FORMULATION OF STANDARD AND HIGH PROTEIN READY TO RECONSTITUTE ENTERAL FORMULA FEEDS

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Formulation of Standard and High Protein Ready to Reconstitute Enteral Formula Feeds

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 *Formulation of standard and high protein ready to reconstitute enteral formula feeds* presented by Sapan Dahal has been accepted as the partial fulfillment of the requirements for the Bachelor degree in Nutrition and Dietetics.

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Regards,

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Sapan Dahal

Abstract

The objective of this study was to formulate two ready to reconstitute (RTR) enteral formula feeds with whole some nutritional approach and ease of administration, for the normal and post-operative patients to overcome the hospital malnutrition. Few food ingredients from the basic food groups were selected. This food products were processed and made into a flour based enteral feeding. The physiochemical analysis of the feeds were carried out and the reconstitution behavior of the feed was done.

The 511 gm sample of the protein feed was fabricated to supply 2051 kcal and 101.12gm protein (19.72% of total energy contents). The carbohydrates and fat contents were 302.10 gm (58.92 % of total energy contents) and 48.68gm(21.36% of total energy) from the total feed. The second standard feed (standard feed) was intended to deliver about 2041 kcal energy and 74.45 gm of protein (14.59 % of total energy) from the total amount of 520 gm of the feed. The amount of major energy constituents i.e. totals carbohydrates and fats were accounted as 335.29 gm (65.71% of total energy content) and 44.69 gm per (19.70% of total energy content) from the total feed. The micro nutrient levels of the sample were detected to be sufficient in amount in both feeds. The developed feeds were checked for their reconstitution behavior as well as their flow behavior through Ryle’s tube. The total plate count was done and found to be 1482 Cfu/gm and 1396 Cfu/gm for the protein feed and standard feed respectively The molds count were 41Cfu/gm and 37Cfu/gm for protein feed and standard feed respectively. Thus the present study speculates that both formulated RTR enteral formula feeds would render appreciably the whole some nutrients to the patients‟ dependent upon enteral nutrition system for long time.

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List of Abbreviations

Abbreviation	Full form
AIDS	Acquired immunodeficiency syndrome
AOAC	Association of analytical communities
ASPEN	American society for parenteral and enteral nutrition
BMI	Body mass index
CFU	Colony forming unit
DAA	Dietitians association of Australia
DFTQC	Department of Food Technology and Quality Control
EF	Enteral feeding
EN	Enteral nutrition
ERAS	Enhanced recovery after surgery
ESPEN	European society for clinical nutrition and metabolism
GI	Gastrointestinal
GIT	Gastrointestinal tract
GRV	Gastric residual volume
HDPE	High Density Polythene
IBW	Ideal Body Weight
ICMR	Indian Council of Medical Research
IV	Intra venous
LCT	Long chain triglycerides
LSD	Least significance difference

MCT	Long chain triglycerides
MOFS	Multi organ failure syndrome
MUFA	Monounsaturated fatty acids
NDT	Nasoduodenal tube
NGT	Nasogastric tube
NJT	Nasojejunal tube
NRI	Nutritional risk index
PEG	Percutaneous endoscopic gastrostomy
PEJ	percutaneous endoscopic jejunostomy
PGI	Post graduate institution
PN	Parenteral nutrition
PPN	Peripheral Parenteral Nutrition
PUFA	Polyunsaturated fatty acids
RDA	Recommended dietary allowance
RTR	Ready to reconstitute
SGA	Subjective global assessment
TPC	Total plate count
TPN	Total parenteral nutrition
TUTH	Tribhuvan University Teaching Hospital
UNICEF	United Nations Children's Fund
WFP	World Food Programme
WHO	World Health Organization

PART I

Introduction

1.1 General introduction

After operation, injury, extensive chemotherapy, radiotherapy, infection and other severe illness including Acquired Immunodeficiency Syndrome (AIDS), and cancer both protein and fat are lost from the body. A 10% or greater loss of body protein will contribute to morbidity and debility. Cerra (1992) reported that hypermetabolism and multi organ failure syndrome (MOFS) after trauma, surgery or sepsis is associated with accelerated catabolism , rapid onset of malnutrition and immune system failure (Cerra *et al.*, 1992).

Malnutrition is a frequent concomitant of surgical illness. Studies have reported 40%-50% of surgical patients to be malnourished on admission to hospital (Beattie *et al.*, 2000; Torosian, 1999). There is a high incidence of malnutrition in hospitalized patients undergoing gastrointestinal surgery. Gastrointestinal patients, especially with underlying malignancy, are at high risk of developing malnutrition, and surgical stress can also accentuate this catabolic problem. Malnutrition can occur in gastrointestinal (GI) cancer due to increased metabolic demands, insufficient nutrient intake, or nutrient loss. Many patients with gastrointestinal cancers will require surgical intervention, which imposes further metabolic demands and compounds preexisting nutritional disorders. Patients who undergo gastrointestinal surgery are at risk of nutritional depletion from inadequate nutritional intake; both preoperatively and postoperatively, the stress of surgery and the subsequent increase in metabolic rate. If a gastrointestinal cancer patient requires surgery during treatment, their metabolic condition should be optimal at the time of intervention (Mariette *et al.*, 2012).Studies reported that up to 40% of patients were malnourished at the time of their admission and the majority of these patients continued to be nutritionally depleted throughout their hospital course (Beattie *et al.*, 2000). In general, surgery-related causes of malnutrition are hypercatabolism, postoperative fasting, prolonged ileus, fistula, malabsorption syndrome, intestinal obstruction, and gastric atony (Senesse *et al.*, 2008). Malnutrition in hospitalized patients often goes unrecognized (McWhirter and Pennington, 1994).

Nutritional support is a vital component of medical care. Enteral nutrition (EN), the provision of nutrients via the gastrointestinal (GI) tract through a feeding tube, catheter or stoma, is the preferred route for the provision of nutrition for patients who cannot meet their nutrition needs through voluntary oral intake. This type of feeding provides many physiologic, metabolic, safety, and cost benefits over other methods of feeding.. According to Reshma and Nilesh (2011) the best way to feed a patient is using their own gastrointestinal tract (stomach and bowel). Those patients who cannot swallow food due to a breathing tube inserted in the throat, they are fed through a feeding tube. The major indicators of the enteral nutrition are the symptoms like unconscious, neuromuscular swallowing disorders, physiological anorexia, upper gastrointestinal obstruction , gastrointestinal dysfunction / malabsorption, increased nutritional requirements, psychological problems and specific treatment (Joshi and Sharma, 2014).

Enteral feeding formulas have been produced in great extent and it reduces the time to be prepared and provide all sorts of nutrients needed for the patients. By ESPEN guideline for surgery 2017, it is said that in most patients, a standard whole protein formula is appropriate. For technical reasons with tube clotgging and the risk of infection the use of kitchen-made (blenderized) diets for tube feeding is not recommended in general (Weimann *et al.*, 2017). Enteral feeding formula is being used with increasing frequency when oral intake is limited. Early support is usually given via a post pyloric naso-jejunal (NJ) tube or surgical jejunostomy placed pre, inter, or postoperatively. Feeding is usually possible for although gastric and colonic function may be impaired for several days after surgery, small bowel function is often normal (Carr *et al.*, 1996). Peri-operative Enteral Feeding may have advantages over parenteral nutrition (PN) feeding. A meta-analysis comparing these methods of support concluded that enteral nutrition (EN) reduced infective risks by about one third (Braunschweig *et al.*, 2001).

1.2 Statement of the problem

During recent years, the diversity of Enteral Feeding (EF) formulas has been rapidly increased. In developed and advanced countries, they prefer enteral formula feed rather than hospital made blenderized feed. In many countries, commercial formulas are routinely used for EF which has been improving the nutritional status of the patients and is decreasing the number of the hospital stay of those patients (Ghomi *et al.*, 2017). According to ESPEN guideline 2017, a standard whole protein formula is appropriate for technical reasons with tube clotgging and the risk of infection (Weimann *et al.*, 2017).

Malnutrition in post-operative patient has been very vulnerable in every part of the world. In developing countries like Nepal the condition is even worst. According to Department of Health Services Report 2072/73, there are 407 hospitals in Nepal. In these hospitals, the enteral feedings are either blenderized diet prepared by hospitals or other foods like milk, fruit juices and even horlicks, viva etc. These types of feeding do not provide all the essential nutrients and energy fulfillment and protein requirements cannot be fulfilled and also influencing the mortality and morbidity of the patients and also on the number of hospital stay days. Post-operative patients require high energy and high protein diet but such foods do not contain the required amount of protein needed for the patients for proper healing of the wounds. The preparation of blenderized feeding are also time consuming and and has a greater chance of producing complications. So use of nutritionally concentrated, easily digestible and palatable instant liquid diet will be very useful in such occurences. Such type of enteral formulas helps to meet patients' nutrients requirement which also decreases the mortality and morbidity of the post-operative patients who are dying due to malnourishment.

Therefore, it is very essential to develop and produce new generation of enteral diets that are prepared as per the nutritional requirement of the post-operative patients (i.e. high calorie and high protein) diet for our country and also for the world. It can also be used as a oral feed who are malnourished during the hospital stay as it is high calorie and high protein diet. Such diets can solve the problems of the hospitals who are using the foods like horlicks, viva for the tube feeding patients.

1.3 Objectives

1.3.1 General objectives

The general objective was to formulate standard and high protein ready to reconstitute enteral formula feeds.

1.3.2 Specific objectives

- To prepare standard and high protein RTR.
- To perform physiochemical analysis of the prepared formula diet.
- To evaluate the microbiological (Total plate count (TPC), Yeast and Mold count) quality of prepared product.

