture was 7, 7.5, 7, 7.4, 6.9, 7, 7.6, 7.4, 7, 6.8 and 6.8 for A, B, C, D, E, F, G, H, I, J and K respectively. There were no significant differences between the samples.

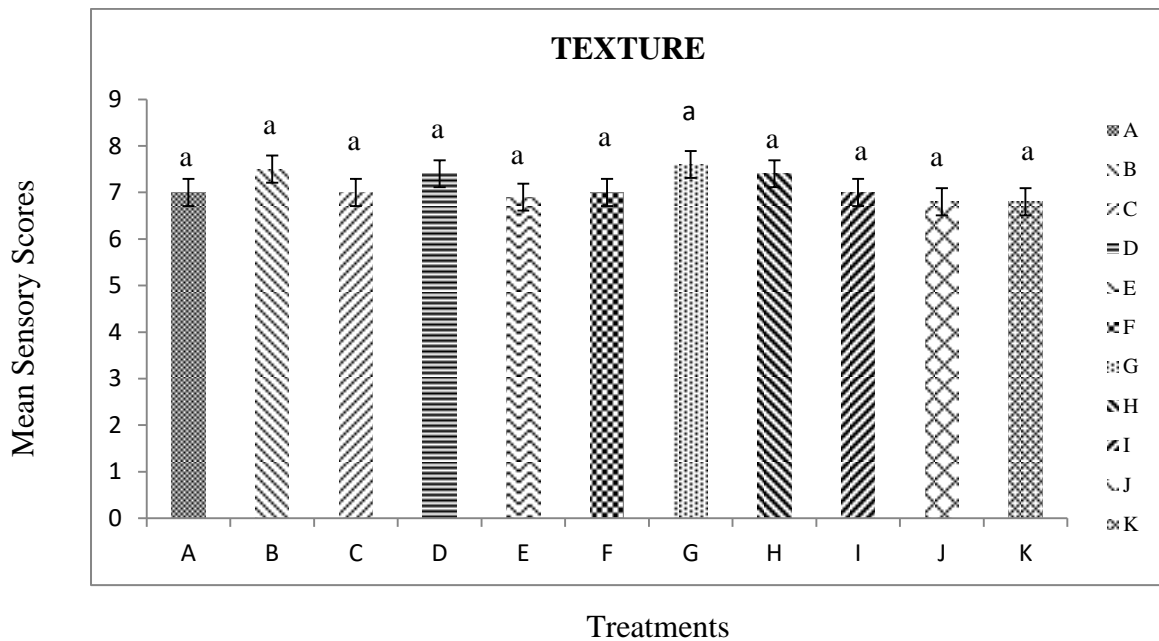


Fig 4.3 Mean sensory score for texture

Figure 4.3 represents the mean sensory scores for flavor of RUTF. Value on top of the bar shows that the samples were not significantly different from each other at 5% level of significance.

4.2.4 Taste

The average sensory score for taste was 7.5, 8, 7.6, 7.1, 7.2, 7.2, 7.4, 7.6, 7.1, 6.9 and 7.1 for A, B, C, D, E, F, G, H, I, J and K respectively. In case of taste also, there were no significant differences between the samples.

