PREPARATION OF READY TO USE SUPPLEMENTARY FOOD (RUSF) FOR TREATING MODERATELY ACUTE MALNUTRITION IN CHILDREN AGED 6-24 MONTHS

by

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Preparation of Ready to Use Supplementary Food (RUSF) For Treating Moderately Acute Malnutrition in Children Aged 6-24 Months

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 of ready to use supplementary food (RUSF) for treating moderately acute malnutrition in children aged 6-24 months* presented by Pratik Niraula has been accepted as the partial fulfillment of the requirements for the Bachelor degree in Nutrition and Dietetics.

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Regards,

Date of submission: January, 04, 2017

Pratik Niraula

Abstract

A well-balanced diet was prepared based on the recommendation of WFP and UNICEF for the treatment of moderate acute malnourished children aged 6-24 months. According to the formulae, three products of RUSF A, B and C were prepared using cereals, legumes, seeds, oil, sugar, SMP and vitamin and mineral premix.

On the basis of sensory evaluation, RUSF B was found to be the best among the three products. The product was analyzed for proximate composition, mineral, vitamin, microbiological quality, organoleptic quality, rate of change in PV and AV, amino acid composition and cost calculation. The protein, fat, carbohydrate, crude fiber, total ash, vitamin C, iron and calcium of the product were found to be 14.77%, 33.87%, 44.73%, 1.67%, 2.70%, 66.95 mg/100gm, 12.27 mg/100gm and 525.58 mg/100gm respectively. The diet can supply 542.83 Kcal/100 gm. The energy contributed by the protein, fat and carbohydrate were found to be 10.83%, 56.05% and 32.8% of total Kcals respectively and contains all the essential amino acids required by the children under 2 years of age. The diet was found to be microbiologically safe. The TPC and yeast and mold count in the product were 1850 cfu/g and 48 cfu/g respectively. The cost of product was calculated as NRs. 30.36/100 gm. The rate of change in AV and PV under three packaging materials, i.e., HDPE, aluminum foil and glass bottles when studied for four consecutive weeks were found within the acceptable limit. Hence, the prepared RUSF is in accordance to the specification given by WFP which could be effective in the treatment of moderate acute malnourished child after the clinical trial.

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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 acute malnutrition
LNS	Lipid nutrient supplement
MAM	Moderate acute malnutrition
MOHP	Ministry of health and population
MUAC	Mid upper arm circumference
MUFA	Mono unsaturated 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

Under nutrition is a prevalent condition in many developing countries and a pre-disposing factor to various forms of diseases. This to a large extent has been a result of poverty which makes it very difficult for vulnerable groups to afford nutritionally adequate food. As a result, they are heavily dependent on high carbohydrate foods with little or inadequate supplies of proteins and essential vitamins. 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).

Under nutrition includes both protein energy malnutrition and micronutrient deficiencies. Undernourishment directly affects many aspects of the children mental functions, growth and development which have adverse effects on children ability to learn and process information and grow into adults. Undernourishment also impairs immune function leaving them more susceptible to infection in children (Jelliffe, 1966).

Moderate acute malnutrition (MAM) is defined as a weight-for-age between -3 and -2 z-scores below the median of the WHO child growth standards. It can be due to a low weight-for-height (wasting) or a low height-for-age (stunting) or to a combination of both. Similarly, moderate wasting and stunting are defined as a weight-for-height and height-for-age, respectively, between -3 and -2 z-scores. Moderate acute malnutrition affects many children in poor countries. Children with moderate malnutrition have an increased risk of mortality and MAM is associated with a high number of nutrition-related deaths. If some of these moderately malnourished children do not receive adequate support, they may progress towards severe acute malnutrition (severe wasting and/or oedema) or severe stunting (height-for-age less than -3 z-scores), which are both life-threatening conditions. Therefore, the management of MAM should be a public health priority (WHO, 2016c).

Malnourished children are highly vulnerable to death and diseases. Children with moderate acute malnutrition have nutritional requirements that differ from non-malnourished and severely malnourished children, that is, they require increased intake of energy and essential nutrients over and above those required by non-malnourished children and, when necessary, treatment for any associated medical conditions. The dietary management of moderate acute malnutrition should normally be based on the optimal use of locally available nutrient-dense foods to improve the nutritional status of children and prevent them from becoming severely acutely malnourished or failing to thrive. Intake of nutrients present in inadequate amounts in the habitual diet can be increased through a number of approaches, including dietary diversification and fortification of certain staple foods with vitamins and minerals (Karakochuk *et al.*, 2012).

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, sesame etc. The treatment may also include commercially produced ready to use (therapeutic and supplementary) food. These foods are high energy dense foods fortified with vitamin and minerals which are very effective in the treatment of SAM and MAM. The supplement of both macro and micro nutrients make ready to use foods more effective (Wagh and Deore, 2015).

The term ready to use 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 (WHO, 2012).

RUTF and RUSF have been developed to provide a nutrient-dense alternative to more conventional supplements. As these supplements have a low water activity, they are safer to use in developing countries where clean water and optimal storage conditions are often problematic. RUTF generally consists of a peanut butter base in vegetable oil with added protein, vitamins and minerals. RUTF has been used in the international setting for the treatment of SAM as an improved alternative to F-100 for a number of years. Recently, calls have gone out to use these energy dense supplements in the treatment of moderate malnutrition as well. This practice was supported by the WHO, which also favors short, aggressive supplementation periods in children with malnutrition that allows for rehabilitation in 14 to 40 days (Steenkamp *et al.*, 2015).

Supplementary feeding programs are designed to treat MAM and prevent the progression from MAM to SAM and thus have the potential to reduce child mortality and morbidity. Supplementary food in general, is any appropriate food product or products, enhanced nutritionally, and thus made to be more energy-dense and more nutrient-dense. When needed, usually in emergency situations, it can be used in effect as medicine, in conjunction with necessary therapy, for as short possible period of time (Latham *et al.*, 2011) .

For the past, several decades, supplementary feeding programs have been used in developing countries to treat MAM despite insufficient evidence on the effectiveness of the treatment foods provided in these programs. Fortified blended flours, such as corn-soya blend (CSB), prepared as porridge, are the most widely used foods in supplementary feeding programs. The goal of supplementary feeding programs is to treat children with MAM and prevent children from deteriorating and developing SAM (Karakochuk *et al.*, 2012).

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 overall (WHO, 2016b).

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 supplementary 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 RUSF could be the best in the management of outpatient care or home based treatment of MAM children (Wagh and Deore, 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 *et al.*, 2011).

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 11% of children aged below 5 years in Nepal, where 3% are severely malnourished and 8% are moderately malnourished (MOHP, 2012). 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 (WHO, 2000). 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, 2015). There is no any commercial production of RUSF in Nepal instead it is imported from India and France. However, WHO recommends the use of locally available foods in production of RUSF for the treatment of MAM children (WHO, 2013). So, this work primarily focuses on the production and quality evaluation of RUSF using locally available raw materials.

1.3 Objectives

1.3.1 General objectives

The main objective of this work is to prepare ready to use supplementary food (RUSF) for the treatment of moderately acute malnutrition in children aged 6-24 months

1.3.2 Specific objectives

a. To prepare RUSF in accordance to the specification given by WFP.
b. To perform sensory and physiochemical analysis of the prepared RUSF.
c. To evaluate microbiological quality of the prepared RUSF.
d. To analyze the essential amino acid composition of the formulated product.
e. To study the shelf life of the product on the selected packaging material at room temperature.

1.4 Significance of the study

RUSF has been effective in the treatment of MAM (Manary and Sandige, 2008) and the use of locally available foods in the preparation of RUSF has been highly recommended (WHO, 2012). This study would be effective in the treatment of MAM. The diet can be prescribed to the children with MAM after the clinical trials. This study can be extended in the wide aspect such as clinical trials of the product in children and even pregnant and lactating women suffering from MAM. If further researched the industrial production of RUSF using different locally available foods in Nepal could be possible. Thus, this study will provide the basis for the further research in this field.

1.5 Limitations

a. Clinical trials using albino rats could not be done.
b. Analysis of vitamin and trace elements, amino acid and fatty acid composition of the product could not be performed due to the limitation of the required facilities in Central Campus of Technology (CCT) laboratory.
c. Shelf life of the RUSF could not be studied for enough period of time

Part II

Literature review

2.1 Food and nutrition

Food is not just something to it but it 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 *et al.*, 2006).

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 (Thompson and Manore, 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. (WHO, 2016a). 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

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, also an essential component in the diet, in resource-poor populations make-up about 10% of the diet. Fats also 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 by-products 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

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).

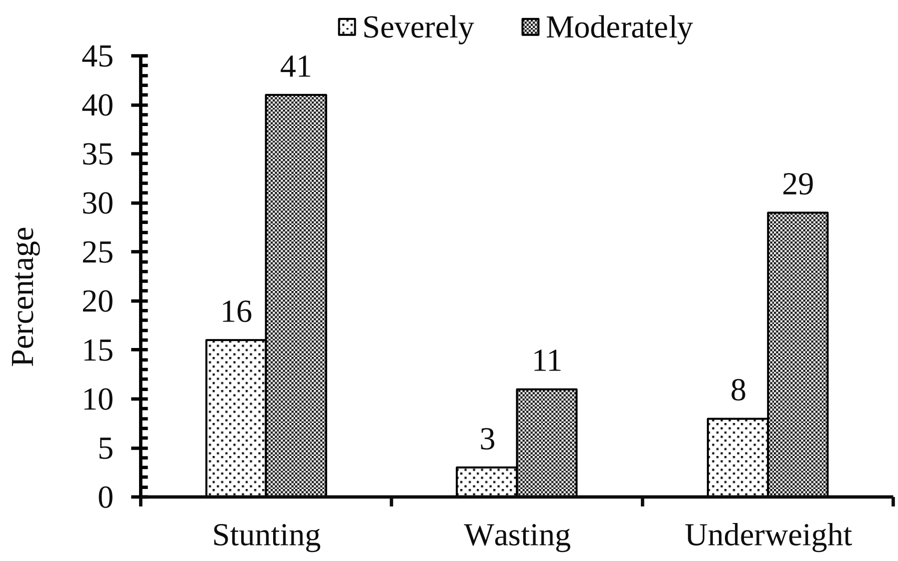
2.3 Nutritional status

Nutritional status is a complex concept that is difficult to define. Adequate nutritional status can perhaps be best defined as maintenance of a normal pattern of growth and a normal body composition by consumption of appropriate amounts and types of food (Foster and Leonard, 2004).

2.3.1 Nutritional status of children in Nepal

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).

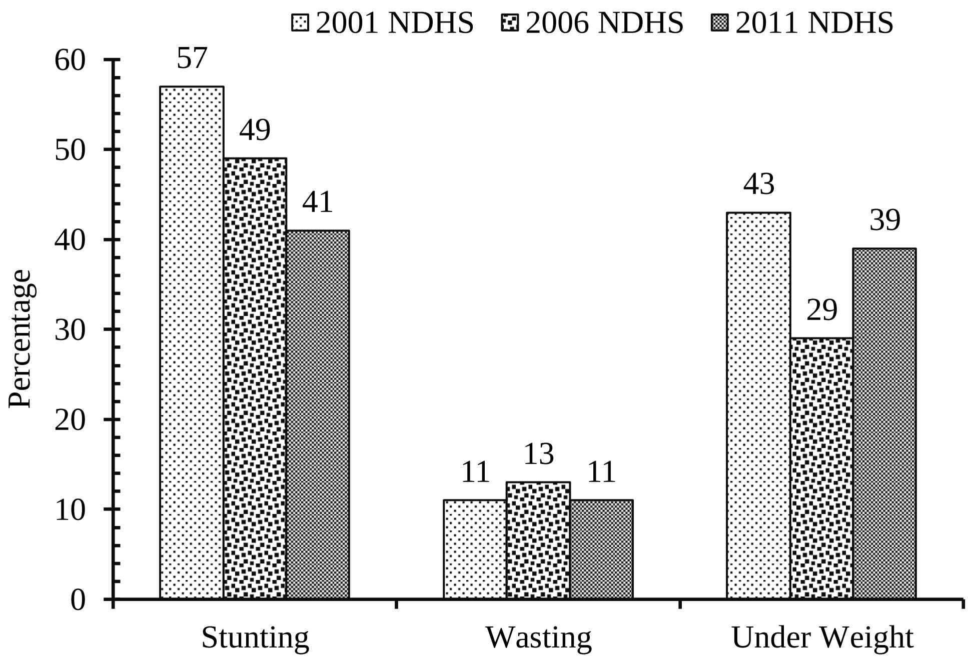
According to NDHS 2011, 41% of the children under five years of age are stunted, out of which 16% are severely stunted. Similarly, 11% of children are wasted, out of which 3% are severely wasted. 29% of the children under five years of age are underweight, out of which 8% are severely underweight (MOHP, 2012).