1.4 Significance of the study

This formula can be used further by researcher to prepare other specific reconstitute feeding formula for post-operative patients and normal patients. It will also provide the basis for the further work in this field to any factory, government agencies, local agencies and others whose primary aim is to improve the nutritional status ofpost-operative patients and can even be used for malnourished patients at the lowest cost. Thus, this study will provide the basis for the further research in this field.

1.5 Limitations

- Clinical trials could not be done.
- Analysis of vitamins and trace elements of the products were not done due to limited facilities.
- Shelf-life of the product could not be studied.

Part II

Literature Review

2.1 Historical background

The history of dietetics can be traced as far back as the writings of Homer, Plato and Hippocrates in ancient Greece. Although diet and nutrition continued to be judged important for health, dietetics did not progress much till the 19th century with the advances in chemistry. Early research focuses on vitamin deficiency diseases while later workers proposed daily requirements for protein, fat and carbohydrates. Dietetics as a profession was given a boost during the Second World War when its importance was recognized by the military. Today, professional dietetic association's can be found on every continent, and registered dietitian are involved in health promotion and treatment, and work alongside physicians. The growing need for dietetics professionals is driven by a growing public interest in nutrition and the potential of functional foods to prevent a variety of diet-related conditions (Hwalla and Koleilat, 2004).

The history of enteral feeding goes back about 3500 years to the ancient Greeks and Egyptians, who infused nutrient solutions into the rectum to treat various bowel disorders. Over the centuries, experimentation and research have contributed to a greater understanding of nutrient requirements; methods to more accurately access the gastrointestinal tract; development of new materials to use in equipment, tubes, and containers; and the digestion, absorption, and use of macro- and micronutrients. It is notable that while advances were made in one area, progress was being made in another. For example, while enteral access and feeding techniques were being developed, essential amino acids were identified. When new information came together, rapid changes opened up the applications for enteral feeding in new directions, sometimes in unexpected ways such as diets designed for the space program, leading to the use of elemental diets as a therapeutic modality. (Ronni, 2006)

Total parenteral nutrition (TPN) has been available for only 30 years. However, history in this field goes back more than 350 years with the first landmark being the description of general blood circulation by William Harvey in 1628. His discovery is the anatomical basis for intravenous infusions. Many investigations were performed during the following centuries showing that solutions containing electrolytes and glucose could be given intravenously in man. The accumulated knowledge of protein metabolism formed the basis for studies on intravenous nutrition with protein hydrolysates, peptides and amino acids. The observation in the late 30-s by Robert Elman that amino acids in the form of protein hydrolysate could be safely administered intravenously in man was the first major step toward TPN (Wretlind and Szczygieł, 1994).

During the following years, major efforts were made to find methods to prepare infusion solutions with a high energy content and low osmotic pressure. The most realistic alternative seemed to be fat in the form of an emulsion. Many studies of a large number of various fat emulsions were made however, all of these emulsions caused severe adverse reactions in man. The first safe fat emulsion, intralipid, was made available in the early 60s. This was the second major step toward TPN. It was then no problem to include vitamins, electrolytes and trace elements in the fat emulsions and the solutions of amino acids and glucose. A few years later it was shown that a central venous catheter could be used to administer the infusion fluid intravenously. Many clinical investigations and reports have shown that the newly developed intravenous nutritional regimens are adequate alternatives to the ordinary diet. In this way it has been possible to maintain or obtain a good nutritional condition in most situations when oral or tube feeding cannot be used. TPN has been shown to be of very great clinical importance to prevent and treat starvation often related to high morbidity and mortality (Wretlind and Szczygieł, 1994).

Jain and Joshi (2005) had also carried a research work on the development of RTR formula feed. The data of that feed composition were presented to be as cereals and millet, pulses, nuts & oil seeds, milk powder, vegetable slurry, sugar and oil. The Chilus (1989) has reported that soaking increases the nutrient availability of the food. Gopaldas and Deshpande (1988) have reveled in his study that oven drying near the temperature range of 500C conserves the amylase activity in germinated grains. Further Phillips 1990, Joshi 2005, Khali et al 2006 reported that germination of seeds enhances thenutrient content of the pulses as well as minimizes the enzymatic activity inside the food components. According to Bau and others (2000), soybean germination leads to substantial increase in certain biochemical and biologically active compounds of the beans. Bhupender Singh (2007) favored the popping of pearl millet as it increases the starch susceptibility.

2.2 Malnutrition in hospitalized patients

The nutritional status of adult and elderly hospitalized patients has been discussed for years. The rates of malnutrition in this population usually depend on disease and assessment criteria and vary from 10% to 50%. (Amaral *et al.*, 2010; Edington *et al.*, 2000; D. Waitzberg *et al.*, 2001). According to a British study it was 27.4% (Stratton *et al.*, 2004), according to a German study it was 46% (Pirlich *et al.*, 2006), and 46% according to a Canadian study (Caccialanza *et al.*, 2010), Finally, a study in Spain found mild, moderate and severe malnutrition rates of 50.7%, 26.4%, and 5.7% respectively. (Cabello *et al.*, 2011)

Different parameters are being developed to assess the nutritional status of hospitalized patients and better map this reality. Nevertheless, malnutrition is still underreported, despite its association with increased morbidity, mortality, and hospital costs (Marco *et al.*, 2011; Westergren *et al.*, 2009).

Malnutrition increases the risk of complications from abdominal surgery (Schiesser *et al.*, 2008), but weight loss, low albumin, and low body mass index (BMI) are not always associated with mortality and morbidity in surgical patients (Pacelli *et al.*, 2008). Although many studies have assessed the nutritional status of hospitalized patients, the relationship between nutritional status and other variables, such as type of disease, type of surgery, and occurrence of complications, among others, should be further explored. Newfound associations may help improve interventional actions and control strategies that aim to prevent malnutrition related intercurrences (Portero-McLellan *et al.*, 2010).

The frequency of any degree of malnutrition on hospital admission varied from 63.3% as assessed by the Subjective global assessment (SGA) to 90% with the Nutritional risk index (NRI). The severity of malnutrition diverged among indices, with the SGA scoring most cases as mild whereas the NRI scored most cases as moderate. On the basis of the SGA, 8.3% of the patients were classified as suffering severe malnutrition, 18.3% moderate malnutrition, and 36.7% mild malnutrition. The severity of malnutrition was not related to the diagnosis. According to the SGA, an elderly population was associated with a higher prevalence of malnutrition (36.67% regarding the total sample), compared with those younger than 64 years (24–44 y: 11.67%; 45–64 y: 15%). However, the SGA did not show any significant differences between sex (male: 4.76%; female: 6.49%). Weight loss during the previous 6 months was reported in 28.9% of patients. Cancer patients more than those with other diseases reported weight loss. Recent bodyweight changes show significant differences between the 25– 44 y old group and the 45–65 y old group (3.8272.68 kg compared with 8.5875.48 kg, respectively) (Pablo *et al.*, 2003).

2.3 Post-operative nutrition

Malnutrition is associated with postoperative complications and an increased risk for death after surgery (Mogensen *et al.*, 2015). Many surgical diseases result in malnutrition, particularly those that are associated with a hypermetabolic state (e.g., malignancy, inflammation, gastrointestinal dysfunction, and burns). Advanced age is also associated with malnutrition in hospitalized patients, many of whom require emergency operations. Although nutritional optimization prior to surgery is an option for a select group of patients, in most cases, this is not possible (Reilly *et al.*, 2015; Santa Mina *et al.*, 2015).

An enteral route for nutrition is usually recommended if available because it stimulates the intestinal brush border, thereby preventing disuse atrophy and bacterial translocation. It also stimulates gallbladder emptying, thereby preventing bacterial stasis and cholecystitis. Enteral access is also less expensive and has fewer side effects and complications compared with central venous access, which is required for parenteral nutrition. Nevertheless, parenteral nutrition may be necessary for those patients with contraindications to enteral nutrition and for postoperative patients who are unable to meet their nutritional goals using the enteral route (Siparsky, 2018).

Postoperative total parenteral nutrition (TPN) is indicated for patients already receiving TPN preoperatively, those severely malnourished prior to major surgery, those unable to eat satisfactorily for 7 days, or patients presenting with severe complications. Postoperative TPN should last for at least 7 days. The total energy requirements are between 30 and 35 kcal/kg/day. About 50% to 70% should be provided in the form of carbohydrates, and 20% to 30% in the form of lipids. The optimal input rates for glucose and lipids are 4 to 5 g/kg/day and 80 mg/kg/hr, respectively. The ideal nitrogen administration is 250 to 300 mg/kg/day, and the optimal calorie/nitrogen ratio is 150 to 200. Some specific amino acids can be added as intravenous dipeptides. An adequate follow-up must include clinical and biochemical parameters. Several trials evaluated the impact of TPN in postoperative patients, but further well designed, controlled clinical trials are still necessary to address a great number of unanswered questions (Waitzberg *et al.*, 1999).

2.4 Enteral feeding

Enteral nutrition can be provided either orally or by tube feeding. By definition enteral means 'within or by the way of gastrointestinal tract'. Enteral nutrition is given to an individual who has functioning gut and unwilling to achieve adequate oral intake. If the gut is functioning, it should be used. Enteral feeding retains the epithelial structure and function. It also increases mucosal immunity. In practice enteral nutrition is generally considered as tube feeding (Srilakshmi, 2014).

Oral Feeding is the best for the nourishment of patients. But in the following conditions it is not possible to give the feeding orally and tube feeding or parenteral feeding is restorted (Srilakshmi, 2014).