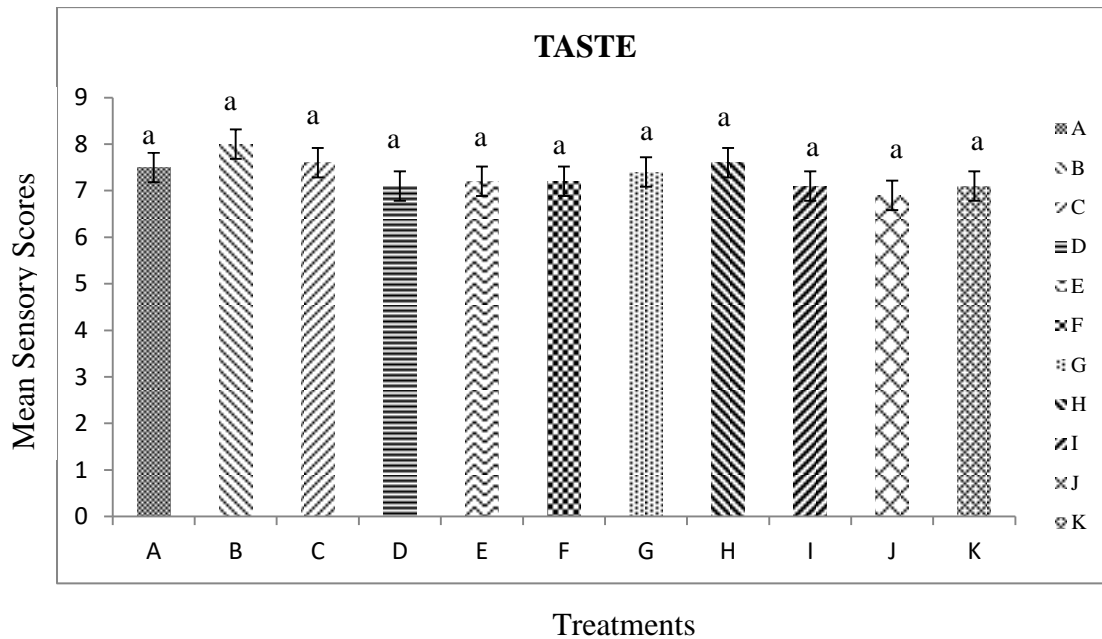


Fig 4.4 Mean sensory score for taste

Figure 4.4 represents the mean sensory scores for flavor of RUTF. Value on top of the bar shows that the samples were not significantly different from each other at 5% level of significance.

4.2.5 Overall acceptability

The average sensory score for overall acceptance was 7.65, 8.1, 7.7, 7.1, 7.3, 7.35, 7.5, 7.4, 6.85, 6.85 and 6.95 for A, B, C, D, E, F, G, H, I, J and K respectively. In case of overall acceptability, sample A, C, D, E, F, G and H had no significant difference between them at 5% level of significance. Similarly, sample I, J and K also had no significant difference between them. But sample B were significantly from all the sample. The overall acceptability mean showed the product B is superior, which might be due to good color, taste and flavor than other products. Hence, from the statistical analysis the overall acceptability of product B with wheat, maize, soybean, Bengal gram, pumpkin seed, peanut, milk powder, sugar and sunflower oil was found to be superior.