Source: (MOHP, 2012)

Fig 2.1 Prevalence of different forms of malnutrition in Nepal

The prevalence of malnutrition imposes significant costs on the Nepalese 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, 2015).



Source: (MOHP, 2012)

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

2.3.2 Recommended dietary allowances (RDAs) for vulnerable groups

Recommended dietary allowance (RDA) is the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group (ICMR, 2010). For normal growth and healthy life people need a well-balanced diet having the nutrient composition that is needed to fully satisfy the metabolic requirements of the body. There are different requirements of nutrients for different groups of people with different characteristics age, gender, health status etc. (Naicker, 2004). Child nutrition is vital for proper growth and development (Campos *et al.*, 2010) 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 (Hermoso *et al.*, 2010).When these needs are not met these people are exposed to malnutrition and its accompanying complications (Brown and Pollitt, 1996).

Table 2.1 Recommended dietary allowances for children <3 years

Nutrients		0-6 months	6-12 months	1-3 years
Body wt.		5.4	8.4	12.9
Net calories (Kcal/day)		92 kcal/kg	80 kcal/kg	1060
Proteins (gm/day)		1.16 gm/kg	1.69 gm/kg	16.7
Visible fat (gm/day)		-	19	27
Calcium (mg/day)		500	500	600
Iron (mg/day)		46 µg/kg	05	09
Vitamin A (µg/day)	Retinol	350	350	400
	β-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)		710 µg/kg	650 µg/kg	8
Pyridoxine (mg/day)		0.1	0.4	0.9
Vit. B_{12} (µg/day)		0.2	0.2	0.2-1.0
Ascorbic acid (mg/day)		25	25	40
Dietary folate (µg/day)		25	25	80

Source: (ICMR, 2010)

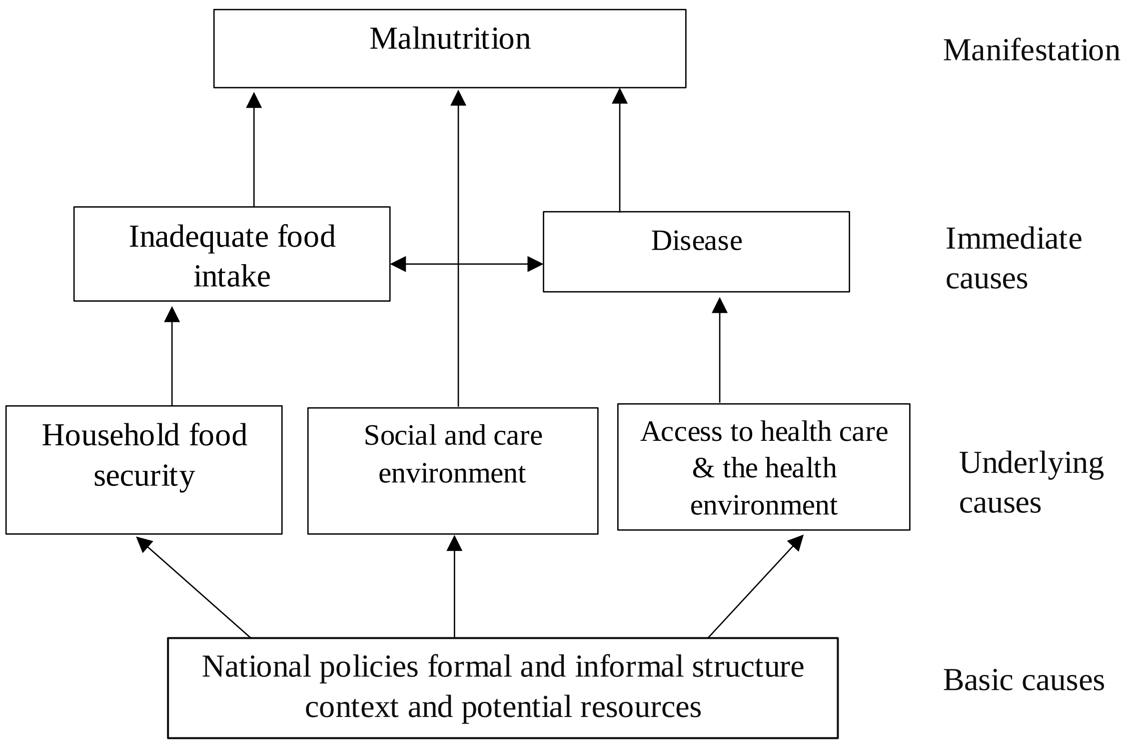
2.4 Malnutrition

Malnutrition or abnormal nutrition may be defined as excess or a deficit of nutrients depending on the physiological requirements of the body and based on the requirements of normal nutrition which occurs in human beings as a result of nutritional imbalance, the body mass index (BMI) is used as an indicator of the risk of an individual being over or under-nourished (Stratton *et al.*, 2003). 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 (Stratton *et al.*, 2003).

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.



Source: (UNICEF, 1990)

Fig 2.3 UNICEF conceptual framework

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 and Fanzo, 2014).

2.4.2.1 Chronic malnutrition

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. However, stunted growth is only one manifestation of 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 and Fanzo, 2014).

2.4.2.2 Acute malnutrition

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 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 *et al.*, 2015). Clinical forms of acute malnutrition can be defined by the characteristics of severe wasting, also called marasmus, and/or bilateral pitting oedema, found in kwashiorkor as well as in a range of other clinical signs (Dibari *et al.*, 2013).

2.4.2.2.1 Marasmus

Marasmus is a form of severe undernutrition, referred to alternatively as non-oedematous malnutrition. The Wellcome 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 *et al.*, 2013).

2.4.2.2.2 Kwashiorkor

Kwashiorkor is a form of severe undernutrition, also referred to as oedematous malnutrition. Kwashiorkor is associated with growth failure (when compared with healthy children) and characterized by oedema, loss of appetite, thin, sparse or discolored hair and skin with discolored patches that may crack and peel. Kwashiorkor is associated with a pitting oedema affecting both sides of the body (bilateral). Oedema is defined as "swelling from excessive accumulation of watery fluid in cells, or tissues" (Dibari *et al.*, 2013).

2.4.2.2.3 Marasmic Kwashiorkor

Marasmic kwashiorkor is a form of severe undernutrition diagnosed by the presence of risk of short-term death is assessed by the circumference of the left middle upper arm (MUAC), whereas nutritional indices referring to z-scores (explained in Table 2.1) are more indicated to define the anthropometry of children presenting MAM or SAM. In addition, the presence of bilateral pitting oedema is considered diagnostic for SAM (Dibari *et al.*, 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 (WHO, 2013).

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

	Measure	Cut-off
Severe acute malnutrition	Weight-for-height	< -3SD
	MUAC	<115mm
	Bilateral pitting oedema	Grades 1, 2 or 3
Moderate acute malnutrition	Weight-for-height	<-2SD and ≥-3SD
	MUAC	<125mm and ≥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, 2015).

2.4.4.1 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 programmes and services focused on the critical 1000-day window (UNOCHA, 2015).

IMAM is based on the same principles as the initial CMAM programme (UNOCHA, 2015). 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 prioritises early case-finding and mobilisation 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 programme until they have recovered.

2.4.4.2 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 include 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, 2007).

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 100 kcal/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 (Manary and Sandige, 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) (Collins *et al.*, 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 (Defourny *et al.*, 2009) due to cost the availability of facilities and their effect on the number of people reached by these interventions (Karakochuk *et al.*, 2012).

Children with severe acute malnutrition need safe, palatable foods with a high-energy content and adequate amounts 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 at will (UNICEF, 2007).

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) 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 *et al.*, 2011).

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, 2013).

2.4.5.3 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, 2007). 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, 2012). Unfortunately, available and affordable foods often fail to meet nutrient needs, particularly when families cannot afford frequent consumption of animal-source foods (Arimond *et al.*, 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 *et al.*, 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 *et al.*, 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, 2015).

Ingredients: 92g of each sachet Plumpy'Sup contains peanuts, sugar, whey, vegetables oil, milk, soy protein, cocoa, vitamins and minerals with 500 kcal, 13g protein (10%) and 31g fat (55%). 92g of each sachet eeZeeRUSF 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, 2015).

Ration: Each admitted individual will be provided 1 sachet of RUSF per day for a period of 60 to 90 days as supplementary food. However, all caregivers, mothers and children will be encouraged to utilize nutritious food available at household level. The RUSF 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, 2015).

Principles of nutritional management of children with moderate acute malnutrition

According to (WHO, 2012) 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 counselling 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 fulfil 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 Alimentary 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 therapeutic food (RUTF)

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 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 and Deore, 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 (Karakochuk *et al.*, 2012); (Briend and Collins, 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 or RUTF include peanuts, oil, sugar, milk powder and vitamin and mineral supplements (UNICEF, 2013).

2.6 Ready-to-use supplementary food (RUSF)

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 *et al.*, 2010). In children with SAM this may come as RUTF whereas in those with MAM it has been described as RUSF (Defourny *et al.*, 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).

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 *et al.*, 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 *et al.*, 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 *et al.*, 2012).

2.7 Raw materials and their nutritive value

2.7.1 Cereals

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.) (Serna-Saldivar, 2010).

2.7.1.1 Wheat

Wheat (*Triticum* spp.) 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 a higher protein content than the other major cereals maize and rice (Shewry, 2009). 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, 2012).

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 (Suhasini and Malleshi, 2003).

2.7.1.2 Maize

Maize *(Zea mays)* is one of the most important and widely distributed cereals crop 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 (Iken and Amusa, 2004).

2.7.2 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 *et al.*, 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, haematogluinins etc. which can be removed by heat treatment before consumption (Prasad *et al.*, 2016).

2.7.2.1 Soyabean

It is the member of the family Leguminosae. 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 and Richard, 2003). The PER of the heat processed soyabean protein ranges from 1.9-2.2 and is increased markedly to 2.8-3.0 by supplementation with methionine. The chief protein of soyabean is a globulin known as glycinin; other proteins present in small amount are phaseotin 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 *et al.*, 2007).

2.7.2.2 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).

2.7.3 Oil seeds

2.7.3.1 Sesame

Sesame *(Sesamum indicum L.)* is a flowering plant in the genus *Sesamum.* Sesame has one of the highest oil contents of any seed. It is called as "queen of oil seeds" because of its excellent qualities of the seed, oil and meal. Sesame seed contains 50% of oil and 25% of protein. Sesame proteins are limited by lysine but rich in tryptophan and methionine. Sesame oil is rich in linoleic and oleic acids, the predominance of gamma-tocopherol over the other isomers of vitamin E and high content of fat-soluble lignans (sesamin and sesamolin). These substances have been shown to have a cholesterol-lowering effect in humans, and to prevent high blood pressure and increase vitamin E supplies in animals. Sesamin has also been found to protect the liver from oxidative damage. Sesame seeds are also an excellent source of copper, a very good source of manganese, and a good source of calcium, phosphorus, magnesium, iron, zinc, molybdenum, vitamin B_1, selenium and dietary fiber. The sesame meal or cake is by-product after oil extraction. The defatted sesame meal is good source of nutrients, containing approximately 50% protein. One of the principal characteristics of this protein is its high methionine and tryptophan content that distinguishes sesame from other oil seeds. This meal has high potential for use as a protein source or as an ingredient in the food industry (Bukya and Vijayakumar, 2013).