- Those who cannot swallow due to paralysis of the muscles of swallowing (diptheria, poliomyelitis) or cancer of the oral cavity or larynx.
- Those who cannot be persuaded to eat.
- Those with persistent anorexia requiring forced feeding.
- Semiconscious or unconscious patient.
- Severe malabsorption requiring administration of unpalatable Formula.
- Short bowel syndrome.
- Those who are undernourished or at risk of becoming so.
- Those who cannot digest and absorb.
- Post-surgery.
- Patients with neurological and renal disorders and those with fevers or diabetes.
- Severe diarrhoea

2.4.1 Tube feeding

This is done by passing a tube into the stomach or duodenum through the nose which is called nasogastric feeding or directly by the surgical operation known as gastrostomy feeding. Short term (3-4 weeks) feedings usually are administered via nasogastric, nasoduodenal or nasojejunal tubes. For long term feedings, a gastostomy or jejunostomy is usually indicated (Srilakshmi, 2014).

In order to minimize the risks of aspiration, tubes should not be placed in the stomach unless there is normal gastric emptying and an intact gag reflux and if possible, nasoenteric rather than nasogastric feeding tubes should be employed. This may be especially valuable in post-operative and critically ill patients in whom gastric motility is impaired (Srilakshmi, 2014).

A satisfactory tube feeding must be (Srilakshmi, 2014)

- Nutritionally adequate
- Well tolerated by patients so that vomiting is not induced
- Easily digested with no unfavourable reaction such as distention, diarrhoea or constipation
- Easily prepared
- Inexpensive

2.4.1.1 Methods of tube feeding administration

Four methods have been developed for enteral feeding

A Continuous Feeding

Continuous feeding, defined as an hourly rate of EN administration using an electric enteral feeding pump, is the preferred method for feeding patients who are critically ill, being incubated for respiratory failure, exhibiting poor glycemic control, being fed via a jejunostomy tube, or intolerant of intermittent feedings (Brantley and Mills, 2012). The typical recommendation for continuous feeding is to start at 20–50 mL/h, advancing by 10-25 mL every 4-24 h (Parrish, 2003). However, while continuous feeding is used in most critical care units, only a few relatively outdated studies have provided evidence to support this practice. In a study of 76 adult burn patients, those receiving continuous tube feeding had lower stool frequency and less time required to reach nutritional goals than those receiving bolus feeding (Hiebert *et al.*, 1981). In neurologically impaired adult patients, aspiration was observed less frequently in those receiving continuous feeding (1/17) than in those receiving intermittent feeding (3/17) (Kocan and Hickisch, 1986). Regarding drug-nutrient interactions, continuous feeding is the most problematic met and frequently requires interruption of tube feeding for the administration of medication. The rate of tube feeding might therefore have to be increased to provide appropriate nutrition during the reduced infusion period (Brantley and Mills, 2012).

B Cyclic Feeding

Cyclic feeding provides EN using an electric enteral feeding pump in less than a 24-h time period. Similar to continuous administration, this delivery method may be used when the feeding tube is located at the stomach or small intestine. Depending on patient tolerance to volume load, infusion time might be decreased from 24 h per day to as short as 8 h. As the patient recovers from illness, this method may be used to transition from the 24-h continuous feeding method to 12-h nocturnal feeding in an effort to enhance the patient's appetite during the day and increase mobility by freeing the patient from tubing and pump connections (Brantley and Mills, 2012).

C Intermittent Feeding

Intermittent feeding is administered via an electric enteral feeding pump or gravity drip. Volume can range from 240 to 720 ml, be administered over a time period ranging from 20 to 60 min, and be provided anywhere from 4 to 6 times per day depending on the volume of formula required to meet the patient specific needs (Brantley and Mills, 2012). However, while this feeding method is more physiological and affords greater patient mobility between feedings, it is not without its disadvantages, such as risk of aspiration (Kocan and Hickisch, 1986), diarrhea (Ciocon et al, 1992), and gastric distention. Some alert and mobile patients may increase the volume of each feeding as tolerated and decrease the number of feedings required per day to improve quality of life (Brantley and Mills, 2012).

D Bolus Feeding

Bolus feeding is defined as formula administered via a syringe or gravity drip over a short period of time. A typical feeding regimen might provide 240 ml of formula over a 4-10-min period infused 3–6 times per day. This rapid delivery method is used for clinically stable patients with normal gastric function and is generally not well tolerated in patients with small bowel access. Further, rapid infusion can cause GI intolerance and discomfort. Bolus feeding offers the advantage of allowing administration of medication to be separated from feeding. In addition, this method closely mimics ordinary eating patterns and can reduce feeding time, thereby enabling the patient to move about freely, participate in rehabilitation therapy, and live a more normal life (Brantley and Mills, 2012)

2.4.1.2 Types of tube feeding

There are two main types of feeding tubes based on the site of placement and they are:

- **Prepylorictubes:** This tubes end in the stomach above the pyloric sphincter. They're preferred for intermittent feeding and to allow gastric absorption (Houston and Fuldauer, 2017).
- **Postpylorictubes:** This tubes end beyond the pyloric sphincter in the jejunum. They're indicated for patients with gastroparesis, acute pancreatitis, gastric outlet stenosis, hyper emesis (including gravida), recurrent aspiration, tracheoesophageal fistula, and stenosis with gastroenterostomy. Postpyloric feedings must be administered on a continuous basis (Houston and Fuldauer, 2017).

On the basis of duration of the use enteral feeding it can be classified into two types as

A Short term enteral feeding

- **Nasogastric Access**
  Nasogastric tubes (NGTs) are used most commonly to access the GIT, for gastric decompression, medication delivery, and/or feeding. They are appropriate only for those patients who require short-term (no more than 3 to 4 weeks) EN. Typically, the tube is inserted at the bedside by a nurse or physician (or a registered dietitian with appropriate clinical privileges) and passed through the nose into the stomach. Polyurethane or silicone tubes of various diameters, lengths, and design features may be used, depending on formula characteristics and feeding requirements. These tubes are soft, flexible, and often well tolerated by patients. Tube placement is verified by aspirating gastric contents in combination with auscultation of air insufflation into the stomach or by radiographic confirmation of the tube tip location (Mahan and Raymond, 2017).
- **Nasoduodenal or Nasojejunal Access**
  Patients who do not tolerate gastric feedings and require relatively short-term enteral nutrition support will benefit from placement of a nasoduodenal tube (NDT) or a nasojejunal tube (NJT), described by the point at which the tube tip terminates. These tubes may be placed with endoscopic or fluoroscopic guidance. In some cases, a feeding tube may be placed intragastrically with the goal of migration into the duodenum by peristalsis; this process is unlikely to result in desired location of the

feeding tube tip and inevitably delays initiation of appropriate EN. Spontaneous migration never achieves jejunal tip placement (Mahan and Raymond, 2017).

B Long-term enteral feeding

- **Gastrostomy or Jejunostomy**

  When EN is required for more than 3 to 4 weeks, a surgically or endoscopically placed gastrostomy or jejunostomy feeding tube should be considered for overall patient comfort and to minimize nasal and upper GIT irritation. Such a tube may be placed during a required surgical or endoscopic procedure to maximize efficiency and cost effectiveness (Mahan and Raymond, 2017).

  Percutaneous endoscopic gastrostomy (PEG) or percutaneous endoscopic jejunostomy (PEJ) is a nonsurgical technique for placing a tube directly into the stomach through the abdominal wall, using an endoscope and local anesthesia. The tube is guided from the mouth into the stomach or the jejunum and brought out through the abdominal wall. The short procedure time and limited anesthesia requirements have contributed to making it a very common method of feeding tube placement (Mahan and Raymond, 2017).

2.4.2 Types of enteral feeds

Enteral feeding can be classified in different ways. A classification is based on the practical considerations according to the clinical indications for the solution. Mahan 2000 has classified the enteral formula feeds as (Joshi and Sharma, 2014).

2.4.2.1 Blenderized feeds

Blenderized feeds are mainly composed of fresh and regular foods with simple processing techniques. It should be considered important for the maintenance of home enteral nutrition because they can be prepared using the foods consumed by the family. Blenderized tube feeding formulas preparation consisted of three stages: stage I: the raw foods were cooked together in the same pot. Stage II: once the raw foods were cooked, they were placed in a blender with half of the milk volume and the other ingredients, and blended for three minutes at medium speed. The remainder of the milk was added, and the mixture was blended again for another three minutes at medium speed. Stage III: the ready-to-eat formula was sieved three times through a fine mesh strainer, placed in a sterile plastic container, and stored in the refrigerator (Bento *et al.*, 2017).

2.4.2.2 Polymeric food solutions

These solutions contain macronutrients in the form of isolates of intact protein, triglycerides and carbohydrate polymers, which can be used through a tube and can provide complete nutrition. These can be categorized as follows (Joshi and Sharma, 2014):

- **Standard adult formulae**: They provide 1 kcal/ml and are suitable for the majority of patients. They are available with and without fiber (Joshi and Sharma, 2014).
- **High energy adultformulae:** These provide 1.2 to 2 kcal /ml and are useful for patients on fluid restriction or with increased nutritional requirements such as burn patients (Joshi and Sharma, 2014).

2.4.2.3 Monomeric solutions

These solutions usually contain proteins as peptides and /or amino acids, fat and long chain triglycerides or mixture of LCTs (Long chain triglycerides) and MCTs (Medium chain triglycerides) and carbohydrates as partially hydrolyzed starch, maltodextrin glucose or oligosaccharides (Joshi and Sharma, 2014).