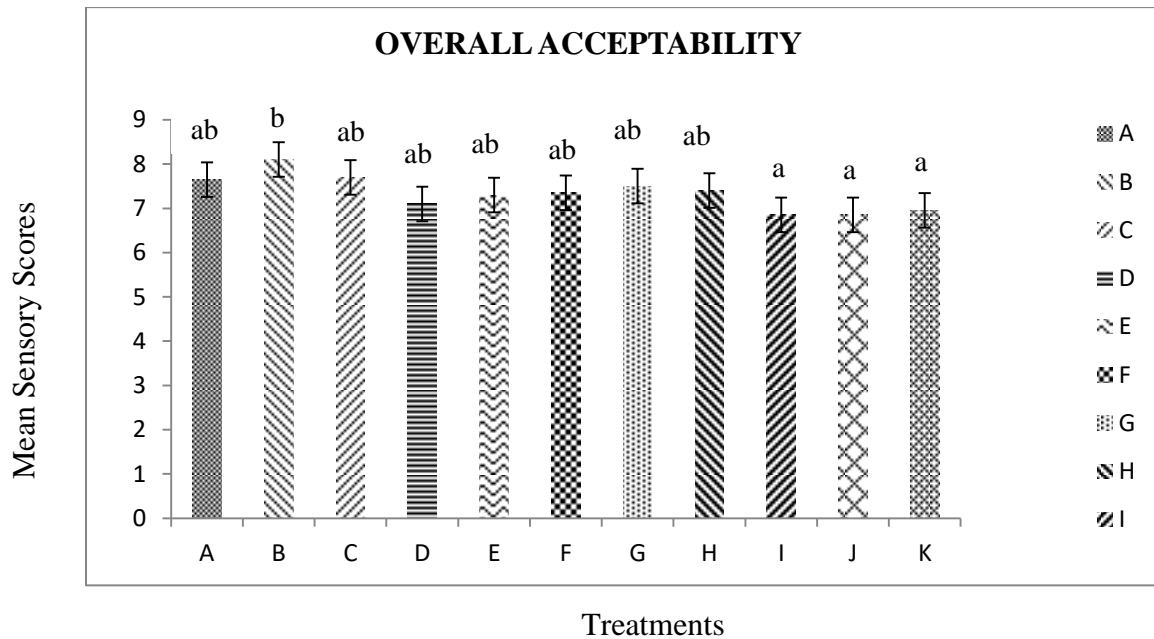


Fig 4.5 Mean sensory score for texture

Figure 4.5 represents the mean sensory scores for flavor of RUTF. Value on top of the bar bearing similar superscript were not significantly different at 5% level of significance.

4.3 Analysis of optimized RUTF

The chemical analysis of sample B which was selected best from sensory analysis was carried out. It may be due to high or low amount of any ingredient. The result is tabulated in Table 4.1.

Table 4.1 Analysis of RUTF

Parameters	Amount
Moisture (%)	5.9
Protein (% db)	13
Fat (% db)	35.76
Ash content (% db)	1.8
Crude fiber (% db)	2.7
Carbohydrate (% db)	45.44
Calcium (mg/100 g)	535.6
Iron (mg/100g)	13.2
Ascorbic acid (mg/100g)	55
Energy (Kcal/100g)	555.6

4.4 Microbiological quality of product

Total plate count (TPC) and yeast and mold count of the product as received by the microbiological assay are shown in table 4.2

Table 4.2 Microbiological assay of the products

Parameters	Cfu/g
TPC	2.13×10^3
Yeast and Mold count	42

The total plate count of the RUTF was found to be 2.13×10^3 cfu/g which was within the acceptable limit as specified on a Joint Statement by the WHO, the WFP, the United Nations System Standing Committee on Nutrition and the UNICEF, the maximum acceptable limit of microorganisms is 10^4 cfu/g (UNICEF, 2007b).

Yeasts and molds are ubiquitous in the environment and can contaminate food through inadequately sanitized equipment or as airborne contaminants. The yeast and mold count in the product was found to be 42/g which was within the acceptable limit of 50/g in the product (UNICEF, 2007b).

4.5 Rate of change in AV and PV and shelf life of the RUTF

The shelf life of the RUTF was studied for 4 weeks. The product was packed in HDPE bags. The acid value and peroxide value of extracted fat of the product was evaluated from the date of manufacture up to 4-week of storage at room temperature.

4.5.1 Changes in acid value

The changes of acid value of RUTF in HDPE bags at every interval of week were observed for four weeks where free fatty acid of the product was found to be increased. The acid value (AV) of the product was observed to be 0.16 mg KOH/g at initial which reached 0.72 mg KOH/g after 28 days. The normal acid value range of sunflower oil is less than 2 mg KOH/g of fat (Hesham R. Lotfy, 2015).