2.7.3.2 Pumpkin seed

Pumpkin has received considerable attention in recent years because of the nutritional and health protective values of the seeds. 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 (Giami *et al.*, 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 can enable product diversification and contribute to aggregate technological and nutritional quality and to reduce environmental impact (Elinge *et al.*, 2012) (Santana *et al.*, 2014).

2.7.4 Skim milk powder

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 RUSF 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 antinutrients (Hoppe *et al.*, 2008).

2.7.5 Sunflower oil

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 *et al.*, 2000).

Sunflower oil is the non-volatile oil compressed from the seeds of sunflower *(Helianthus annuus)*. Sunflower oil is commonly used in food as a frying oil. Sunflower oil is a monounsaturated (MUFA)/polyunsaturated (PUFA) mixture of mostly oleic acid (omega-9)-linoleic acid (omega-6) group of oils. The oil contains appreciable quantities of vitamin E, sterols, squalene, and other aliphatic hydrocarbons (Swaminathan, 2004).

2.7.6 Sucrose

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).

2.7.7 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 and 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 and FAO, 2006). (Beigler, 1976) listed the following minerals salts for elemental diet:

- Calcium gluconate
- Calcium citrate
- Calcium chloride.$2H_2O$
- Ferrous gluconate
- Ferrous ammonium sulphate.$6H_2O$
- Cupric acetate.$2H_2O$
- Manganous acetate.$4H_2O$
- Magnesium oxide
- Ferrous sulphate
- Potassium chloride
- Potassium Iodide
- Sodium chloride
- Zinc benzoate
- Zinc acetate.$2H_2O$

2.8 Technology of processing of RUSF

Traditional treatments such as soaking, cooking, germinating and fermenting have been used to improve nutritional quality of the cereals and legumes. Processing of food such as soaking, germination and fermentation leads to a reduction in phytic acid and increases of the minerals solubility in foods and also improves the bioavailability of minerals in cereals and legumes. Processing techniques reduce the levels of ant 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).

2.8.1 Soaking or steeping

Soaking or steeping is a pretreatment for decertification of grain facilitate the removal of the husk or skin. 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 antinutrients. 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 *et al.*, 2000).

The malting process begins when the cereal grain is steeped in water. Steeping is arranged so that sufficient moisture enters the grin to initiate germination. 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 (Kent, 1994).

2.8.2 Germination

Germination or sprouting of legumes and cereals increase their palatability and nutritional value, particularly through the breakdown of certain antinutrients, such as phytates and protease inhibitors. Germination was more effective in reducing phytic acid than heat treatment, and therefore it improves the nutritional quality of cereals and legumes. Germination also slightly increases the total essential amino acids in cereals and legumes. Dehusking, germination, cooking, and roasting have been shown to produce beneficial effects on nutritional quality of legumes (Kadam and Salunkhe, 1985).

The desirable nutritional changes that occur during sprouting are mainly due to the breakdown of complex compounds into a simpler form, transformation into essential constituents and breakdown of nutritionally undesirable constituents. The metabolic activity of resting seeds increases as soon as they are hydrated during soaking. Complex biochemical changes occur during hydration and subsequent sprouting. The reserve chemical constituents, such as protein, starch and lipids, are broken down by enzymes into simple compounds that are used to make new compounds. Sprouting causes increased activities of hydrolytic enzymes, improvements in the contents of total proteins, fat, certain essential amino acids, total sugars, B-group vitamins, and a decrease in dry matter, starch and anti-nutrients. The increased contents of protein, fat, fiber and total ash are only apparent and attributable to the disappearance of starch. However, improvements in amino acid composition, B-group vitamins, sugars, protein and starch digestibilities, and decrease in phytates and protease inhibitors are the metabolic effects of the sprouting process (Chavan *et al.,* 1989).

2.8.3 Drying

Drying produce a friable, readily milled stable product that may be stored for long periods, and from which roots may easily be removed. In drying green malt, the removal of moisture at low temperature allows the maximal survival of enzyme and the least development of aroma and color. Diastatic enzyme survives if the green malt is dried in a rapid air-flow at 40°C, to not less than 10% moisture (Hough *et al.*, 1982).

2.8.4 Roasting

Roasting is a cooking method that uses dry heat where hot air envelops the food, cooking it evenly on all sides with temperatures of at least 150 °C (~300 °F) from an open flame, oven, or another heat source. 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 and Sehgal, 1994a).

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 (Ndidi *et al.*, 2014). 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 and Sehgal, 1994b).

2.8.5 Milling and sieving

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 *et al.*, 1971).

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 *et al.*, 2012).

2.8.7 Packaging of RUSF

RUSF 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 RUSF 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 technology of enclosing or protecting products for distribution, storage, sale, and use. Packaging also refers to the process of designing, evaluating, and producing packages. 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 *et al.*, 2003).

2.9.1 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 and Paine, 1992).

2.9.2 Special feature required for RUSF packaging

RUSF are lipid based paste products. The fat content is generally high so, it is very susceptible to oxidative rancidity. Thus, RUSF 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).

2.9.3 HDPE

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 (Kumar and Singh, 2013). 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, i.e. the lower the value of WVTR, but the oxygen transmission rate is high (Coles *et al.*, 2003). According to (Marsh and Bugusu, 2007) main advantages of HDPE are:

a) Water proofness, low gas and water vapor permeability.
b) Good aroma retention.
c) It is heat sealable, can be oriented and made into bags.
d) It is useful in wrapping meat, fish and dried foods.

2.9.4 Aluminum foil

Aluminum foil provides a complete barrier to light, oxygen, moisture and bacteria. For this reason, foil is used extensively in food and pharmaceutical packaging. It is also used to make aseptic packaging that enables storage of perishable goods without refrigeration. Aluminum is used for packaging as it is highly malleable and can be easily converted to thin sheets and can be easily folded, rolled and packed. Aluminum foil acts as a total barrier to light and oxygen (which cause fats to oxidize or become rancid), odors and flavors, moistness, and germs, and so it is used broadly in food and pharmaceutical packaging. The purpose of aluminum is to make long-life packs (aseptic packaging) for drinks and dairy goods, which allows storing without refrigeration. Aluminum foil is made by rolling pure aluminum metal into very thin sheets, followed by annealing to achieve dead-folding properties (a crease or fold made in the film will stay in place), which allows it to be folded tightly. Moreover, aluminum foil is available in a wide range of thicknesses, with thinner foils used to wrap food and thicker foils used for trays. Like all aluminum packaging, foil provides an excellent barrier to moisture, air, odors, light, and microorganisms. It is inert to acidic foods and does not require lacquer or other protection. Although aluminum is easily recyclable, foils cannot be made from recycled aluminum without pinhole formation in the thin sheets (Scott and Brock, 2006).

Aluminum, when used as a component of food packaging, is in most cases covered by a polymeric film (surface coating or laminated plastic film) the level of migration of aluminum even into acidic foodstuff is extremely low. There is no indication of any adverse health effects caused by aluminum in concentrations that may occur due to migration from packaging material (ILSI, 2007).

2.9.5 Glass bottles

A glass bottle is a bottle created from glass. Glass bottles can vary in size considerably, but are most commonly found in sizes ranging between about 10ml and 5 liters. Glass bottles and jars still offer advantages over other materials, though they are being increasingly displaced by plastics for packaging condiments and oils however they can be reuse and recycled. Glass bottles and jars are easy to clean, sterilize and re-use. Glass bottles and jars are available in various color choices and a multitude of design options. With high shelf appeal and wide decorating possibilities, packaging your product in glass projects quality and substance (Coles *et al.*, 2003).

Glass is entirely made from natural raw materials, which are toxicologically inert. The major constituents, i.e. sodium/potassium silicates are non-toxic and chemically highly inert. The transfer of silicates and cations into food is marginal and even if it occurs, is toxicologically irrelevant, since the cations usually present are non-toxic. Virtually no traces of problematic migrants originating from the glass are found in glass-bottled food products (Schrenk, 2014).

2.10 Shelf life

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 (Gyesley, 1991).

Shelf life depends on the degradation mechanism of the specific product. Most can be influenced by several factors: exposure to light, heat, moisture, transmission of gases, mechanical stress, and contamination by thins such as microorganisms. Product quality is often mathematically modelled around a parameter (concentration of a chemical compound, a microbiological index or moisture content (Azanha and Faria, 2005).

2.11 Rancidity of fat and oils

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).

2.11.1 Types of rancidity

Two types of lipid oxidation cause the most concern for causing fats and oils to be rancid. These are oxidative rancidity and hydrolytic rancidity (Koon, 2009).

2.11.1.1 Hydrolytic rancidity

Hydrolytic rancidity results in the formation of free fatty acids and soaps (salts of free fatty acids) and is caused by either the reaction of lipid and water in the presence of a catalyst or by the action of lipase enzymes (Flavia *et al.*, 2016).

2.11.1.2 Oxidative rancidity

Oxidative rancidity results from more complex lipid oxidation processes. The processes are generally considered to occur in three phases: an initiation or induction phase, a propagation phase, and a termination phase. In complex systems, the products of each of these phases will increase and decrease over time, making it difficult to quantitatively measure lipid oxidation. During the initiation phase, molecular oxygen combines with unsaturated fatty acids to produce hydroperoxides and free radicals, both of which are very reactive. For this phase to occur at any meaningful rate, some type of oxidative initiators must also be present, such as chemical oxidizers, transition metals (i.e., iron or copper), or enzymes (i.e., lipoxygenases). Heat and light also increase the rate of this and other phases of lipid oxidation. The reactive products of this initiation phase will, in turn, react with additional lipid molecules to form other reactive chemical species. The propagation of further oxidation by lipid oxidation products gives rise to the term "auto-oxidation" that is often used to refer to this process. In the final, termination phase of lipid oxidation, relatively unreactive compounds are formed including hydrocarbons, aldehydes, and ketones (Flavia *et al.*, 2016).

2.11.2 Test for rancidity of fats and oils

2.11.2.1 Peroxide value

One of the most widely used tests for oxidative rancidity, peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Milliequivalents of peroxide per kg of fat are measured by titration with iodide ion. High peroxide values are a definite indication of a rancid fat, but moderate values may be the result of depletion of peroxides after reaching high concentrations (Pizarro *et al.*, 2013).

2.11.2.2 Acid Value

The acid value is a common parameter in the specification of fats and oils. It is the amount of free fatty acid content in the product. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil. Free fatty acids are the source of flavors and aromas. These undesirable flavors and aromas are considered as defects in fats and oils and foods that contain them. High acid value is the indication of rancidity of fats and oils (Koczon *et al.*, 2008).

Part III

Materials and methods

3.1 Materials

3.1.1 Wheat

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

3.1.2 Maize

Maize was collected from Jhapa. Scientific name of maize is *Zea mays* and it is locally known as ‘makai’.

3.1.3 Soybean

Brown variety of soyabean was collected from Dharan market. Soybean’s scientific name is *Glycine max* and is locally known as ‘Nepali bhatmas’.

3.1.4 Bengal gram

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

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

3.1.6 Sesame

White variety of sesame was collected from Jhapa market. Scientific name of sesame is *Sesamum indicum* and it is locally known as ‘seto til’.

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 sugar available in Dharan market was used. It was stored safely at room temperature.

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.

3.1.11 Packaging material

HDPE (20μ), aluminum foil (30μ) and glass bottles that are used as packaging material for instant coffee by Nestle was used for the packaging of the product. HDPE and aluminum foil available in Dharan market was used and glass bottles that are used as packaging material for instant coffee by Nestle are reused.