2.4.2.4 Solutions for specific metabolic needs

These nutritional solutions are intended for patients who have unique metabolic requirements- Inborn error of metabolism, renal failure, hepatic failure etc. (Joshi and Sharma, 2014)

2.4.2.5 Modular solutions

Solutions which contain nutritional components that can be given by themselves or can be mixed with other enteral products to meet special nutritional or metabolic needs of a given patient like increased calories, increased minerals etc. (Joshi and Sharma, 2014).

2.4.2.6 Hydration solution

Solutions which provide minerals, water and small amounts of carbohydrates (Joshi and Sharma, 2014).

2.4.3 Complications of tube feeding

Patients with feeding tubes are at risk for such complications as aspiration, tube malpositioning or dislodgment, refeeding syndrome, medication-related complications, fluid imbalance, insertion-site infection, and agitation. To identify these problems, thoroughly assess the patient before tube feeding begins and monitor closely during feedings (Houston and Fuldauer, 2017).

2.4.3.1 Aspiration

Gastrostomy (G) tube feedings can cause pulmonary aspiration. Multiple factors contribute to aspiration, including recent hemorrhagic stroke, high gastric residual volume (GRV), high bolus feeding volumes, supine positioning, and conditions that affect the esophageal sphincters (such as an indwelling endotracheal or tracheostomy tube with dysfunction of the upper esophageal sphincter and a nasogastric or an enteral tube traversing both esophageal sphincters (Houston and Fuldauer, 2017).

Studies show that patients who received tube feedings of 500 to 1,500 mL/day didn't have a higher aspiration risk than those fed lower daily volumes; even some who received low volumes aspirated. However, relatively fast feeding rates with volumes exceeding 1,500 mL/day did place patients at higher risk for aspiration (Houston and Fuldauer, 2017).

To help reduce risk, GRV should be monitored every 4 hours in patients receiving continuous tube feedings. A.S.P.E.N. and the Society of Critical Care Medicine guidelines for critically ill patients advise against halting tube feedings for GRVs below 500 mL unless the patient has other signs and symptoms of intolerance. Sometimes, healthcare providers order withholding of tube feedings at lower GRVs because of specific risk factors (Houston and Fuldauer, 2017).

During patient transport or when placing the head of the bed flat for patient repositioning, turn the tube feeding off, especially if the patient has a high aspiration risk. However, be aware that no conclusive evidence shows that pausing tube feeding during repositioning reduces aspiration risk for patients with high GRVs (Houston and Fuldauer, 2017).

2.4.3.2 Tube malpositioning or dislodgment

During initial placement, the feeding tube may be positioned improperly. To prevent this problem, the tube should be placed by experienced personnel and its position confirmed radiographically. After initial placement, the tube may become fully or partially dislodged, causing such problems as bleeding, tracheal or parenchymal perforation, and GI tract perforation (Houston and Fuldauer, 2017).

To help prevent malpositioning and dislodgment, verify feeding tube integrity at the beginning of each shift. Be aware that verbal patients with dislodged tubes may complain of new-onset pain at or near the insertion site of a percutaneous endoscopic gastrostomy (PEG) tube, G tube, gastric-jejunal (GJ) tube, or J tube. Nonverbal patients may respond with vital-sign changes (such as increased blood pressure or heart rate), increased agitation, and restlessness (Houston and Fuldauer, 2017).

2.4.3.3 Refeeding syndrome

Patients with sustained malnutrition are at risk for refeeding syndrome—the body's reaction to digestion after depleted electrolytes shift from the serum to the intracellular space. This syndrome may trigger life-threatening arrhythmias and multisystemic dysfunction. It occurs when a depleted metabolic system with little to no mineral reserve (for instance, from vitamin B deficiency) becomes exhausted by the body's increased demands to process proteins and produce glycogen. An insulin response to reintroducing nutrition causes an anaerobic state, as the body can't meet the demand for oxygen and other resources needed to metabolize nutrients. Serum electrolytes then move into the intracellular space to help satisfy the higher demands, resulting in acute electrolyte abnormalities (Houston and Fuldauer, 2017).

In patients with long-term malnutrition, monitor for intolerance at the onset of enteral feedings by checking heart rate and rhythm and electrolyte levels. Although refeeding syndrome incidence is low, failure to recognize the sudden drop in potassium and magnesium levels can have catastrophic consequences (Houston and Fuldauer, 2017).

To reduce the risk of refeeding syndrome in patients with vitamin and mineral deficiencies, supplements may be ordered for parenteral administration before enteral feedings begin. Refer to specific guidelines based on total energy needs and specific micronutrient deficiencies; thiamine and other B-vitamin deficiencies are the most pressing ones to address before initiating enteral feeding. As the tube-feeding goal rate is achieved, taper micronutrient supplement dosages as indicated (Houston and Fuldauer, 2017).

2.4.3.4 Fluid imbalance

Most patients need supplemental free-water flushes to maintain adequate hydration; on average, they need 30 mL/kg of water per day, given either as free-water flushes or I.V. hydration (Houston and Fuldauer, 2017).

If a free-water flush is ordered, calculate its volume by subtracting the volume of water in the feeding formula from the patient's total daily requirement; then divide the remaining volume over a regular routine of tube flushing. Before and after medication administration, flush the tube with about 30 mL of fluid or more, depending on drug characteristics (Houston and Fuldauer, 2017).

2.5 Parenteral nutrition

In parenteral nutrition a sterile, nutrient dense solution is infused intravenously by a peripheral or a central venous access, entirely bypassing the digestive tract. Parenteral nutrition was originally developed to nourish those patients who are not capable of digesting and absorbing nutrients. Peripheral Parenteral Nutrition (PPN) is a mean of nutrition support in which the parenteral solution is directly administered into the peripheral vein. PPN is indicated for anticipated short-term use(less than 10 days) because PPN usually does not meet all the nutritional needs of the patients. Central venous access is used when higher concentration solution to be administered and for longer period (Srilakshmi, 2014).

2.5.1 Total parenteral nutrition

Total Parenteral Nutrition is defined as provision of all nutrients essential for normal homeostasis and growth in the required amounts through parenteral route, that is, directly into a vein (Srilakshmi, 2014). So, TPN has been used when patients are unable to feed normally. However, it is essential to know whether its use would benefit the patient, because constant bowel rest can impair its function and integrity. TPN is always indicated in following cases (Reghim and Zeitoun, 2012):

- Malnutrition indicated by a loss of body mass index greater than 15%.
- Interference diseases based on ingestion, digestion and absorption of foods.
- Hypermetabolic conditions including large burns, septic patients, extensive multiple trauma, acute pancreatitis and intestinal fistula.

An outline of basic TPN solution is given below (McCarthy, 1991):

- **Fluid requirements**: 100 mL/kg body weight for the first 10 kg, 50 mL/kg for next 10 kg and 20 mL/kg for each additional kg of body weight. Compensations should be made for additional losses e.g., from a fistula.

- **Calories**: Glucose is the major carbohydrate which supplies calories, and this is administered in the form of 25% or 50% solution. Total energy requirement may vary considerably between 2000 to 4500 or more calories daily.

- **Fats**: In order to avoid essential fatty acid deficiency at least 4% of calories should be supplied as fats.

- **Proteins**:Protein requirement varies from 1.5 to 2.5 g/kg of body weight per day. The ratio of nitrogen to calories should be 1: 100-150. Branched-chain amino acids have been recommended as an integral part of TPN. However, their benefits have so far not been conclusively proved.

- **Electrolytes**: Daily maintenance requirements of sodium are 1-1.5 mEq/kg; potassium 1 mEq/kg; chloride 1.5-2 mEq/kg; calcium 0.2 mEq/kg and magnesium 0.35 - 0.45 mEq/kg.

- **Micronutrients**: Trace elements are an important component of TPN. Zinc 5 mg, copper 1 mg, chromium 10 mcg, manganese 0.5 mg and iron 1-2 mg are required daily.

- **Vitamins**: Vit K-1 10 mg and folic acid 5 mg should be administered intramuscularly once a week. Vit B-12 1 mg is given once a month. Water soluble vitamins should be given daily.

2.6 Principles of metabolic and nutritional care in surgery patients

In order to make proper plans for the nutritional support of patients undergoing surgery, it is essential to understand the basic changes in metabolism that occur as a result of injury, and that a compromised nutritional status is a risk factor for postoperative complications. Starvation during metabolic stress from any type of injury differs from fasting under physiological conditions (Soeters *et al.*, 2016). Surgery itself leads to inflammation corresponding with the extent of the surgical trauma, and leads to a metabolic stress response. To achieve appropriate healing and functional recovery ("restitutio ad integrum") a metabolic response is necessary, but this requires nutritional therapy especially when the patient is malnourished and the stress/inflammatory response is prolonged. The negative effect of long term caloric and protein deficits on outcome for critically ill surgical patients has been shown again recently (Yeh *et al.*, 2016). The success of surgery does not depend exclusively on technical surgical skills, but also on metabolic interventional therapy, taking into account the ability of the patient to carry a metabolic load and to provide appropriate nutritional support. In patients with cancer, management during the perioperative period may be crucial for long-term outcome (Gustafsson *et al.*, 2016; Horowitz *et al.*, 2015).

Patients undergoing surgery may suffer from chronic low-grade inflammation as in cancer, diabetes, renal and hepatic failure. Other non-nutritional metabolic factors interfering with an adequate immune response have to be taken into account and, whenever possible, corrected or ameliorated before surgery. These are diminished cardio-respiratory organ function, anaemia, acute and chronic intoxications (e.g. alcohol, recreational drugs), medical treatment with anti-inflammatory and cytotoxic drugs (Schols and Soeters, 2009)

In order to optimize the mildly malnourished patient short-term (7-10 days) nutritional conditioning has to be considered. In severely malnourished patients longer periods of nutritional conditioning are necessary and this should be combined with resistance exercise. In the truly infected patient immediately dealing with the focus of sepsis (“source control”) should have priority and no major surgery should be performed (risky anastomoses, extensive dissections etc.). Definitive surgery should be performed at a later stage when sepsis has been treated adequately (Bakker *et al.*, 2015).