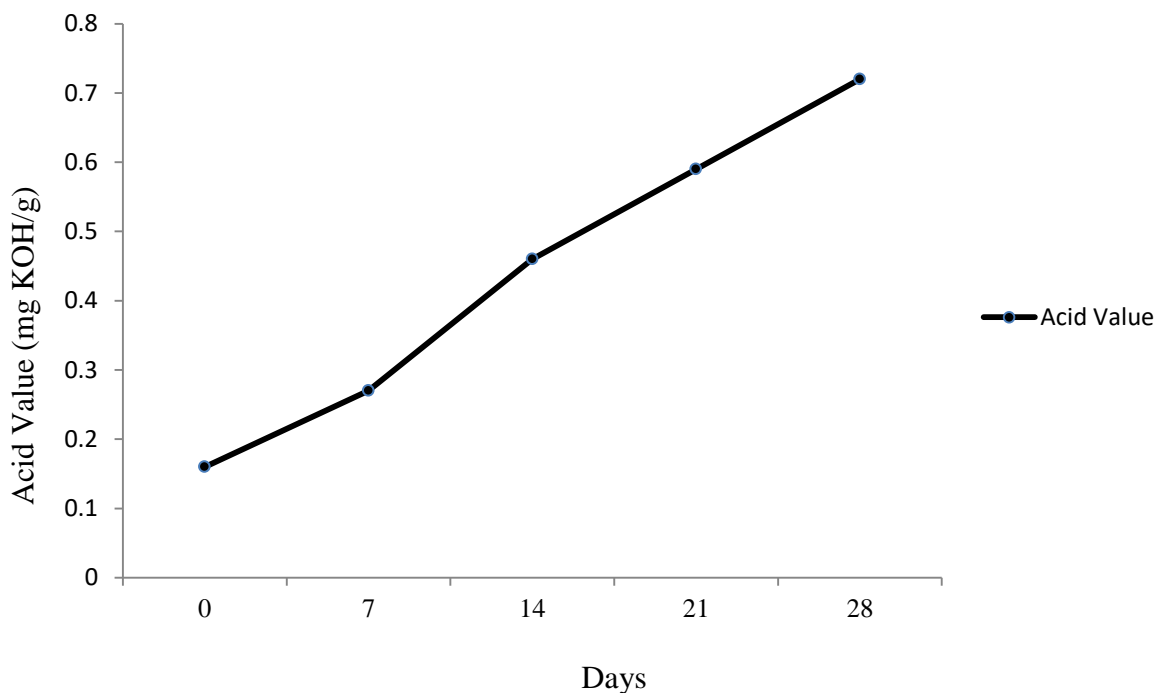


Fig 4.6 Changes in acid value during storage at room temperature

In general, the acid value is the amount of free fatty acid content in the product. The acid content in fat or oil is given by the quantity of the free fatty acids deriving from the hydrolytic deterioration of the triacylglycerol. This alteration occurs under unsuitable conditions of treatment and preservation of the fats and thus acidity represents a basic indicator of the genuineness of the product. The determination of acid value is one of the more common analyses that fats are subjected to in terms of evaluating lipolysis after extraction and of defining and monitoring refining procedures. The acid content in an alimentary fat or oil is given by the quantity of free fatty acids deriving from the hydrolytic deterioration (rancidity) of the triacylglycerols. This alteration occurs under unsuitable conditions of treatment and preservation of the fats and thus the acidity represents a basic indicator of the genuineness of the product (Piotr Koczon, 2008).

4.5.2 Changes in peroxide value

The changes of peroxide value of RUTF in HDPE bags at every interval of week were observed for four weeks where PV of the product was found to be increased with storage time. The peroxide value of the product was found to be increased with storage time. The peroxide value (PV) of the product was observed to be 1.3meq/kg at initial

which reached 2.5meq/kg after 28 days. The normal peroxide value of sunflower oil is less than 10meq/kg fat (Hesham R. Lotfy, 2015).