3.2 Methods

3.2.1 Processing of raw materials

3.2.1.1 Wheat

It was sorted, cleaned and soaked in water for 12 hours and then drained and left in a container to germinate for 3-4 days by covering with wet muslin cloth. At regular interval of 3-4 hours' water was sprinkled on the surface of muslin cloth. They were germinated at 25 ± 2°C and watered 2 to 3 times a day for 72 hours. It was then dried in cabinet drier at 50°C for 3 hours and 70°C for 1 hour until moisture was sufficiently reduced to about 5%. The germinated part was removed, then roasted and ground into flour and packed in air tight plastic bags (Yaseen *et al.*, 2014)

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). Then the surplus water was drained off and the grain was spread at room temperature (25- 27 °C) by covering with wet muslin cloth to germinate. To prevent drying out, the grain was sprinkled with water at the interval of every 12 hours and the grain was turned and mix from time to time to equalize the moisture and for aeration. The germination was cut off when the acrospires were ½ of the length of the grain, after 72 hours. Then first dried at 50°C unless moisture reduces about 12-15% and then it was again dried in cabinet drier to reduce the moisture to 4-6%. The rootlets and acrospires were removed by agitation and screening. It was then roasted and ground in a mixture into flour. The flour was sieved and put into air tight plastic bags (Pokhrel, 2011).

3.2.1.3 Soyabean

It was cleaned and soaked for 8 hours with 1% w/v sodium bicarbonate at room temperature and then drained and put in the form of heap on the double layer of filter paper by covering with wet muslin cloth. At regular interval of 3-4 hours' water was sprinkled on the surface of the heap and was kept for 3 days for germination. In the middle of germination period, the heap was flattened, agitated and again made into heap as before. After 72 hours, the length of radical was increased to about length of the grain. It was then dried in cabinet drier at 50°C for 3 hours and 70°C for 1 hour until moisture was sufficiently reduced to about 6%. It was roasted, dehusked, splitted cotyledons were ground into flour. The flour was sieved and stored into airtight plastic bags (Pulami, 1998).

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 put in the form of heap on the double layer of filter paper by covering with wet muslin cloth. At regular interval of 3-4 hours' water was sprinkled on the surface of the heap and was kept for 3 days for germination. In the middle of germination period, the heap was flattened, agitated and again made into heap as before. After 72 hours, the length of radical was increased to about length of the grain. It was then dried in cabinet drier at 50°C for 3 hours and 70°C for 1 hour until moisture was sufficiently reduced to about 6%. It was roasted, dehusked, splitted cotyledons were ground into flour. The flour was sieved and stored into airtight plastic bags (Pulami, 1998).

3.2.1.5 Pumpkin seed

Pumpkin seed was cleaned sorted and soaked in hot water for 10-20 minutes to remove the impurities. Then it was dried in cabinet drier at 50°C for 3 hours and 70°c for 1 hour. It was then roasted and grinded in mixture and finally the flour was sieved and stored in the airtight plastic bags (Ikujenlola *et al.*, 2013).

3.2.1.6 Sesame

Sesame was sorted and cleaned and dried in cabinet drier at 50°C for 3 hours and 70°c for 1 hour. It was grinded in mixture to form paste which was in airtight plastic bags and stored in refrigerator (Onabanjo *et al.*, 2009).

3.2.1.7 Sugar

White sugar crystals were ground in grinder and then packed in airtight plastic bags (Pokhrel, 2011).

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 RUSF.

Table 3.1 Nutrient requirement in RUSF

Nutrients	Nutritional values per 100g 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) (μg)	550-1150
Thiamin (Vit B_1) (mg)	1
Riboflavin (Vit B_2) (mg)	2.1
Niacin (Vit B_3) (mg)	13
Pantothenic acid (Vit B_5) (mg)	4
Pyridoxine (Vit B_6) (mg)	1.8
Biotin (Vit B_7) (μg)	60
Folic acid (Vit B_9) (μg)	330
Cobalamin (Vit $_{B12}$) (μg)	2.7
Ascorbic Acid (Vit C) (mg)	60
Calcium (Ca) (mg)	535-750
Copper (Cu) (mg)	1.4-1.9
Iodine (I) (μg)	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 RUSF, 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 RUSF. Finally, from the calculation, three formula mixes were developed.

3.2.3 Product preparation

The calculated amounts of ingredients for three different formula (A, B and C) were calculated on dry basis. Flow chart diagram of different ingredients used for the preparation of RUSF is shown in Fig 3.1

3.2.3.1 Grinding and milling

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

3.2.3.2 Sieving of the ground powder product

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

3.2.3.3 Mixing

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

3.2.3.4 Packaging

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

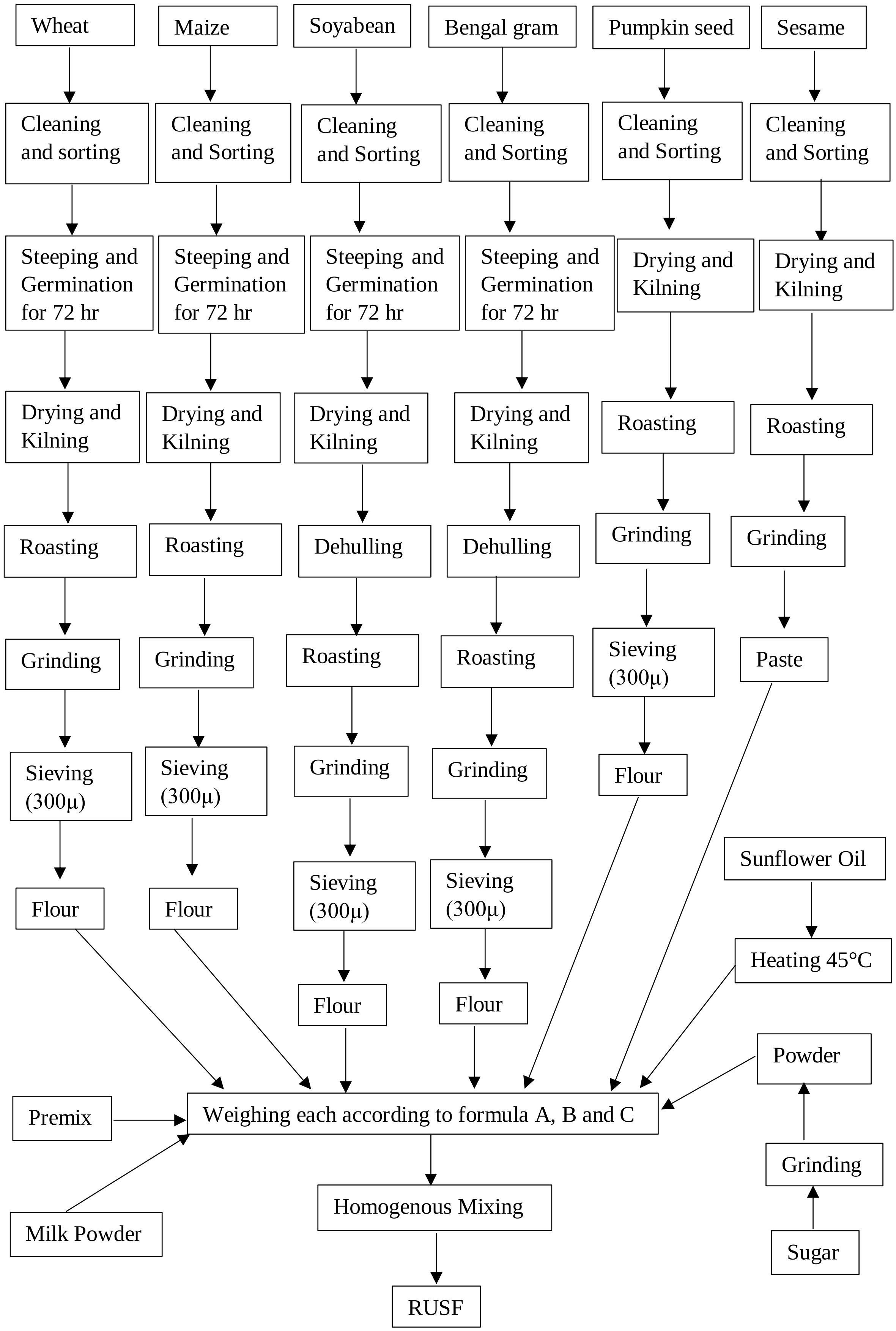


Fig 3.1 Outline for the preparation of RUSF (Pilot plant scale)

3.2.4 Evaluation of prepared RUSF

3.2.4.1 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 3 females and 7 males. Sensory evaluation was carried out in individual booth with adequate light and free from obnoxious odors. Each panelist was provided with 3 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.

3.2.4.2 Physicochemical analysis of product

3.2.4.2.1 Moisture content

Moisture content was determined by using hot air oven (ambassador, working temperature 0 to 300ºC) as per Rangana, 2001.

3.2.4.2.2 Crude fat

The fat content was determined by Soxhlet method as per Rangana, 2001.

3.2.4.2.3 Crude protein

The crude protein was determined by using Kjeldahl's method as per Rangana, 2001.

3.2.4.2.4 Crude fiber

Crude fiber was determined as per Rangana, 2001.

3.2.4.2.5 Total ash

Total ash content was determined by ashing in electric muffle furnace (ambassador, working temperature 900ºC, UK) as per Rangana, 2001.

3.2.4.2.6 Total carbohydrate

Total carbohydrate was determined by difference method Rangana, 2001.

3.2.4.2.6 Calcium

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

3.2.4.2.7 Vitamin C

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

3.2.4.2.8 Iron

Iron content was determined as per Rangana, 2001.

3.2.4.3 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 (Bassey *et al.*, 2013).

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

3.2.4.4 Evaluation of microbiological quality

It is done to assess bacterial, fungal and yeast load under laboratory condition. For analysis, 10 gm of each sample is aseptically weighed and diluted to (1:10), i.e., 10 gm 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.

3.2.4.4.1 Total plate count (TPC)

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

3.2.4.4.2 Yeast and mold

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

3.2.4.5 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.

3.2.4.5.1 Acid value

Acid value was determined according to Rangana, 2001.

3.2.4.5.2 Peroxide value

Peroxide value was determined according to Rangana, 2001.

3.3 Cost calculation

The cost of the best RUSF from three different formulations were calculated including a profit of 10% (Appendix D).

3.4 Data analysis

Data on sensory analysis were tabulated for comparison and were graphically represented using Microsoft excel-2016. Data were statistically processed by IBM statistical package for social science (SPSS) version 20.0 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 RUSF. 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, seasame 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, three products were developed on the basis of WHO/UN specification of RUSF. Table 4.1 shows the amount of ingredients in each product. The ingredients content in product-A and product-B are same, however, product-A is formulated from non-germinated ingredients while, product-B from germinated ingredients. Product-C is formulated from mixes of both germinated and non germinated raw materials on equal basis.

Table 4.1 Formulae mixes on dry basis

Ingredients	Product-A	Product-B	Product-C
Wheat (g)	90	90	60
Maize (g)	40	40	80
Soyabean (g)	80	80	110
Bengal gram (g)	40	40	40
Pumpkin seed (g)	140	140	130
Seasame (g)	140	140	140
Milk Powder (g)	160	160	140
Sunflower oil (g)	170	170	180
Sugar (g)	124	124	104
Vitamin & Mineral Premix(g)	16	16	16
Total	1000	1000	1000

4.2 Organoleptic quality of the products

The prepared three RUSF formulae were subjected to sensory evaluation. The samples were provided to 10 semi trained panelists. The semi trained panelists evaluated for various parameters of RUSF 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.