In elective surgery it has been shown that measures to reduce the stress of surgery can minimize catabolism and support anabolism throughout surgical treatment and allow patients to recover substantially better and faster, even after major surgical operations. Such programmes for Fast Track surgery (Kehlet, 1997) later developed into Enhanced Recovery after Surgery (ERAS). A series of components that combine to minimize stress and to facilitate the return of function have been described: these include preoperative preparation and medication, fluid balance, anesthesia and postoperative analgesia, pre- and postoperative nutrition, and mobilization (Bakker *et al.*, 2015; Ljungqvist, 2014).

The ERAS programmes have now become a standard in perioperative management that has been adopted in many countries across several surgical specialties. They were developed in colonic operations (Gustafsson *et al.*; Lassen *et al.*, 2009; Varadhan *et al.*, 2010) and are now being applied to all major operations. ERAS programmes have been also successful in promoting rapid “functional” recovery after gastrectomy (Mortensen *et al.*, 2014), pancreatic resections (Balzano *et al.*, 2008; Braga *et al.*, 2014), , pelvic surgery (Nygren *et al.*, 2012; Patel *et al.*, 2014), hysterectomy (Wijk *et al.*, 2014), gynaecologic oncology (Nelson *et al.*, 2016). In times of limitations in health care economy ERAS is also a reasonable contribution for the purpose of saving resources (Bond *et al.*, 2016). ERAS protocols have been also shown to be safe and beneficial in the elderly (Slieker *et al.*, 2017). . High adherence to ERAS protocols may be associated with improved 5-year cancer specific survival after major colorectal surgery (Gustafsson *et al.*, 2016).

As a key component of ERAS, nutritional management is an inter-professional challenge. The ERAS programmes also include a metabolic strategy to reduce perioperative stress and improve outcomes (Ljungqvist, 2014). While early oral feeding is the preferred mode of nutrition, avoidance of any nutritional therapy bears the risk of underfeeding during the postoperative course after major surgery. Keeping in mind that the nutritional status is a risk factor for postoperative complications, this is especially relevant for patients at nutritional risk and those undergoing upper gastrointestinal (GI) surgery. For this reason, ERAS guidelines recommend liberal subscription of oral supplements pre- and postoperatively (Weimann *et al.*, 2017). Equally ERAS protocols support early oral intake for the return of gut function.

- integration of nutrition into the overall management of the patient
- avoidance of long periods of preoperative fasting
- re-establishment of oral feeding as early as possible after surgery
- start of nutritional therapy early, as soon as a nutritional risk becomes apparent
- metabolic control e.g. of blood glucose
- reduction of factors which exacerbate stress-related catabolism or impair gastrointestinal function
- minimize time on paralytic agents for ventilator management in the postoperative period
- early mobilisation to facilitate protein synthesis and muscle function.

2.7 Undernutrition associated with poor clinical outcomes

There is a clear relationship between inadequate food intake and poor outcomes for surgical patients. In 1932, Cuthbertson (Cuthbertson, 1932) drew attention to the link between wound healing and negative nitrogen balance of patients after trauma. At about the same time, Studley (Studley, 1936) noted that patients with chronic peptic ulcers who had lost > 20% of their body weight had an almost 10 times greater risk of dying after operations than other patients. Since then, a number of studies have documented the link between undernutrition and poor outcomes after operation (DeWys *et al.*, 1980; Larsson *et al.*, 1994; Sungurtekin *et al.*, 2004; Windsor and Hill, 1988)

No single nutritional marker is of consistent value in nutritional assessment of critically ill patients (Manning and Shenkin, 1995). This had led to use of combinations of indicators from several categories to measure nutritional status (Klein *et al.*, 1997). Buzby and colleagues developed a multiparameter index of nutritional status (the Prognostic Nutritional Index) based on the assessment of 161 patients undergoing elective operations. They then applied it prospectively on another group of patients and concluded that "This index provided an accurate, quantitative estimate of operative risk, permitting rational selection of patients to receive preoperative nutritional support" (Buzby *et al.*, 1980). Other combined measures, such as the Index of Undernutrition (Hall, 1990) and the Mini Nutrition Assessment (Thorsdottir *et al.*, 2005), have produced similar results. Attempts have been made to simplify the measurements. In 1987, Detsky and colleagues (Detsky *et al.*, 1987) 2 introduced the concept of subjective global assessment of nutritional status. A recent study supported the use of unintentional weight loss of > 10% and subjective global assessment as being appropriate measures to identify undernourished patients at risk (Mourao *et al.*, 2004).

Hypoalbuminemia is often associated with poor outcomes. When Reinhardt and colleagues (Reinhardt *et al.*, 1980) evaluated 509 patients admitted to a Veterans Affairs Hospital, they found that 25% of the patients with a serum albumin concentration < 34 g/L died, which compared with 2% for the normal albuminemic patients. The National Veterans Affairs Surgical Risk Study found that the preoperative serum albumin concentration is a predictor of operative mortality and morbidity (Gibbs *et al.*, 1999). In acutely ill surgical patients, it is difficult to attribute this risk solely to undernutrition. Serum albumin concentrations are influenced by fluid redistributions during critical illness and the acute phase response (Franch-Arcas, 2001). Hence, short-term fluctuations in the concentration of serum albumin, which has a half-life of 28 days, cannot be solely ascribed to nutritional deprivation (Klein, 1990).

2.8 Biology of undernutrition in surgical patients

Starvation is a component of undernutrition that is observed in surgical patients. This is particularly so for patients with obstructing oropharyngeal and esophageal cancers (Huckleberry, 2004; Piquet *et al.*, 2002) and elderly patients with a fractured neck of femur

(Bastow *et al.*, 1983; Delmi *et al.*, 1990). Many surgical patients have cancer. Cancer cachexia is a syndrome characterized by a marked weight loss, anorexia, asthenia, and anemia. It involves loss of weight, mainly in skeletal muscle and adipose tissue, which is not caused simply by anorexia. Palesty and Dudrick (Palesty and Dudrick, 2003) have estimated that cachexia eventually leads to about 30% of cancer-related deaths overall, 30% to 50% of deaths in patients with gastrointestinal tract cancers, and up to 80% of deaths in patients with advanced pancreatic cancer. The same authors commented that: "Despite trials of conventional and/or aggressive nutritional support by a myriad of feeding techniques, patients with cancer cachexia have failed to gain consistent significant benefits in terms of weight gain, functional ability, quality of life, or survival (John, 2006).

One explanation for such findings might be the negative effects of cytokines and other biologic modifiers. For example, atrophy of skeletal muscle is common to cancer, sepsis, trauma, and burns. It is characterized by depression of protein synthesis in skeletal muscle, which is accompanied by increased protein degradation through the ubiquitin-proteasome proteolytic pathway (John, 2006). In a article by Tisdale noted that this pathway is not responsive to simple nutritional intervention, but rather, it is influenced by agents such as glucocorticoids, cytokines, proteolysis-inducing factor, and oxidative stress (Tisdale, 2005).

2.9 Nutritional assessment of post-operative patients

It is well known that malnourished surgical patients have a higher risk of developing postoperative complications (e.g. infections, delayed wound healing, suture dehiscence, fistula formation, pulmonary embolism and respiratory insufficiency) ; they also have a higher mortality rate. To identify patients at high surgical risk, a number of nutritional indicators were proposed. These reflect functional changes related to malnutrition, as well as their recovery under nutritional management. In addition, several prognostic indices and methods were proposed to predict surgical risk. Although these methods were correlated with post-operative morbidity, it has been reported that clinical evaluation on its own provided just as good prognostic information in the surgical patient. Others, however, suggested that weight loss alone can be used as a rapid and inexpensive method for identifying patients at a higher risk of developing post-operative complications (Ardawi *et al.*, 1991).

Some methods used to access nutritional status of post-operative are:

2.9.1 Subjective global assessment

Subjective Global Assessment (SGA) is a method for evaluating nutritional status based on a practitioner's clinical judgment rather than objective, quantitative measurements. Encompassing historical, symptomatic, and physical parameters, SGA aims to identify an individual's initial nutrition state and consider the interplay of factors influencing the progression or regression of nutrition abnormalities. SGA has been widely used for more than 25 years to assess the nutritional status of adults in clinical settings. Guidelines are given for performing and interpreting physical examinations that look for evidence of loss of subcutaneous fat, muscle wasting, and/or edema in children of different ages. Age related questionnaires are offered to guide history taking and the rating of growth, weight changes, dietary intake, gastrointestinal symptoms, functional capacity, and any metabolic stress. Finally, the associated rating form is provided, along with direction for how to consider all components of a physical exam and history in the context of each other, to assign an overall rating of normal/well nourished, moderate malnutrition, or severe malnutrition (Secker and Jeejeebhoy, 2012).

Subjective nutritional evaluation of the patients was carried out using the Subjective Global Assessment, recommended by Detsky and coworkers (Detsky *et al.*, 1987). Subjective Global Assessment is a validated nutritional assessment tool, considered as a gold standard method, using a range of medical factors including body weight, body weight change, dietary intake and change, gastrointestinal symptoms and functionality; along with a physical examination of sites related to subcutaneous fat and muscle mass. This method was performed by an experienced dietician previously trained in the technique (Detsky *et al.*, 1987). The patients are classified as:

A: Well nourished

B: Moderately or possibly malnourished

C: Severely malnourished

2.9.2 Anthropometric measures

Height was measured with a portable stadiometer. When height was not possible to measure, stature was calculated from knee height or arm span and estimated by validated equations. Body weight was measured with digital scales determining body weight in kilograms to the nearest 0.1 kg. Patients should be in light day clothing. Height and weight were used to determine body mass index (BMI) (Buzby *et al.*, 1988).