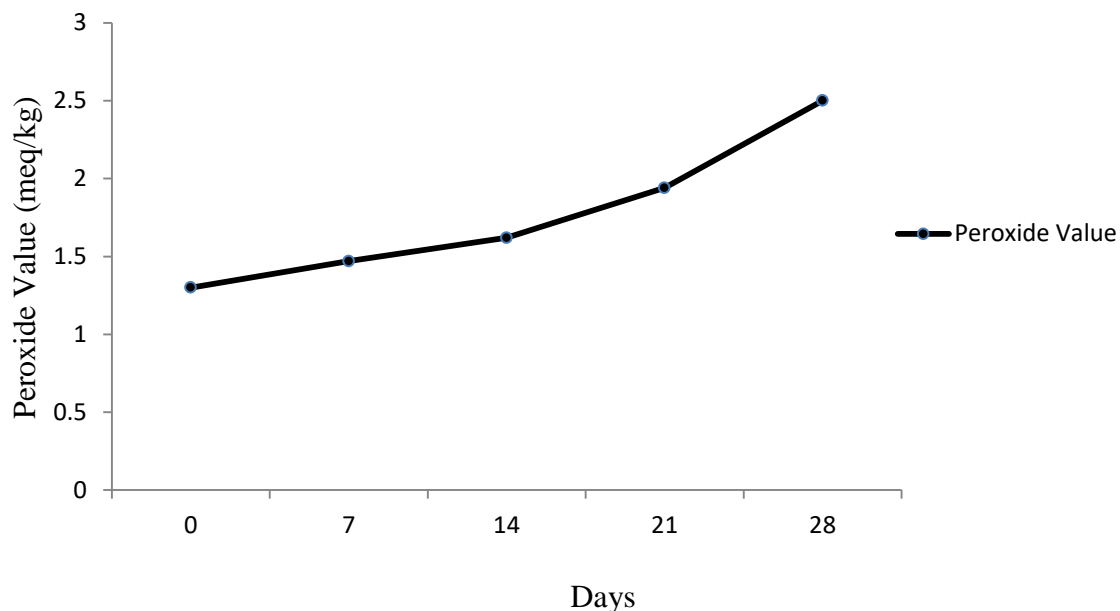


Fig 4.7 Changes in peroxide value during storage at room temperature

The peroxide value is a useful indicator of the extent of oxidation of lipids, fats, and oils. The oxidation of food lipids is undesirable due to off-flavors, toxins, and loss of fat-soluble vitamins. The peroxide value shows the degree of peroxidation and measures the amount of total peroxides in the substance. It has been associated with the rancidity in lipid-containing food products. It is one of the main causes of generation of off-flavors, deterioration, and production of toxins in oils. Therefore, it negatively affects the quality and storage life of oils. The peroxide value is widely used as a measurement of these unwanted reactions in food stuffs and oils, as well as in biological samples where such reactions are implicated in physiological processes related to the modification of macromolecules causing the initiation of degenerative diseases, cancer and aging (Saadet Dermiş, 2012).

4.6 Cost of product

The cost of the best formulated and superior RUTF analyzed from sensory evaluation is NRs. 28.10 per 100 gm. (calculation is given in Appendix C). The cost of the product

may vary as there may be increase or decrease in the cost of raw materials as per the season.

4.7 Comparison of the prepared product with CG sarbottam pitho

The prepared RUTF was compared with Chaudhary's Group sarbottam pitho. The protein, carbohydrate and iron content were found to be slightly more in CG sarbottam pitho. The amount of energy, calcium and fat were more in the prepared RUTF.

Part V

Conclusions and recommendations

5.1 Conclusions

From the above result and discussions, it can be concluded that:

- a. Among all in terms of sensory analysis, the sample with 11 parts wheat, 13 parts maize, 11 parts soybean, 1 part Bengal gram, 10 parts pumpkin seed, 10 parts peanut, 11 parts milk powder, 17 parts sunflower oil, 15 parts sugar and 1 part vitamin and mineral premix was found to be the best.
- b. From analysis of the best product, it fulfills the basic requirement of RUTF given by WHO. The prepared RUTF provides 13 g protein, 35.76 g fat, 45.44 g carbohydrate, 535.6 mg calcium, 13.2 mg iron, 55 mg ascorbic acid and 555.6 kcal energy per 100 g of the product. The moisture, ash, crude fiber content of the product was found to be 5.9%, 1.8% and 2.7 % respectively.
- c. The total cost of the RUTF was calculated and found to be NRs. 28.10 per 100 gm.
- d. The total plate count, and yeast and mold count in RUTF was 2.13×10^3 cfu/g, 42 cfu/g respectively. The product was safe from microbiological point of view.
- e. The product is safe till 28 days in HDPE on the basis of acid value and peroxide value.
- f. The prepared RUTF should be served to the children by mixing the RUTF in warm water.

5.2 Recommendations

This study can be further continued with the following recommendations.

- a. The formulation having different cereals, legumes, oilseeds and vitamin and mineral premix can be used to make low cost RUTF to treat severe acute malnutrition in children.
- b. In vivo evaluation using albino rats could be done.
- c. Shelf life study using different packaging materials can be done.