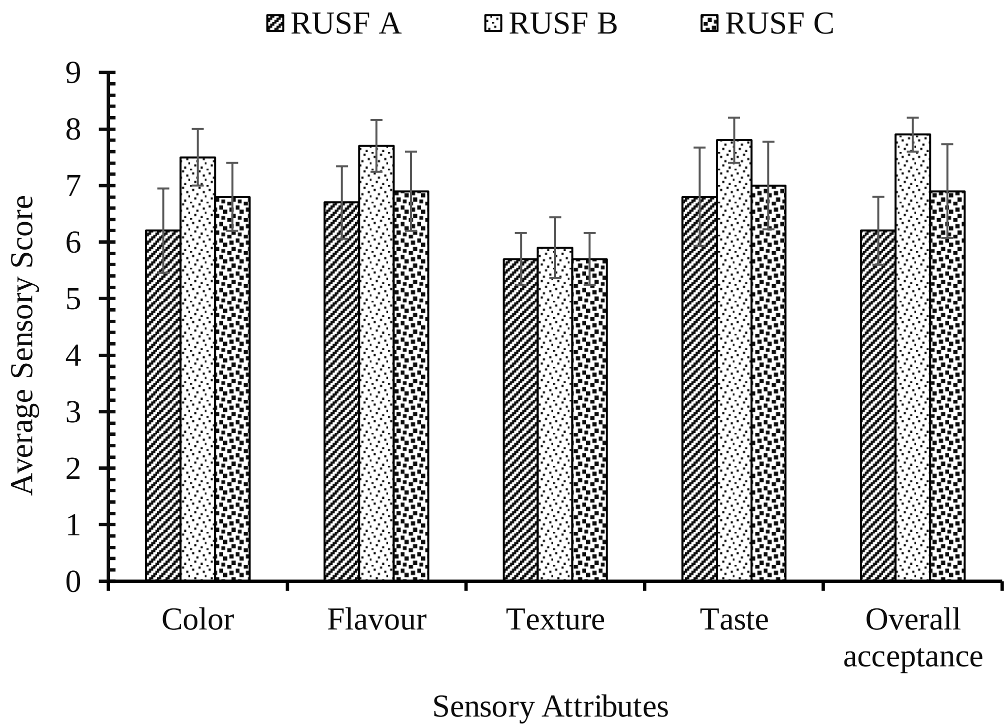


Fig 4.1 Average sensory score for three different formula

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

4.2.1 Color

The average sensory score for color was 6.2, 7.5 and 6.8 for A, B and C respectively. The analysis of variance showed that in case of color, A and B showed significant difference

($p<0.05$), also B and C showed significant difference ($p<0.05$), while A and C were not significantly different ($p>0.05$).

4.2.2 Flavor

The average sensory score for flavor was 6.7, 7.7 and 6.9 for A, B and C respectively. In case of flavor, A and B showed significant difference ($p<0.05$), also B and C showed significant difference ($p<0.05$), while A and C were not significantly different ($p>0.05$).

4.2.3 Texture

The average sensory score for texture was 5.7, 5.9 and 5.7 for A, B and C respectively. There was no significant difference ($p>0.05$) between A, B and C.

4.2.4 Taste

The average sensory score for taste was 6.8, 7.8 and 7 for A, B and C respectively. In case of taste also, A and B and B and C showed significant difference ($p<0.05$), while A and C were not significantly different ($p>0.05$).

4.2.5 Overall acceptability

The average sensory score for overall acceptance was 6.2, 7.9 and 6.9 for A, B and C respectively. There is significant difference ($p<0.05$) in A, B and C in case of overall acceptability. The overall acceptability mean showed the product B is superior, which might be due to good color, taste and flavor than product B and C. Hence, from the statistical analysis the overall acceptability of product B with malted wheat, maize, soybean, bengal gram, pumpkin seed, sesame, milk powder, sugar and sunflower oil was found to be superior.

4.3 Analysis of RUSF B

The chemical analysis of the superior product found from sensory analysis was carried out. The result is tabulated in Table 4.2

Table 4.2 Analysis of RUSF.

Parameters	Amount
Moisture (%)	2.24 (0.11)
Protein (% db)	14.77 (0.78)
Fat (% db)	33.87 (0.56)
Ash content (% db)	2.70 (0.13)
Crude fiber (% db)	1.67 (0.07)
Carbohydrate (% db)	44.73 (0.76)
Calcium (mg/100 gm)	525.58 (3.07)
Iron (mg/100 gm)	12.27 (0.81)
Ascorbic acid (mg/100 gm)	66.95 (2.39)
Energy (K cal./100 gm)	542.83

The values are the means of triplicate samples and the values in the parenthesis are standard deviation.

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 4.3

Table 4.3 Microbiological assay of the products

Parameters	**Cfu/g**
TPC	1850
Yeast and mold count	48

The total plate count of the RUSF was found to be 1850 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, 2007).

Yeasts and molds are ubiquitous in the environment and can contaminate food through inadequately sanitized equipment or as airborne contaminants. Yeast and mold counts frequently predominate when conditions for bacterial growth are less favorable, such as lower water activity, low pH, high salt, or high sugar content (OECD, 2011). The yeast and mold count in the product was found to be 48/g which was within the acceptable limit of 50/g in the product (UNICEF, 2007).

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

The shelf life of the RUSF was studied for 4 weeks. The product was packed in HDPE bags, aluminum foils and glass bottles. 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 RUSF in different packaging material at every interval of week was observed for four weeks where free fatty acid of the product was found to be increased with storage time depending upon the type of packaging material used. The increment in acid value after four weeks was found to be minimum in glass bottles, and then aluminum foil and maximum in HDPE bags. The acid value (AV) of the product was observed to be 0.23 at initial which reached 0.67, 0.58 and 0.49 in HDPE bags, aluminum foils and glass bottles respectively.

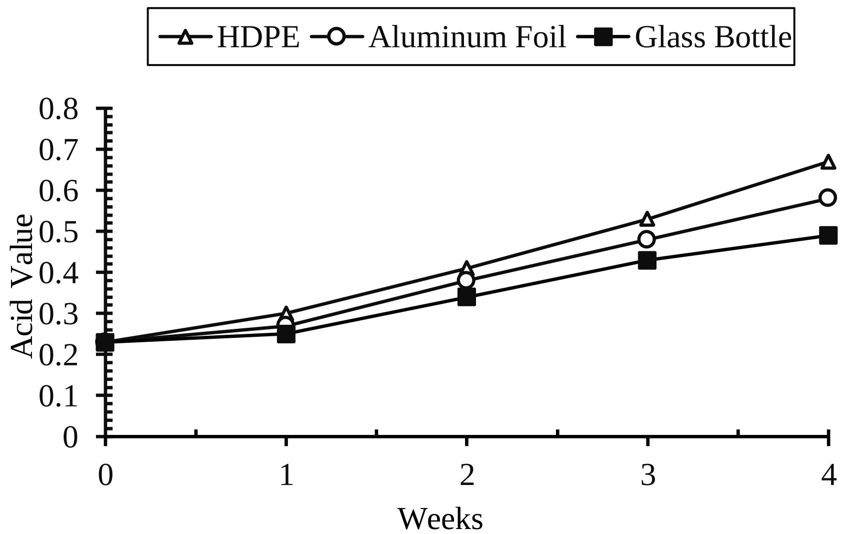


Fig 4.2 Changes in acid value during storage at room temperature

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 (Koczon *et al.*, 2008).

The rate of increase in the AV was calculated to be 0.015 mg KOH/gm oil per day in case of HDPE, 0.0125 mg KOH/gm oil per day in case of aluminum foil, and 0.01 mg KOH/gm oil per day in case of glass bottles. The unacceptable value of AV which is 3 mg KOH/ gm oil per day (Bako *et al.*, 2014). So, the product was within the acceptable value, when shelf life was studied for four weeks. Pokhrel in 2011 observed similar increment in the AV of the product on different packaging material when AV was studied for 4 consecutive weeks (Pokhrel, 2011).

From the result of statistical analysis (Appendix C), there is significant difference ($p<0.05$) in AV and packaging material and duration of time. In case of packaging material, AV in HDPE and glass bottles are found to be significantly different ($p<0.05$), while, AV in HDPE and aluminum foil and glass bottles and aluminum foil were not significantly different ($p>0.05$) with each other.

Similarly, in case of time, there is significant difference ($p<0.05$) in change in AV during the time of manufacture and different storage time.

4.5.2 Changes in peroxide value

The changes of peroxide value of RUSF in different packaging material at every interval of week was observed for four weeks where PV of the product was found to be increased with storage time depending upon the type of packaging material used. The peroxide value of the product was found to be increased with storage time depending upon the type of packaging material used. The increment in peroxide value after four weeks was found to be minimum in glass bottles, and then aluminum foil and maximum in HDPE bags. The peroxide value (PV) of the product was observed to be 1.21 at initial which reached 2.54, 2.12 and 2.01 in HDPE bags, aluminum foils and glass bottles respectively.

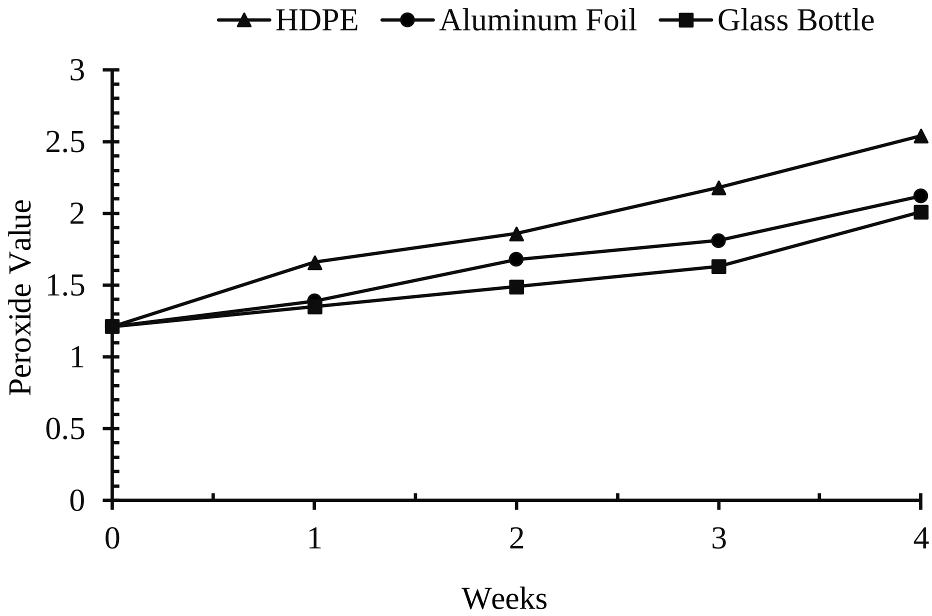


Fig 4.3 Changes in peroxide value during storage at room temperature

Peroxide value is commonly used to determine the rancidity of a sample containing fat or oil subject to oxidation. It is a very sensitive indicator of the early stages of oxidative deterioration of fats and oil. Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils (Pizarro *et al.,* 2013). The rate of increase in the PV was calculated to be 0.0475 meq. peroxide/kg fat per day in case of HDPE, 0.0325 meq. peroxide/kg fat per day in case of aluminum foil and 0.03 meq. peroxide/kg fat per day in glass bottles respectively. Pokhrel in 2011 also observed similar increment in PV of the product, when observed at four consecutive weeks in different packaging materials (Pokhrel, 2011). The unacceptable value of PV is 10 meq. peroxide/kg oil per day (Gotoh and Wada, 2006). So, the product was within the acceptable limit, when shelf life was studied for four weeks.

Again, from the result of statistical analysis (Appendix C), there is significant difference ($p<0.05$) in change in PV and packaging material and duration of time. In case of packaging material, PV in HDPE and glass bottles and HDPE and aluminum foil are found to be significantly different ($p<0.05$), while, PV in glass bottles and aluminum foil are not significantly different ($p>0.05$) from each other.

Similarly, in case of time, it is observed that the change in PV from the time of manufacture up to 4 weeks is found to be significantly different ($p<0.05$), except the PV in

1st and 2nd week and 2nd and 3rd week, which are not significantly different from each other ($p>0.05$).