BMI was obtained by dividing body weight by the square of the height and classified for adults: underweight if BMI ≤18.4 kg/m^2; normal weight, if 18.5 ≤BMI ≤24.9 kg/m^2; pre-obese, if 25.0 ≤BMI ≤29.9 kg/m^2 and obese if BMI ≥30.0 kg/m^2.17 For the elderly (≥60 years of age), BMI was classified: underweight if BMI ≤22 kg/m^2; normal weight if 22 <BMI <27 kg/ m^2; and overweight if BMI ≥27 kg/m^2 (WHO).

Subjects were asked about their history of weight change in the past six months. Percentage of weight loss over the six months before hospital admission was estimated by the equation: usual weight (kg) – actual weight (kg) x 100/usual weight (kg). Unintentional body weight loss of more than 10% was considered as evidence of malnutrition.

Mid-arm Circumference (cm) was measured with an inelastic tape, midway between the tip of the acromion and olecranon process, with the right arm hanging relaxed at the subject's side. Triceps skinfold thickness (mm) was measured in a vertical fold midway at the same level as the Mid upper arm circumference, on the posterior aspect of the arm, with the right arm held vertically, with skinfold caliper, which applies 10 g/mm2 pressure to the applied surface. These values were used to calculate Mid-arm Muscle Mass(cm)=Mid-arm Circumference (Triceps Skinfold Thickness (mm)X0.314). Values were compared with normal values for Triceps Skinfold Thickness and Mid-arm circumference measurements standardized for sex and age and percentage of adequacy was calculated, considering well-nourished >90%, mild malnourished 80 - 90%, moderately malnourished 70 - 80% and <70% severely malnourished (Thieme *et al.*, 2013).

2.9.3 Laboratory parameters

A fasting blood sample was obtained to measure complete blood count, serum albumin, and lymphocyte count. Total Lymphocyte Count was calculated from the total, and the differential white count was obtained by an automated analyzer. To serum albumin, normal values were considered >3.5 g/dL and depletion was considered mild between 3-3.5g/dL, moderate 2.4-2.9g/dL, and severe <2.4g/ dL. Total Lymphocyte Count was considered adequate when >2000mm3, and mild depletion between 1200-2000mm^3, moderate depletion between 800-1199mm^3, and severe depletion <800mm^3 (Blackburn, 2011; Lohsiriwat *et al.*, 2008).

2.9.4 Nutritional risk index

It was calculated by a simple equation that uses serum albumin and recent body weight loss.

NRI= (1.519 X serum albumin, g/L) + 0.417 X (present weight/usual weight x 100).

Nutritional Risk Index>100 indicates that the patient is not malnourished,

97.5–100 indicates mild malnourishment,

83.5-<97.5 indicates moderate malnourishment and

<83.5 indicates severe malnourishment (Thieme *et al.*, 2013).

2.10 Nutritional requirements of Post-operative patients

2.10.1 Energy

Without sufficient calorie intake, tissue proteins cannot be synthesized. Excess metabolism of body fat may leads to acidosis, whereas depletion of the glucose may increase the likelihood of damage to the liver (Srilakshmi, 2014). Daily calorie required in post-operative patient is 35-40kcal/kg/day (Weimann *et al.*, 2017).

i.e. Energy Requirement = (35-40) × IBW

Obesity delays healing, whenever possible, it should be corrected. Rapid weight loss results in loss of lean body mass and it should be avoided (Srilakshmi, 2014).

2.10.2 Protein

A satisfactory state of protein nutrition ensures:

- Rapid wound healing.
- Increases the resistance to infection.
- Exerts a protective action upon the liver against the toxic effects of anesthesia and
- Reduces the possibility of oedema at the sites of the wound.

When protein is depleted in post-operative condition complications are increased. Protein catabolism is increased for several days immediately following surgery or injury, patients undergo negative nitrogen balance even though the protein intake may be appreciable. The degree of negative balance can be reduced through higher intakes of protein and calories (Srilakshmi, 2014).

Postoperative protein requirements typically range between 1.2 and 2 g/kg, with the lower end appropriate for patients after uncomplicated elective surgery and the higher end recommended after major surgery. Severe renal or hepatic dysfunction may require short-term protein restriction below this range, whereas large open wounds or burns can increase protein needs to >2 g/kg (Huckleberry, 2004).

2.10.3 Fats

Fats are typically provided as 15-30% of non-protein calories. Lipids provide the essential long-chain fatty acids, linoleic acid, and α-linolenic acid. A minimum of 1-2% of calories as linoleic acid is recommended to prevent essential fatty acid deficiency. A trend toward lower postsurgical infection rates and a significantly shorter hospital stay for patients fed omega-3 fatty acids (Huckleberry, 2004).

2.10.4 Minerals

Phosphorus and potassium are lost in proportion to the breakdown of body tissue. In addition, derangements of sodium and chloride metabolism may occur subsequent to vomiting, diarrhea, perspiration, drain of fluids, anorexia and dieresis or renal failure. Iron-deficiency anemia occurs in association with malabsorption or excessive blood loss. Diet alone is ineffective in correction of anemia, but liberal intake of protein and ascorbic acid, together with administration of iron salt is of value in convalescence. Transfusion are usually required to overcome severe reduction in haemoglobin level (Srilakshmi, 2014).

2.10.5 Vitamins

Ascorbic acid is especially important for wound healing and should be provided in increased amounts prior to and following the surgery. Vitamin K is of concern to the surgeon since there is evidence of failure to synthesise vitamin K in the small intestine since the inability to absorb and /or the defect in conversion to prothrombin which is likely to result in bleeding. Haemorrhage is especially likely to occur in patients who have diseases of the liver (Srilakshmi, 2014).

2.10.6 Fluids

The fluid balance may be upset prior to and following surgery owing to failure to ingest normal quantities of fluids and to increased loss from vomiting, exudates, haemorrhage, dieresis and fever. A patient should not be operated in a state of dehydration since the subsequent dangers of acidosis are great. When dehydration exists prior to operation, parenteral fluids are administered, if the patient is unable to ingest sufficient liquid by mouth (Srilakshmi, 2014).

2.11 Nutritional requirements of normal patients

For the normal patients a diet which meets RDA of a reference man can be provided. The RDA by ICMR (2010) shows following nutrients requirements (Joshi and Sharma, 2014)

Table 2.1: RDA of a reference man

Nutrients	RDA
Energy (Kcal/day)	2425
Protein (g/day)	60 (10%)
Fat (g/day)	20 (8-10%)
Carbohydrate (g/day)	485 (80%)
Fiber (g/day)	-
β Carotene (μg/day)	2400
Iron (mg/day)	28
Folic Acid(μg/day)	100
Sodium (mg/day)	500
Calcium (mg/day)	400
Ascorbic Acid(mg/day)	40

Source: ICMR (2010)

2.12 Raw materials used in enteral feed formula and their nutritive value

2.12.1 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).

2.12.1.1 Pearl millet

Pearl millet (*Pennisetum typhoides*), a hardy cereal crop compared with wheat and rice, is grown in regions with relatively low rainfall owing to its ability to tolerate and survive under continuous or intermit- tent drought. The principal homes of this crop are Ethiopia, Malawi, Sudan, Zimbabwe, Kenya, Tanzania, Uganda, Zambia, Somalia, Botswana, India and Pakistan. It is a short duration crop (88 to 96 days) and suits many crop rotation programme (Bal and Jain, 1997).

Pearl millet had higher protein (14.0%), fat (5.7%), fiber (2.0%), and ash (2.1%) content (Sade, 2009) when compared to the major cultivated cereal crops such as wheat, rice, sorghum. Superior protein quality in term of its tryptophan and threonine content (Elyas *et al.*, 2002) along with higher content of calcium, iron as well as zinc (Sade, 2009; Yadav *et al.*, 2014) makes this crop very useful for human. Energy content of pearl millet is greater than sorghum and equivalent to brown rice due to its rich unsaturated fatty acids (75%) and linoleic acid (46.3%) contents (Jaybhaye *et al.*, 2014).

Pearl millet is very nutritious food, but contains certain anti-nutritional factors (phytate, tannins and polyphenols) (Ranasalva and Visvanathan, 2014). Presence of these factors leads to chelation of dietary minerals in the gastrointestinal tract, thereby reducing their bioaccessibility and bioavailability (Nour *et al.*, 2014). So in order to reduce this factors blanching is done (Rani *et al.*, 2017). Blanching slow down the enzymatic activity without having any significant effect on its nutritional composition. It is done by boiling water at 98 °C, followed by immersing of the grains in the boiling water (1:5 ratio of seeds to boiling water) for 30 sec and drying at 50 °C for 60 min (Rai *et al.*, 2008).Archana (1998) reported that pearl millet when blanched at 98 °C for 30 sec showed a significant reduction in polyphenols (from 764.45 to 544.45 mg/100 g) and phytic acid content (from 833.42 to 512.10 mg/100 g) which might be due to the leaching of polyphenols and phytate ions into the soaking medium under the effect of concentration gradient (Archana *et al.*, 1998).

2.12.1.2 Rice flakes

Rice flake is a processed form of cereal derived from paddy (*Oyza sativa*). Rice is a very widely used staple cereal and is eaten either directly after cooking in boiling water or after processing to various products such as rice flakes. In South East Asia, flaked rice is usually produced by roasting fully soaked paddy in hot fine sand and then flaking in a unit called an edge-runner flaker or in a heavy duty cereal roller-flaker. Rice flakes contains 90% carbohydrate, 7% protein and 3% fat (Mujoo *et al.*, 1998).