Part VI

Summary

Malnutrition, in all its forms, includes under nutrition (wasting, stunting, underweight), inadequate vitamins or minerals, overweight, obesity, and resulting diet-related non communicable diseases. Though malnutrition can imply inappropriate intake of any of the essential nutrients, protein energy malnutrition (PEM) which is due to an inappropriate intake of the macro-nutrients especially protein and fats is the commonest form of malnutrition. Acute malnutrition is the leading causes of morbidity and mortality of children aged 6–59 months as risk of death is nine times higher than that of children without it. The severely malnourished child with an adequate appetite is best managed at home with ready to use therapeutic food (RUTF). RUTF are soft or crushable foods that can be consumed easily by children from the age of six months without adding water. It is very energy dense and does not need to be mixed with water. RUTF is highly effective in the treatment of various forms of severe acute malnutrition, including kwashiorkor, nutritional marasmus, and several forms of severe wasting.

Eleven different formulations were made from locally available raw materials based on the WFP specification. The raw materials were processed and the products were prepared in laboratory and sensory evaluation was performed by 10 semi trained panelists. On the basis of results from sensory evaluation the product B was taken for further chemical analysis. The analysis includes the proximate analysis of the product and ultimate analysis of some vitamins and minerals. The protein, fat, carbohydrate, crude fiber, total ash, vitamin C, iron and calcium of the product were found to be 13%, 35.76%, 45.44%, 2.7%, 1.8%, 55 mg/100gm, 13.2 mg/100gm and 535.6 mg/100gm respectively. The diet can supply 555.6 Kcal/100 gm. The energy contributed by the protein, fat and carbohydrate were found to be 9.35%, 57.9% and 32.71% of total Kcals respectively and contains all the essential amino acids required by the children under 5 years of age.

The microbiological analysis of the product was carried out where TPC and yeast and mold count were tested. The TPC and yeast and mold count in the product were 2.13×10^3 cfu/g and 42 cfu/g respectively. The rate of change in AV and PV in HDPE packaging material, when studied for 30 days showed that the product was within the acceptable limit. The average cost of the product was calculated. Finally, the data

obtained from chemical analysis and sensory evaluations were analyzed using standard statistical software.

Thus, the prepared RUTF using locally available raw materials which contain all the essential nutrients required for the children could be effective for the treatment of severe acute malnutrition (SAM).

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Appendices
Appendix- A

1. Sensory evaluation card

Sensory analysis of ready to use therapeutic food (RUTF)

Name of the panelist:

Date:

Name of the product: Ready to use therapeutic food (RUTF)

Type of product: High energy nutrient dense food for children with severely acute malnutrition

Dear panelist, you are given 11 samples of RUTF, please conduct the sensory analysis based on the following parameter using the table given;

Sample	Color	Flavor	Taste	Texture	Overall acceptability
A					
B					
C					
D					
E					
F					
G					
H					
I					
J					
K					

Perceptions	Point
Like extremely	
Like very much	
Like moderately	
Like slightly	
Neither like nor dislike	
Dislike slightly	
Dislike moderately	
Dislike very much	
Dislike extremely	

Comments (if any)

.....
.....

.....

Signature

Appendix-B

1) Sensory evaluation of the product

Table B.1.1 Two-way ANOVA (no blocking) for color

Source of variation	d.f	s.s	m.s	v.r	Fpr.
Sample	10	3.3636	0.3364	1.69	0.095
Panelists	9	78.9909	8.7768	44.11	<.001
Residual	90	17.9091	0.1990		
Total	109	100.2636			

Table B.1.2 Two way ANOVA (no blocking) for flavor

Source of variation	d.f	s.s	m.s	v.r	Fpr.
Sample	10	11.3636	1.1364	1.90	0.056
Panelists	9	37.4909	4.1657	6.95	<.001
Residual	90	53.9091	0.5990		
Total	109	102.7636			