4.6 Essential amino acid composition of the product

The essential amino acid content of raw materials is analyzed on the basis of data from the report published by FAO, and the essential amino acids, chemical score of the superior product was calculated. The limiting amino acid was determined on the basis of chemical score and the essential amino acid in the product was compared to the essential amino acid requirements for children below 2 years given by FAO.

The quality of a protein is primarily a function of its essential amino acid composition. Block and Mitchell in 1946 proposed a method for assessing the quality of nutritional proteins by means of chemical score. This method includes calculation of quantity of each essential amino acid contained in the protein mixture. The calculated values are then expressed as percentage of the amino acid content of a similar quantity of egg protein or human milk protein. Egg and human milk proteins, for their very high biological value, have been considered as reference standards. The amino acid showing the lowest percentage is called the limiting amino acid and this percentage is the chemical score (Block and Mitchell, 1946). Based on chemical score, the first limiting amino acid in the formulated product was lysine followed by isoleucine. Similarly, comparing the amount of essential amino acids with RDA by FAO, the 100 grams of the product will provide all the essential amino acids required by child less than 2 years of age (Appendix E.)

4.7 Cost of product

The cost of the best formulated and superior RUSF analyzed from sensory evaluation is NRs. 303.6 per kg i.e. NRs. 30.36 per 100 gm. (calculation is given in Appendix D). The cost of the product may vary as there may be increase or decrease in the cost of raw materials as per the season.

Part V

Conclusions and recommendations

5.1 Conclusions

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

a) Protein, calorie and micronutrients rich RUSF can be prepared by using easily and locally available raw materials; i.e., wheat, maize, soyabean, bengal gram, pumpkin seed, sesame, milk powder, sugar with the addition of vitamin and mineral premix. The food may be prepared using traditional techniques not requiring special equipment and it could be very much effective in treating the MAM children. If this product is manufactured in industrial scale in Nepal, the cost of importing RUSF from foreign countries can be reduced.

b) From the sensory evaluation and statistical analysis of the product, formula-B was found to be the best among all, it contains 9 parts wheat, 4 parts maize, 8 parts soyabean, 4 parts bengal gram, 14 parts pumpkin seed, 14 parts sesame, 16 parts milk powder, 17 parts sunflower oil, 12.4 parts sugar and 1.6 parts vitamin and mineral premix.

c) From the analysis of product-B, it was concluded that it provides 14.77 gm protein, 33.87 gm fat, 44.73 gm carbohydrate, 325.58 mg Calcium, 12.27 mg Iron, 56.95 mg ascorbic acid and 542.83 Kcal energy per 100 gm of the product. The moisture, ash and crude fiber content of the product were found to be 2.24%, 1.67% and 2.70% respectively.

d) The amount of essential amino acid found in formulation-B (in 100 gm) was higher than the RDA of the children below 2 years of age; from this it can be concluded that the 100 gm of the product provides the complete protein for children suffering from moderately acute malnutrition.

e) The total cost of the RUSF was calculated and found to be NRs. 303.6 per kg i.e. NRs. 30.36 per 100 gm.

f) The total plate count, and yeast and mold count in RUSF was 1850 cfu/g, 48 cfu/g respectively. The product was safe from microbiological point of view.

g) The shelf life of the RUSF was found to be 200 days (6.7 months) in HDPE, 240 days (8 months) in aluminum foil and 300 days (10 months) in glass bottle.

5.2 Recommendations

This study can be further continued with the following recommendations.

a) Evaluation of nutritional quality (e.g. PER, NPU etc.) of the product.
b) Clinical trials in Albino rats can be done to check the efficiency of the product.
c) Study of the fatty acid composition and amino acid profile of the prepared products can be done.

Part VI

Summary

Acute childhood malnutrition affects about a tenth of the world's children under 5 years of age, particularly those living in circumstances of extreme poverty in the developing world. Malnutrition is typically the result of an inadequate diet and is one of the most common diagnoses in children of developing countries. Children usually suffer from acute malnutrition due to inadequate dietary intake and poor dietary management. So, management of those malnourished children can be done with specially formulated high protein and high calorie, energy densed food. This work describes how best to manage cases of moderately acute childhood malnutrition using RUSF prepared from locally available foods.

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. It is generally made with heat treated oil seeds/pulses/cereals, sugar, milk powder, vegetable oils, vitamins and minerals.

Three 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 14.77%, 33.87%, 44.73%, 1.67%, 2.70%, 66.95 mg/100gm, 12.27 mg/100gm and 525.58 mg/100gm respectively. The diet can supply 542.83 Kcal/100 gm. The energy contributed by the protein, fat and carbohydrate were found to be 10.83%, 56.05% and 32.8% of total Kcals respectively and contains all the essential amino acids required by the children under 2 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 1850 cfu/g and 48 cfu/g respectively. The rate of change in AV and PV under three packaging materials, i.e., HDPE, aluminum foil and glass bottles, when studied for four consecutive weeks showed that the product was within the acceptable limit. However due to limitation in time

shelf life could not be studied for longer time. The essential amino acid content in the product was analyzed and compared with the RDA of the children below 2 years of age. The 100 grams of the product supplies all the essential amino acid required for the children below 2 years. The average cost of the product was calculated. Finally, the data obtained from chemical analysis and sensory evaluation were analyzed using standard statistical softwares.

This study where RUSF has been prepared from locally available food with contains all the essential nutrients required for the children, could be effective in the treatment of moderate acute malnourished child after the clinical trial. If further researched the industrial production of RUSF using different locally available foods in Nepal could be possible.

References

Amagloh, F. K., Weber, J. L., Brough, L., Hardacre, A., Mutukumira, A. N. and Coad, J. (2012). Complementary food blends and malnutrition among infants in Ghana–A review and a proposed solution. **7** (9), 972-988.

Arimond, M., Zeilani, M., Jungjohann, S., Brown K.H., Ashorn, P., Allen, L. H. and Dewey, K. G. (2013). Considerations in developing lipid-based nutrient supplements for prevention of undernutrition: experience from the International Lipid-Based Nutrient Supplements (iLiNS) Project.

Ashworth, A. (2006). Efficacy and effectiveness of community-based treatment of severe malnutrition. **27**.

Asif, M. (2011). General Chemistry, Composition, Identification and Qualitative Tests of Fats or Oils. **1** (2), 52-64.

Ayana, A. B., Hailemariam, T. W. and Melke, A. S. (2015). Determinants of acute malnutrition among children aged 6–59 months in Public Hospitals, Oromia region, West Ethiopia: a case–control study. **1** (1), 1-11.

Azanha, A. B. and Faria. (2005). Use of mathematical models for estimatin the shelf life of cornflakes in flexible packaging. **18** (4), 161-222.

Bako, T., Umogbai. V.I. and S.E., O. (2014). Extraction and Characterization of Mackery (Scomber scombrus) Oil for Industrial Use. **6** (8), 80-85.

Bassey, F. I., McWatters, K. H., Edem, C. A. and Iwegbue, C. M. A. (2013). Formulation and nutritional evaluation of weaning food processed from cooking banana, supplemented with cowpea and peanut. *Blackwell Publishing Ltd*. **1** (5), 384-391.

Becker, J. (2013). Macro and Micro Nutrients. Retrieved from http://peakpilates.com/media/peak_pilates_news/2013-8-2.html. [Accessed 2016].

Beigler, M. A. (1976). Complete Synthetic Foods. *In:* "In New Protein Foods" (Vol. 2).). pp. 62-85. Elsevier Inc.

Block, E. J. and Mitchell, H. (1946). Some Relationships between the Amino Acid Contents of Proteins and their Nutritive Value for the Rat. **163**, 599-620.

Briend, A. and Collins, S. (2010). Therapeutic nutrition for children with severe acute malnutrition summary of African experience.

Brown, J. L. and Pollitt, E. (1996). Malnutrition, poverty and intellectual development. **274** (2), 38-43.

Bukya, A. and Vijayakumar, T. P. (2013). Properties of industrial fractions of sesame seed (Sesamum indicum L.). **3** (3), 86-89.

Campos, R., Hernandez, L., Soto-Mendez, M. J., Vossenaar, M. and Solomons, N. W. (2010). Contribution of complementary food nutrients to estimated total nutrient intakes for rural Guatemalan infants in the second semester of life. **19** (4), 481-490.

CBS. (2015). Nepal Multiple Indicator Cluster Survey 2014, Final Report [Report]. Central Bureau of Statistics and UNICEF Nepal. Kathmandu, Nepal,

Chavan, J. K., Kadam, S. S. and Beuchat, L. R. (1989). Nutritional improvement of cereals by sprouting. **28** (5), 401–437.

Coles, R., Mcdowell, D. and Kirwan, M. J. (2003). "Food Packaging Technology". Blackwell Publishing Ltd. Paris, France.

Collins, S., Dent, N., Binns, P., Bahwere, P., Sadler, K. and Hallam, A. (2006). Management of severe acute malnutrition in children. **368** (9551), 1992-2000.

Defourny, I., Minetti, A., Harczi, G., Doyon, S., Shepherd, S., Tectonidis, M. and Golden, M. (2009). A large-scale distribution of milk-based fortified spreads: evidence for a new approach in regions with high burden of acute malnutrition **4**(5).

Dewan, A. P. (1994). "Food for Health". AC Specialist Publishers.

DFTQC. (2012). Food Composition Table for Nepal [Report]. Nepal Government, Ministry of Agriculture Development, DFTQC, National Nutrition Program. Kathmandu,

Dibari, F., Bahwere, P., Huerga, H., Irena, A. H., Owino, V., Collins, S. and Seal, A. (2013). Development of a cross-over randomized trial method to determine the acceptability and safety of novel ready-to-use therapeutic foods. **29** (1), 107-112.

el-Adawy, T. A., Rahma, E. H., el-Bedawy, A. A. and Sobihah, T. Y. (2000). Effect of soaking process on nutritional quality and protein solubility of some legume seeds. **44** (5), 339-343.

Elinge, C. M., Muhammad, A., Atiku, F. A., Itodo, A. U., Peni, I. J., Sanni, O. M. and Mbongo, A. N. (2012). Proximate, Mineral and Anti-nutrient Composition of Pumpkin (Cucurbitapepo L) Seeds Extract. **2** (5), 146-150.

FAO. (1970). Amino-acid content of foods and biological data on proteins [Report]. Food Policy and Food Science Service, Nutrition Division, FAO.

FAO/WHO/UNU. (2002). Protein and amino acid requirements in human nutrition : report of a joint FAO/WHO/UNU expert consultation. [Report]. Vol. 935. Geneva, Fennema, O. R. (1996). "Food Chemistry" (3rd ed.). Library of Congress Cataloging-in-Publication Data. New York.

Flavia, P., Anamaria, S. C. and Lucia, M. (2016). Oxidative and hydrolytic stability of alimentary poultry fats under refrigeration and freezing. **118** (11), 1795-1798.

Foster, B. J. and Leonard, M. B. (2004). Measuring nutritional status in children with chronic kidney disease. **80** (4), 801-814.

Gahlawat, P. and Sehgal, S. (1994a). Formulation and nutritional value of home made weaning foods. *Elsevier*. **12** (10), 1171-1180.

Gahlawat, P. and Sehgal, S. (1994b). Protein and starch digestibility and iron availability in developed weaning foods as affected by roasting. *Blackwell Publishing Ltd*. **7** (2), 121-126.

Giami, S. Y., Mepba, H. D., Kiin-Kabari, D. B. and Achinewhu, S. C. (2003). Evaluation of the nutritional quality of the breads prepared from the wheat-fluted pumpokin (Telfaria occidentalis Hook) seed flour blends. **58**, 1-8.

Glew, R. H., Glew, R. S., Chaung, L. T., Huang, Y. S., Millson, M., Constans, D. and Vanderjagt, D. J. (2006). Amino Acid, Mineral and Fatty Acid Content of Pumpkin Seeds (Cucurbita spp) and Cyperus esculentus Nuts in the Republic of Niger. **61** (2), 49-54.