2.12.1.3 Semolina

Semolina is processed from durum wheat (*Triticum turgidumspp.durum*) (Vinci *et al.*, 2013). Wheat (*Triticumspp*.) is a cereal grain (botanically, a type of fruit called a caryopsis) originally from the Levant region of the near east but now cultivated worldwide. Globally, wheat is the leading source of vegetal protein in human food, having higher protein content than the other major cereals maize and rice (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 proteins are deficient in the essential amino acid, lysine and contain adequate amounts of the other essential amino acids, at least for adults. Because the proteins present in the wheat endosperm (gluten proteins) are particularly poor in lysine, white flours are more deficient in lysine compared with whole grains. Supplementation with proteins from other food sources (mainly legumes) is commonly used to compensate for this deficiency, science the limitation of a single essential amino acid causes the others to break down and become excreted, which is especially important during the period of growth. Further, wheat is a major source for natural and bio-fortified nutrient supplementation, including dietary fiber, protein and dietary minerals (Suhasini and Malleshi, 2003).

2.12.2 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. Like other plant-based foods, pulses contain no cholesterol and little fat or sodium. Legumes are also an excellent source of resistant starch which is broken down by bacteria in the large intestine to produce short-chain fatty acids (such as butyrate) used by intestinal cells for food energy. Preliminary studies in humans include the potential for regular consumption of legumes in a vegetarian diet to affect metabolic syndrome. There is evidence that a portion of pulses (roughly one cup daily) in a diet may help lower blood pressure and reduce LDL cholesterol levels, though there is a concern about the quality of the supporting data (Niraula, 2017).

2.12.2.1 Green gram

Green gram (*Vigna radiata*) is an important legume widely used in Nepal and India. It has a protein content comparable to that of chick pea (*Cicer arietinum*) but contains less anti-nutritional (Chitra *et al.*, 1995). It is rich in micronutrients and can be used to deliver minerals to malnourished populations if processed well to retain them in the diet. Green gram varieties are grown in wide agro-climatic zones and have diverse agronomical, processing and nutritional characteristics (Bisht *et al.*, 2005).

According to DFTQC 2012, green gram consist of 24.5% of protein, 1.2% of fat and 59.9% of carbohydrates and 3.5 % of minerals (DFTQC., 2012).Sprouted green gram which is good source of protein, iron, calcium and also has lots of medicinal values. Sprouted green gram incorporated food product have high biological values, calorie dense, low dietary bulk and cost effective in reducing the nutritional problem (Bisht *et al.*, 2005).

2.12.2.2 Soybean

Soybean is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. 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. 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 source of plant protein (WHO, 1998).

2.12.3 Oil and oil seeds

2.12.3.1 Sesame seed

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 B1, 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).

2.12.3.2 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 frying oil. Sunflower oil is a monounsaturated (MUFA)/polyunsaturated (PUFA) mixture of mostly oleic acid (omega9)-linoleic acid (omega-6) group of oils. The oil contains appreciable quantities of vitamin E, sterols, squalene, and other aliphatic hydrocarbons (Swaminathan, 2004).

2.12.4 Fruits

2.12.4.1 Banana

A banana (*Musa paradisiaca)*is an edible fruit, botanically a bery produced by several kinds of large herbaceous flowering plant in the genus Musa. Bananas are a healthy source of fiber, potassium, vitamin B6, vitamin C, and various antioxidants and phytonutrients. One medium-sized banana contains about 105 calories, most of which come from carbs (Arnarson, 2014).

Table 2.2 The nutrients in 100 gm bananas (Arnarson, 2014)

Nutrients	**Amounts**
Calories	89 kcal
Water	75 %
Protein	1.1 gm
Carbohydrate	22.8 gm
Fibre	2.6 gm
Fat	0.3 gm
Sugar	12.2 gm

2.12.5 Vegetables

2.12.5.1 Carrot

The carrot (*Daucus carota* subsp. *sativus*) is a root vegetable, usually orange in color. Carrot is the most widely grown member of the *Apiaceae* or *Umbelliferae*. This diverse and complex plant family includes several other vegetables, such as parsnip, fennel, celery, root parsley, celeriac, arracacha, and many herbs and spices. Carrots are a particularly good source of beta-carotene, fiber, vitamin K, potassium and antioxidants (Karki *et al.*, 2012).The water content can vary from around 86-95%, and the edible portion consists of around 10% carbohydrates. Carrots contain very little fat and protein (Bjarnadottir, 2015).

Table 2.3 Nutritional fact of 100 gm carrot (Bjarnadottir, 2015)

Nutrients	Amounts
Calories	41 kcal
Water	88%
Protein	0.9 gm
Carbohydrate	9.6 gm
Fibre	2.8 gm
Fat	0.2 gm
Sugar	4.7 gm

2.12.5.2 Spinach

Spinach (*Spinacia oleracea L*) is the most nutritious vegetable and is very versatile since it is commonly used as a salad, a cooked vegetable or as a component of many other cooked meat and vegetable dishes (Prohens and Nuez, 2008). Eating spinach may benefit eye health, reduce oxidative stress, help prevent cancer and reduce blood pressure levels.By weight, spinach consists of 91.4% water, 3.6% carbohydrate and 2.9%protein (Gunnars, 2015).

Table 2.4 The nutrients in spinach of 100 gm (Gunnars, 2015)

Nutrients	Amounts
Calories	23 kcal
Water	91%
Protein	2.9 gm
Carbohydrate	3.6 gm
Fibre	2.2 gm
Fat	0.4gm
Sugar	0.4 gm

`2.12.6 Protinex

Protinex is a protein supplement fortified with vitamins and minerals. It contains ingredients such as skim milk powder, soya protein isolate, sucrose, wheat flour, corn flour, malt extract, vitamins such as ascorbic acid, nicotinamide, folic acid, biotin, etc) and minerals (ferric pyrophosphate, zinc sulphate, potassium iodate) etc (Protinex label).

Table 2.5 Nutritional labeling information of protinex per 100 gm

Nutrients	Amounts
Calories	367 kcal
Protein	32 gm
Carbohydrate	57.5 gm
Sugar	31.9 gm
Fat	1 gm
Iron	20 mg
Calcium	1300 mg

2.13 Technology of enteral formula feed preparation

Traditional treatments such as soaking, cooking, germinating have been used to improve nutritional quality of the cereals and legumes. Processing of food such as soaking, germination and fermentation leads to reduction in phytic acid and increases of the mineral solubility in foods and also improves the bioavailability of the minerals in cereals and legumes. Processing technique reduces the level of anti-nutritional organic factors, which including phytates, phenols, tannins and enzyme inhibitors by releasing exogenous and endogenous enzymes such as phytase enzyme formed during processing (Tarek, 2002).

2.13.1 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. All anti-nutritional factors such as phytic acid, tannin, trypsin inhibitor and hemagglutinin activity decreases 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 grain 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).

2.13.2 Germination

Germination or sprouting of legumes and cereals increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytase 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 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 activity of hydrolytic enzymes, improvements in the content of total proteins, fat and certain essential amino acids, total sugars, B- group vitamins and a decrease in dry matter, starch and anti- nutrients. The increased content of protein, fat, fiber and total ash are only apparent and attributable to the disappearance of starch. However, improvement in amino acid composition, B- group vitamins, sugars, protein and starch digestibility's, and decrease in phytates and protease inhibitors are the metabolic effects of the sprouting process (Chavan *et al.*, 1989).

2.13.3 Drying

Drying produce a friable, readily milled stable product that may be store for long periods, and from which roots may easily be removed. In drying green malt, the removal of moisture at low temperature allows the maximum survival of enzymes and the least development of aroma and color. Diastasic 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).

2.13.4 Roasting

Roasting is a cooking method that uses dry heat where hot air envelops the food, cooking it evenly on all sides with temperature 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 food stuffs 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. 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).

2.13.5 Milling and sieving

The outer bran in coarse grains are 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).

2.13.6 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).

2.14 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).

2.14.1 Food packaging materials

Food packaging is used to protect food from external environment from spoilage. A package provides protection, tempering resistance, and special physical, chemical or biological needs. It may bear a nutrition facts label and other information about food being offered for sale (Kumar and Singh, 2013).

2.14.1.1 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. According to (Marsh and Bugusu, 2007) main advantages of HDPE are:

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

2.14.1.2 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 is 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 causes fats to oxidize or become rancid), odors, flavor, moistness and germs, 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 a very thin sheet, 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 thickness, with thinner foils used to wrap foods 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 can't be made from recycled aluminum without pinhole formation in 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 food stuff 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).

2.14.1.3 Glass bottles

Glass bottles can vary in size considerably, but are most commonly found in sizes ranging between about 10 ml 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 nontoxic 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).

2.15 The microbiological quality of food

Food-borne Spoilers specifically addresses the role of spoilers in food technology and how they affect the quality of food. Food spoilers represent a great challenge in food quality, determining the shelf-life of many products as they impact consumer acceptability of taste, texture, aroma, and other perceptions. Spoilage microorganisms, including pseudomonas, yeasts, and molds and spore formers, as well as less-common spoilers, including lactic acid bacteria are responsible for the deterioration of food product.

Bacterial colonisation, infection, and septicaemia with organisms such as Enterobacter cloacae and Kiebsiella spp have been reported in patients receiving enteral feeds contaminated during handling. The practice of modifying an enteral feed increases handling and therefore increases the risk of microbial contamination; this may be more common in patients fed at home. To date no studies have compared the contamination of a modified feed with a ready to use feed in hospital and at home. In 1988 a joint working party of the parenteral and enteral nutrition group and the paediatric group of the British Dietetic Association published guidelines for the desired nutritional analysis of a sterile, ready to use enteral feed for children aged between 1 and 5 years (Patchell *et al.*, 1994).