Table B.1.3 Two-way ANOVA (no blocking) for texture

Source of variation	d.f	s.s	m.s	v.r	Fpr.
Sample	10	8.2545	0.8255	1.94	0.050
Panelists	9	80.4091	8.9343	21.00	<.001
Residual	90	38.2909	0.4255		
Total	109	126.9545			

Table B.1.4 Two-way ANOVA (no blocking) for taste

Source of variation	d.f	s.s	m.s	v.r	Fpr.
Sample	10	9.6182	0.9618	1.50	0.152
Panelists	9	36.9455	4.1051	6.41	<.001
Residual	90	57.6545	0.6406		
Total	109	104.2182			

Table B.1.5 One-way ANOVA (no blocking) for overall acceptability

Source of variation	d.f	s.s	m.s	v.r	Fpr.
Sample	10	15.2409	1.5241	3.02	0.002
Panelists	9	51.8750	5.7639	11.44	<.001
Residual	90	45.3500	0.5039		
Total	109	112.4659			

Appendix-C

Table C.1 Chemical composition of selected ingredients

Parameters	Wheat	maize	Soybean	Bengal gram	Pumpkin seed	peanut	Milk powder	Sunflower oil	sugar
Moisture(g/100g)	12.2	14.9	10.2	9.8	8	3	-	-	0.4
Protein(g/100g)	12.1	11.1	33.3	17.1	24.3	25.3	17.4	-	0.1
Fat(g/100g)	1.7	3.6	17.7	5.3	47.2	40.1	18.1	100	
Carbohydrate(g/100g)	69.4	66.2	29.6	60.9	15.6	26.1	53.1	-	99.4
Crude fiber(g/100g)	1.9	2.7	4.2	3.9	0.2	3.1	0	-	0
Ash(g/100g)	-	-	-	-	-	-	-	-	-
Energy(Kcal/100g)	341	342	411	360	584	567	445	900	398
Calcium(mg/100g)	48	10	226	44.8	50	90	-	-	-
Phosphorous(mg/100g)	355	348	546	312	830	350	-	-	-
Iron(mg/100g)	4.9	3.3	8.5	4.6	5.5	2.5	-	-	-
Carotene(μ g/100g)	29	90	10	189	38	37	-	-	-
Vit. C(mg/100g)	0	0	0	3	1	0	-	-	-
Thiamine(mg/100g)	0.49	0.42	0.66	-	0.33	-	-	-	-
Riboflavin(mg/100g)	0.17	0.1	0.22	-	0.16	-	-	-	-
Niacin(mg/100g)	4.3	1.8	2.2	-	3.1	-	-	-	-

Source: (DFTQC, 2017)

Table C.2 Cost calculation of the product (Formula B)

Particulars	Cost (NRs/kg)	Weight (g)	Cost (NRs)
Wheat	50	11	0.55
Maize	40	13	0.52
Soybean	100	11	1.1
Bengal gram	110	1	0.11
Pumpkin seed	-	10	-
Peanut	180	10	1.8
SMP	900	10	9
Sugar	80	15	1.2
Oil	170	17	2.89
Vitamin premix	-	1	-
Total raw material cost			17.17
Processing and labor cost (20% profit)			3.434
Packaging cost			5
Profit (10%)			2.5
Grand total cost			28.10

Cost of material varies with season and time

Appendix-D

Table D.1 Composition of Micronutrient Premix (Fortivit-FBF-v-13)

Ingredient	Label claim / 2kg of premix
Vitamin-A	34.60 min
Vitamin-D	4.416 min
Vitamin-E	83.00 g
Vitamin-K1	0.30 g
Vitamin-B1	2.00 g
Vitamin-B2	14.00 g
Vitamin-B6	10.00 g
Vitamin C	900 g
Pantothenic acid	16.00 g
Folic acid	1.10 g
Niacin	80.00 g
Vitamin B12	0.020 g
Biotin	0.082 g
Iodine	0.400 g
Iron	65.00 g
Zinc	50.00 g

Appendix-E
Photo gallery



Photo 1 and 2: Determination of total protein



Photo 2: Prepared RUTF