Gotoh, N. and Wada, S. (2006). The importance of peroxide value in assessing food quality and food safety. **83** (5), 473-474.

Gyesley, S. W. (1991). "Total systems Approach to Predict shelf life of Packaged foods". ASTM International.

Hartog, A. P., Staveren, W. A. and Brouwer, I. D. (2006). "Food habits and consumption in developing countries" (1st ed.). Wageningen Academic Publishers Netherlands.

Hermoso, M., Tabacchi, G., Iglesia-Altaba, I., Bel-Serrat, S., Moreno-Aznar, L. A., Garcia-Santos, Y., Garcia-Luzardo Mdel, R., Santana-Salguero, B., Pena-Quintana, L., Serra-Majem, L., Moran, V. H., Dykes, F., Decsi, T., Benetou, V., Plada, M., Trichopoulou, A., Raats, M. M., Doets, E. L., Berti, C., Cetin, I. and Koletzko, B. (2010). The nutritional requirements of infants. Towards EU alignment of reference values: the EURRECA network. **6 Suppl 2**, 55-83.

Hoppe, C., Andersen, G. S., Jacobsen, S., Mølgaard, C., Friis, H., Sangild, P. T. and Michaelsen, K. F. (2008). The Use of Whey or Skimmed Milk Powder in Fortified Blended Foods for Vulnerable Groups. **138** (1), 145S-161S.

Hough, J. S., Briggs, D. E., Stevens, R. and Young, T. W. (1982). "Malting and Brewing Science". Vol. 2. Chapman-Hall, Ltd. London, England.

ICMR. (2010). Nutrient Requirements and Recommended Dietary Allowances for Indians [Report]. National Institute of Nutrition, Indian Council of Medical Research. Jamai-Osmania PO, Hyderabad,

IFPRI. (2014). Global Nutrition Report 2014, Actions and Accountability to Accelerate the World's Progress on Nutrition [Report]. Washington, DC,

Iken, J. E. and Amusa, N. A. (2004). Review: Maize research and production in Nigeria. **3** (6), 302-307.

Ikujenlola, A. V., Oguntuase, S. O. and Omosuli, S. V. (2013). Physico-Chemical Properties of Complementary Food from Malted Quality Protein Maize(Zea mays L.) and Defatted Fluted Pumpkin Flour (Telfairia occidentalis Hook, F). **3** (6), 323-328.

ILSI. (2007). Packaging materials; Metal packaging for food stuffs [Report]. International Life Sciences Institute. Belgium,

Islam, T., Chowdhury, A., Islam, M. and Islam, S. (2007). Standardization of Bread Preparation from Soy Flour. **2** (6), 15-20.

Jelliffe. (1966). The Assessment of Nutritional Status of the Community. [Report]. World Health Organization. Geneva Printing Press, Switzerland,

Kadam, S. S. and Salunkhe, D. K. (1985). Nutritional composition, processing, and utilization of horse gram and moth bean. **22** (1), 1-26.

Karakochuk, C., Briel, T. V. D., Stephens, D. and Zlotkin, S. (2012). Treatment of moderate acute malnutrition with ready-to-use supplementary food results in higher overall recovery rates compared with a corn-soya blend in children in southern Ethiopia: an operations research trial.

Kent, N. L. (1994). "Kent's Technology of Cereals". Vol. 4. Woodhead Publishing.

Khokhar, S. and Richard, A. K. O. (2003). Antinutritional factors in food legumes and effects of processing. *In:* "The Role of Food, Agriculture, Forestry and Fishery in Human Nutrition" (Vol. 4).). Encyclopedia of life support systems (EOLSS).

Koczon, P., Gruczynska, E. and Kowalski, B. (2008). Changes in the Acid Value of Butter During Storage at Different Temperatures as Assessed by Standard Methods or by FT-IR Spectroscopy **3**(3), 154-163.

Koon, R. (2009). Understanding Rancidity of Nutritional Lipids Retrieved from https://www.naturalproductsinsider.com/articles/2009/08/understanding-rancidity-of-nutritional-lipids.aspx.

Krah, A. A. (2014). Stabilisation and Characterisation of a Peanut based Ready-To-Use Supplementary Food (RUSF) Masters in the Philosophy of Food Science University of Ghana,

Kumar, S. and Singh, R. K. (2013). Thermolysis of High-Density Polyethylene to Petroleum Products. **2013**, 7.

Latham, M., Jonsson, U., Sterken, E. and Kent, G. (2011). RUTF stuff. Can the children be saved with fortified peanut paste? . **2** (2), 62-85

Manary, M. J. and Sandige, H. L. (2008). Management of acute moderate and severe childhood malnutrition. **337**.

Marsh, K. and Bugusu, B. (2007). Food Packaging—Roles, Materials, and Environmental Issues. *Blackwell Publishing Inc*. **72** (3), R39-R55.

MOHP. (2012). Nepal Demographic and Health Survey , 2011 [Report]. Ministry of Health and Population, New ERA, and ICF International. Kathmandu, Nepal,

Nackers, F., Broillet, F., Oumarou, D., Djibo, A., Gaboulaud, V., Guerin, P. J. and Captier, V. (2010). Effectiveness of ready-to-use therapeutic food compared to a corn/soyblend-based pre-mix for the treatment of childhood moderate acute malnutrition in Niger. **56** (6), 407-413.

Naicker, A. (2004). The incidence of malnutrition and the identification of determinants affecting the nutritional status of rural black children aged 0 to 24 months in Umbumbulu, Kwa-Zulu Natal Masters Durban University of Technology,

Ndidi, U. S., Ndidi, C. U., Aimola, I. A., Bassa, O. Y., Mankilik, M. and Adamu, Z. (2014). Effects of Processing (Boiling and Roasting) on the Nutritional and Antinutritional Properties of Bambara Groundnuts (Vigna subterranea [L.] Verdc.) from Southern Kaduna, Nigeria. **2014**, 9.

NPC. (2015). Sustainable Development Goals, 2016-2030, National (Preliminary) Report. [Report]. Government of Nepal, National Planning Commission. Kathmandu, Nepal,

OECD. (2011). Working Document on Microbial Contaminant Limits for Microbial Pest Control Products [Report]. Vol. 43. Organisation for Economic Co-operation and Development.

Onabanjo, O. O., Akinyemi, C. O. and Agbon, C. A. (2009). Characteristics of complementary foods produced from sorghum, sesame, carrot and crayfish. **8** (1), 71-83.

Osendarp, S., Rogers, B., Ryan, K., Manary, M., Akomo, P., Bahwere, P., Belete, H., Zeilani, M., Islam, M., Dibari, F. and Pee, S. (2015). Ready-to-use foods for management of moderate acute malnutrition: Considerations for scaling up production and use in programs. **36** (1).

Paine, F. A. and Paine, H. Y. A. (1992). "Handbook of Food Packaging" (2 ed.). Blackie Academic & Professional. London, UK.

Pizarro, C., Esteban-Díez, I., Rodríguez-Tecedor, S. and González-Sáiz, J. M. (2013). Determination of the peroxide value in extra virgin olive oils through the application of the stepwise orthogonalisation of predictors to mid-infrared spectra. **34** (1), 158-167.

Pokhrel, P. R. (2011). Preparation and quality evaluation of ready to use therapeutic food (RUTF) for children with severe acute malnutrition. Bachelor Tribhuvan University,

Prasad, P., Kochhar, A. and Javed, M. (2016). Development of ready-to-eat supplementary foods using germinated cereal-pulse mix, potato flour and green leafy vegetable for malnourished children. **46** (1), 30-42.

Pulami, B. P. (1998). Formulation and quality evaluation of balanced , enriched weaning food at low cost. Bachelor Tribhuvan University,

Reinhardt, K. and Fanzo, J. (2014). Addressing Chronic Malnutrition through Multi-Sectoral, Sustainable Approaches: A Review of the Causes and Consequences. *Frontiers Media S.A.* **1**, 13.

Santana, J., Rezende, T., Assaid, A., Duarte, A., Carla, A. and Leal, C. (2014). Development and chemical and sensory characterization of pumpkin seed flour-based cereal bars **34** (2), 346-352.

Schrenk, D. (2014). Literature report on food packaging materials and their potential impact on human health [Report]. Technische Universität Kaiserslautern. Germany,

Scott, G. B. and Brock, H. (2006). North America aluminum industry—a quick review [Report]. Almunium Association. Retrieved from http://www.aluminum.org/Content/NavigationMenu/News_and_Stats/Statistics_Reports/Facts_At_A_Glance/factsataglance05.pdf. .

Serna-Saldivar, S. O. (2010). "Cereal Grains: Properties, Processing, and Nutritional Attributes". Taylor and Francis Group.

Shenkin, A. (2006). Micronutrients in health and disease. *BMJ Group*. **82** (971), 559-567.

Shewry, P. R. (2009). Wheat. **60** (6), 1537-1553.

Soroka, W. (2002). "Fundamentals of Packaging Technology" (5 ed.). Institute of Packaging Professionals.

Steenkamp, L., Lategan, R. and Raubenheimer, J. (2015). The impact of Ready-to-Use Supplementary Food (RUSF) in targeted supplementation of children with moderate acute malnutrition (MAM) in South Africa. *Taylor & Francis*. **57** (5), 322-325.

Stratton, R. J., Green, C. J. and Elia, M. (2003). "Disease-related malnutrition: an evidencebased approach to treatment". Cabi University

Suhasini, A. W. and Malleshi, N. G. (2003). Nutritional and carbohydrate characteristics of wheat and chickpea based weaning foods. **54** (3), 181-187.

Swaminathan, M. (2004). "Essential of Food and Nutrition" (2 ed.). Vol. 1. The Banglore Printing and Publishing Co. Ltd. Mysore Road, Banglore.

Tarek, A. E. (2002). Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. **57**, 83-97.

Thomas, A., Matthäus, B. and Fiebig, H. (2000). Fats and Fatty Oils. *In:* "Ullmann's Encyclopedia of Industrial Chemistry".). Wiley-VCH Verlag GmbH & Co. KGaA.

Thompson, J. and Manore, M. (2012). "Nutrition: An Applied Approach " (3rd ed.). Benjamin Cummings. San Francisco.

UNICEF. (1990). Conceptual framework on causes of malnutrition [Report]. UNICEF. Retrieved from http://www.unicef.org/nutrition/training/2.5/4.html.

UNICEF. (2007). Community-based Management of Severe Acute Malnutrition. Geneva: WHO, WFP, United Nations System Standing Committee on Nutrition, UNICEF.

UNICEF. (2013). Ready-To-Use Therapeutic Food for Children with Severe Acute Malnutrition [Report]. UNICEF.

UNOCHA. (2015). Nepal Integrated Management of Acute Malnutrition (IMAM) Guidelines *Nutrition*: UNICEF.

Van Der Kam, S., Swarthout, T., Niragira, O., Froud, A., Sompwe, E. M., Mills, C. and Roll, S. (2012). Ready-to-use therapeutic food for catch-up growth in children after anepisode of plasmodium falciparum malaria: An open randomised controlled trial. **7** (4).

Viraktamath, C. S., Raghavendra, G. and Desikachar, H. S. R. (1971). "Use of Milling Machinery for Commercial Pearling of Grain Sorghum (Jowar) and Culinary Uses for Pearled Sorghum".

Wagh, V. D. and Deore, B. R. (2015). Ready to Use Therapeutic Food (RUTF): An Overview. **2** (11-15).

WFP. (2016). Technical Specifications for-Ready-to-Use Supplementary Food-RUSF WFP.

WHO. (1998). Complementary Feeding of Young Children in Developing Countries: A Review of Current Scientific Knowledge [Report]. WHO. Geneva,

WHO. (2000). The Management of Nutrition in Major Emergencies [Report]. WHO. Retrieved from http://www.who.int/nutrition/publications/emergencies/9241545208/en/.

WHO. (2012). Technical note: supplementary foods for the management of moderate acute malnutrition in infants and children 6–59 months of age [Report]. World Health Organization. Geneva,

WHO. (2013). Guideline: Updates on the management of severe acute malnutrition in infants and children. [Report]. World Health Organization. Geneva,

WHO. (2016a). 10 facts on nutrition. Department of Nutrition for Health and Development (NHD), WHO. Retrieved from http://www.who.int/features/factfiles/nutrition/en/. (Last update October, 2012).

WHO. (2016b). Infant and young child feeding. WHO. Retrieved from http://who.int/mediacentre/factsheets/fs342/en/. (Last update September 2016).

WHO. (2016c). Nutrition. WHO. Retrieved from http://www.who.int/nutrition/topics/moderate_malnutrition/en/. (Last update 2016).

WHO and FAO. (2006). Guidelines on food fortification with micronutrients [Report].

WHO/UNICEF. (2003). Global strategy for infant and young child feeding. [Report]. WHO. Geneva, Retrieved from http://www.who.int/nutrition/publications/infantfeeding/9241562218/en/. [Accessed 5 November 2014].

Whyte, R. O., Nilsson-Leissner, G. and Trumble, H. C. (1953). Legumes in Agriculture. **76** (5), 403.

Yaseen, T., Yasmeen, A., Nasreen, Z., Usman, S., Ali, S. and Shamshad, A. (2014). Development and formulation of ready to eat baby food from cereals. **24** (3), 121-125.

Appendices

Appendix-A

1. Sensory evaluation card

Sensory analysis of ready to use supplementary food (RUSF)

Name of the panelist: …………………………   Date: ………………

Name of the product: Ready to use supplementary food (RUSF)

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

Dear panelist, you are given 3 sample of RUSF, please conduct the sensory analysis based on the following parameter using the table given

Sample	Color	Flavor	Texture	Taste	Overall acceptance
A					
B					
C					

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Comments (if any)

………………………………………………………………………………………………

……………………………………………………………………………………..................

…………………………….

Signature

Appendix-B

1. Sensory evaluation of the product

Table B.1.1 One-way ANOVA (no contrast) for color

		Sum of squares	d.f.	Mean square	F	Sig.
Color	Between groups	8.476	2	4.233	9.679	0.01
	Within groups	11.7	27	0.433		
	Total	20.167	29			

Since p<0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.2 LSD for color

Sample code	Mean difference	Standard error	Sig.
A	B–A = -1.3	0.294	0.000146*
B	B–C = 0.70		0.025*
C	C–A = 0.60		0.051

(* = Significantly different)

Table B.1.3 One-way ANOVA (no contrast) for flavor

		Sum of squares	d.f.	Mean square	F	Sig.
Flavor	Between groups	5.6	2	2.8	6.811	0.04
	Within groups	11.1	27	0.411		
	Total	16.7	29			

Since p<0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.4 LSD for flavor

Sample code	Mean difference	Standard error	Sig.
A	B–A = 1.0	0.294	0.002*
B	B–C = 0.80		0.010*
C	C–A = 0.20		0.491

(* = Significantly different)

Table B.1.5 One-way ANOVA (no contrast) for texture

		Sum of squares	d.f.	Mean square	F	Sig.
Texture	Between groups	0.267	2	0.133	0.507	0.608
	Within groups	7.100	27	0.263		
	Total	7.367	29			

Since p>0.05, there is no significant difference between the samples so LSD testing is not necessary.

Table B.1.6 One-way ANOVA (no contrast) for taste

		Sum of squares	d.f.	Mean square	F	Sig.
Taste	Between groups	5.600	2	2.800	4.974	0.014
	Within groups	15.200	27	0.563		
	Total	20.800	29			

Since p<0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.7 LSD for taste

Sample code	Mean difference	Standard error	Sig.
A	B–A = 1.0	0.336	0.006*
B	B–C = 0.80		0.024*
C	C–A = 0.20		0.556

(* = Significantly different)

Table B.1.8 One-way ANOVA (no contrast) for overall acceptability

		Sum of squares	d.f.	Mean square	F	Sig.
Overall acceptability	Between groups	14.600	2	7.300	17.289	0.000015
	Within groups	11.400	27	0.422		
	Total	26.000	29			

Since p<0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.9 LSD for overall acceptability

Sample code	Mean difference	Standard error	Sig.
A	B–A = 1.70		0.000003*
B	B–C = 1.00	0.291	0.02*
C	C–A = 0.70		0.023*

(* = Significantly different)

Appendix-C

1. Shelf life of the product

Two-way ANOVA (no contrast) for AV

Table C.1.1 Tests of between-subjects effects for AV

Source	Sum of squares	d.f.	Mean square	F	Sig.
Corrected model	0.258[a]	6	0.043	34.544	0.0000272*
Intercept	2.212	1	2.212	1774.203	0.000
Time	0.241	4	0.060	48.254	0.000012*
Material	0.018	2	0.009	7.123	0.017*
Error	0.010	8	0.001		
Total	2.480	15			

[a] R Squared = .963 (Adjusted R Squared = .935), (* = Significantly different)

Table C.1.2 LSD for pairwise comparison of packaging material for AV

Packaging material	Mean difference	Standard error	Sig.
HDPE (A)	B–A = 0.048		0.064
Aluminum (B)	B–C = 0.036	0.022	0.146
Glass bottles (C)	C–A = 0.084		0.006*

(* = Significantly different)

Two-way ANOVA (no contrast) for AV

Table C.1.3 Tests of between-subjects effects for PV

Source	Sum of squares	d.f.	Mean square	F	Sig.
Corrected model	2.123[a]	6	0.354	25.263	0.000087*
Intercept	42.841	1	42.841	3059.379	0.000
Time	1.796	4	0.449	32.055	0.000057*
Material	0.327	2	0.164	11.677	0.04*
Error	0.112	8	0.014		
Total	45.076	15			
Corrected total	2.235	14			

[a] R Squared = .950 (Adjusted R Squared = .912), (* = Significantly different)

Table C.1.4 LSD for pairwise comparison of packaging material for PV

Packaging material	Mean difference	Standard error	Sig.
HDPE (A)	B–A = 0.248		0.011*
		0.074842	
Aluminum (B)	B–C = 0.104		0.146
Glass bottles (C)	C–A = 0.352		0.202*

(* = Significantly different)

Appendix-D

Table D.1 Chemical composition of selected ingredients

Parameters	Wheat	Maize	Soybean	Bengal gram	Pumpkin seed	Sesame	Milk powder	Sunflower oil	Sugar
Moisture (gm/100gm)	12.2	14.9	10.2	9.8	8	4.28	-	-	0.5
Protein (gm/100gm)	12.1	11.1	33.3	17.1	24.3	25.9	17.4	-	0.1
Fat (gm/100gm)	1.7	3.6	17.7	5.3	47.2	49.57	18.1	100	0
Carbohydrate (gm/100gm)	69.4	66.2	29.6	60.9	15.6	12.4	53.1	-	99.4
Crude fiber (gm/100gm)	1.9	2.7	4.2	3.9	0.2	2.53	0	-	0
Ash (gm/100gm)	-	-	-	-	-	-	-	-	-
Energy Kcal/100gm	341	342	411	360	584	599	445	900	398
Calcium (mg/100 gm)	48	10	226	44.8	50	-	-	-	-
Phosphorus (mg/100 gm)	355	348	546	312	830	442	-	-	-
Iron (mg/100 gm)	4.9	3.3	8.5	4.6	5.5	4.59	-	-	-
Carotene (μg/100 gm)	29	90	10	189	38	-	-	-	-
Vit. C (mg/100 gm)	0	0	0	3	1	-	-	-	-
Thiamine (mg/100 gm)	0.49	0.42	0.66	-	0.33	-	-	-	-
Riboflavin (mg/100 gm)	0.17	0.1	0.22	-	0.16	-	-	-	-
Niacin (mg/100 gm)	4.3	1.8	2.2	-	3.1	-	-	-	-

Source: (DFTQC, 2012)

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

Particulars	Cost (NRs/kg)	Weight (gm)	Cost (NRs)
Wheat	50	90	4.5
Maize	30	40	1.2
Soybean	95	80	7.6
Bengal gram	100	40	4
Sesame	200	140	28
Milk powder	887.5	140	124.25
Sunflower oil	150	160	24
Sugar	70	170	11.9
Pumpkin seed		124	-
Vitamin and mineral premix		16	-
Total raw material cost		1000	205.45
Processing and labor cost (10% of raw material cost)			20.55
Packaging cost			50
Profit (10 %)			27.6
Grand total cost		1000	303.6

Cost of raw materials varies with season and time

Appendix-E

Table E.1 Protein and essential amino acid content of raw materials and egg

Component	Protein (%)	Essential amino acids mg/gm protein								
		Try	Thr	Iso	Leu	Lys	Met	Phe	Val	His
Wheat*	12.2	22.72	29.28	32.64	66.72	28.64	15.04	45.12	44.16	22.88
Maize*	9.5	19.52	38.56	40.96	95.68	34.24	24.64	48.16	55.2	24.48
Soybean*	38	14.01	42.20	49.73	85.11	69.87	13.83	54.11	52.53	27.67
Bengal gram*	20.1	27.84	37.42	44.32	74.88	68.48	33.44	57.28	45.44	26.4
Sesame*	18.1	54.15	42.07	42.64	32.08	32.26	33.21	52.26	54.34	28.86
Milk powder*	26.0	56.89	41.22	51.72	97.02	71	25.23	47.49	63.01	28.06
Pumpkin seed**	30.2	53.27	42.74	55.55	90.76	39.75	34.50	75.66	62.02	30.48
Egg*	12.4	29.44	51.2	62.88	88.16	69.76	33.6	57.28	68.48	24.32

Source: *(FAO, 1970), **(Glew *et al.*, 2006)

Table E.2 Formula-B Calculation of protein and essential amino acid content (worksheet)

Component	wt (gm)	Protein (gm)	Essential amino acids (mg/ gm protein)								
			Try	Thr	Iso	Leu	Lys	Met	Phe	Val	His
Wheat*	9	1.09	24.77	31.92	35.58	72.72	31.22	16.39	49.18	48.13	24.94
Maize*	4	0.38	7.42	14.65	15.57	36.36	13.01	9.36	18.30	20.98	9.30
Soybean*	8	1.52	21.30	64.14	75.59	129.37	106.20	21.02	82.25	79.85	42.06
Bengal gram*	4	0.8	22.27	29.94	35.46	59.90	54.78	26.75	45.82	36.35	21.12
Sesame*	14	2.53	136.99	106.44	107.88	81.16	81.62	84.02	132.22	137.48	73.02
Milk powder*	16	4.16	236.66	171.48	215.16	403.60	295.36	104.96	197.56	262.12	116.73
Pumpkin seed*	14	4.23	225.33	180.79	234.98	383.92	168.14	145.94	320.04	262.34	128.93
Sugar	12.4	0.01	-	-	-	-	-	-	-	-	-
Sunflower oil	17	-	-	-	-	-	-	-	-	-	-
Premix	16	-	-	-	-	-	-	-	-	-	-
Total	100	14.72	674.74	599.36	720.22	1167.03	750.33	408.44	845.37	847.25	416.1
Total mg/gm protein			45.84	40.72	48.93	79.28	50.97	27.75	57.43	57.56	28.27
Egg**			29.44	51.2	62.88	88.16	69.76	33.6	57.28	68.48	24.32
Chemical score			156	80	79	90	73	83	100	84	116
RDA (mg/kg body wt) for child <2 year*			6.4	23	27	54	45	22	40	36	15
RDA (mg/gm protein) for child <2 year*			7.4	27	31	63	52	26	46	42	18

Source: *(FAO/WHO/UNU, 2002), ** (FAO, 1970).

Appendix-F

Photo gallery

Photo 1: Product for shelf life study

Photo 2: Crude fiber determination

Photo 3: Determination of total protein