Part III

Materials and Methods

3.1 Materials

3.1.1 Semolina

Semolina manufactured by Laxmi Food Packaging, Khanar, Sunsari available in Dharan was used. It is locally known as '*Suji*'.

3.1.2 Rice flakes

Rice flake was collected from Dharan market and is locally known as '*chiura'*.

3.1.2 Pearl millet

Pearl millet was collected from Dharan. Its scientific name is *Pennisetum typhoides* and locally known as '*junelo'*.

3.1.3 Green gram

Green gram was used by collecting from market of Dharan. Its scientific name is *Vigna radiata* and locally known as '*moong* dal'.

3.1.4 Soybean

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

3.1.5 Banana

Banana was collected from market of Dharan. Its scientific name *Musaparadisiacaand locally known as 'Kera'*. The variety of banana used was cavendish banana.

3.1.6 Carrot

Carrot used was collected from vegetable vendors of Dharan. Its scientific name is*Daucus carota*and locally known as '*gajaar'*.

3.1.7 Spinach

Spinach was bought from vegetable vendors of Dharan. Its scientific name is *Spinacia oleracea* and locally called as '*palungo ko saag'*.

3.1.8 Protinex

Protinex manufactured by Nutricia International Pvt. Ltd., India available at Dharan market was used.

3.1.9 Sunflower Oil

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

3.1.10 Butter

Butter manufactured from Amul Dairy, India available at Dharan market was used.

3.1.11 Sesame seed

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

3.1.12 Sugar

White sugar available in Dharan market was used.

3.1.13 Salt

Salt manufactured from Salt Trading Corporation Ltd. available at Dharan market was used.

3.1.14 Equipments

- Electronic balance
- Kjeldahl digestion and distillation set
- Soxhlet assembly
- Buchner filter assembly
- Suction pump
- Muffle furnace
- Spectrophotometer

- Flame photometer
- Drying oven

3.1.15 Chemicals

- Petroleum ether
- Acetone
- Phenopthalein indicator
- Methyl orange indicator
- Bromocresol green
- Boric acid
- Digestion mixture (catalyst mixture)
- Methyl red indicator
- Potassium permanganate
- Potassium thiocyanide
- Saturated. Potassium. Persulfate
- Reagent grade ferrous ammonium. Sulfate. $6H_2O$
- Analytical grade Potassium. Chloride
- Conc. Hydrochloric Acid (HCL)
- Conc. Sulphuric Acid (H_2SO_4)
- Conc. Ammonia, Acetic acid
- Silver Nitrate (AgNO3)
- TBHQ (Tertiary Butylated Hydroxy quinine)
- DPPH (2, 2-Diphenyl-1-picril hydrazyl radical)

3.1.16 Glasswares

- Petri dish
- Silica crucible
- Conical flask
- Glass rod
- Burette
- Pipette
- Test tubes
- Volumetric flask
- Digestion flask

- photometer,
- Dessicator
- Hotair oven

3.2 Methods

3.2.1 Processing of raw materials

3.2.1.1 Semolina

It was cleaned and seived. After that roasting for some time so that the white color of semolina just start converting to brown. The roasted product was cooled for some times and it is soaked in warm water for about an hour. The soaked semolina should be dried in a cabinet dryer at 55°C for 2 hrs until the moisture was reduced to 5%. The dried product should be grinded, sieved and packed in air tight plastic bag (Joshi and Sharma, 2014) .

3.2.1.2 Rice flakes

Rice flake was cleaned to remove impurities such as stones, other grains, broken kernels, etc. The cleaned product was roasted for 2-3 minutes at temperature about 70°C to change the white color of rice flakes to brown. The roasted rice was cooled at room temperature (about 22 °C) and is grinded, sieved and packed in air tight plastic bag (Joshi and Sharma, 2014).

3.2.1.3 Pearl millet

At first the pearl millet is cleaned and blanched in water at 98 °C for 30sec.Then the product is oven dried at 50°C for 60 min. After drying, it is popped up which is then cooled at room temperature. After popping, it is grinded, sieved and packed in air tight plastic bag (Joshi and Sharma, 2014) .

3.2.1.4 Green 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%. The product was grinded, seived and stored in air tight bag (Joshi and Sharma, 2014).

3.2.1.5 Soybean

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 (Niraula, 2017).

3.2.1.6 Sesame seed

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 Banana

Banana was cleaned, sorted, peeled, sliced and then oven dried for 4-5 hrs at 60°C. It is then grinded, sieved and stored in air tight plastic bag (Joshi and Sharma, 2014).

3.2.1.8 Carrot

Carrot was cleaned, sorted and washed under the tap. It is than peeled and blanching was carried out at 80±2°C for 3 minutes. Shredding was carried out and oven drying of the product at 60±2°C for 5 hours was done(Sharma *et al.*, 2012). Then, grinding and seiving of the product was done and was packaged in air tight plastic bag (Joshi and Sharma, 2014).

3.2.1.9 Spinach

Spinach was cleaned, sorted and washed under tap. It is than blanched at 80±2°C for 3 minutes. Slurry formation and oven drying at 50°C for 3 hrs was done. Then, grinding and sieving of the product was done with packaging in air tight plastic bag (Joshi and Sharma, 2014).

3.2.1.10 Sugar

White sugar crystals were ground in grinder and then packed in airtight plastic bags (Niraula, 2017).

3.2.2 Development of RTR enteral formula feed

A Selection of the food ingredients

Fourteen ingredients from all the food groups were selected for developing the enteral formula feeds. The selection was based on the nutritional potential, adaptability to the processing, keeping quality and availability of the ingredient.

Table 3.1 Selected food ingredients to develop the feeds

Food groups	Ingredients
Cereals	Semolina, Rice flakes, Pearl millet.
Legumes and pulses	Whole green gram, Soybean
Fruits and Vegetables	Banana, Carrot, Spinach
Milk and milk products	Protinex (whey protein)
Nuts and oil seeds	Sesame seeds, Butter, Oil
Others	Sugar, Salts

B Formulation of the product

Ready to reconstitute enteral formula feeds were formulated to suffice the nutritional requirements of reference man. These feeds would provide complete nutrients for the patients with inability to swallow or any other disability which could lead to malfunctioning of buccal cavity. The major consideration while formulating the feeds was incorporation of food ingredients from all food groups in right proportion and amounts to reach the level of balanced diet. The amounts of ingredients were calculated on dry weight basis, for the formulation of enteral feeding formula. Cereals were taken as staple source of food. Legumes were taken as source of plant protein and protinex as a source of protein. Similarly, butter, sesame and sunflower oil as source of energy and to maintain essential fatty acids, fruits and vegetables were taken as a source of vitamins and minerals.

B I Amounts of ingredients

All the processed food ingredients were mixed in the accurate amounts and proportion to meet out the nutrient requirement of patients. On the basis of RDA, two basic formula feeds were developed. The standard formula (named Standard) was planned to provide the RDA of a person which requires all the nutrients in the proportion given under normal physiological condition. In the second protein formula (named Protein Plus) the ingredients were planned in such a way that provides protein content more than the standard formula. For developing both the feeds the RDA was modified according to the nutritional requirements of critical, bed ridden patients with long term need of enteral nutrition. Both of these feed provide same energy with normal protein content in normal feed and high protein content in protein feed. Table 3.2 shows the amount of ingredients in each product.

Table 3.2 Compositional details of developed formula feeds.

Ingredients	**Product A(Protein plus) (gm)**	**Product B(Standard) (gm)**
Semolina	80	110
Rice flakes	60	100
Pearl millet	25	60
Whole green gram	50	50
Soybean	60	40
Carrot	10	10
Spinach	10	10
Banana	10	10
Sesame seed	50	30
Protinex	135	50
Oil	10	10
Butter	4	4
Sugar	30	60
Salt	1	1
Total amounts	**535**	**545**

B II Basis of formulation

The preparation of standard post-operative diet was done on the basis of tube feeding composition of standard formula which contains following nutrients (Mills, 2004):

Protein content : 10-15%

Carbohydrate content: 50-60%

Fat content : 30%

On the basis of RDA (recommended dietary allowances) for adult male two basic RTR enteral feed were developed. All the processed food ingredients were mixed in the accurate amount and proportion to meet out the nutrient requirement of the patient. The standard formula (named Standard) was planned to provide the RDA of a person which requires all the nutrients in the proportion given under normal physiological condition as in table 3.1. In the second formula (named Protein Plus) the ingredients were planned in such a way that provides protein content more then the standard formula. For developing both the feeds the RDA was modified according to the nutritional requirements of critical, bed ridden patients with long term need of enteral nutrition.

Table 3.3 Modified RDA for the enteral feed

Nutrients	RDA(ICMR)	*Modified nutritional guidelines
Energy (Kcal/day)	2425	2000
Protein (g/day)	60 (10%)	75 and 100
Fat (g/day)	20 (8-10%)	33-66
Carbohydrate (g/day)	485 (80%)	320
Fiber (g/day)	-	8.5
β Carotene (µg/day)	2400	-
Iron (mg/day)	28	4.5-24
Folic Acid(µg/day)	100	-
Sodium (mg/day)	500	-
Calcium (mg/day)	400	Above 600
Ascorbic Acid(mg/day)	40	56-317

*Modifications in the RDA is on the basis of the literatures provided by Joshi and Sharma(2014), Srilakshmi(2014) and ICMR(2010)

Flow sheet for the processing of products

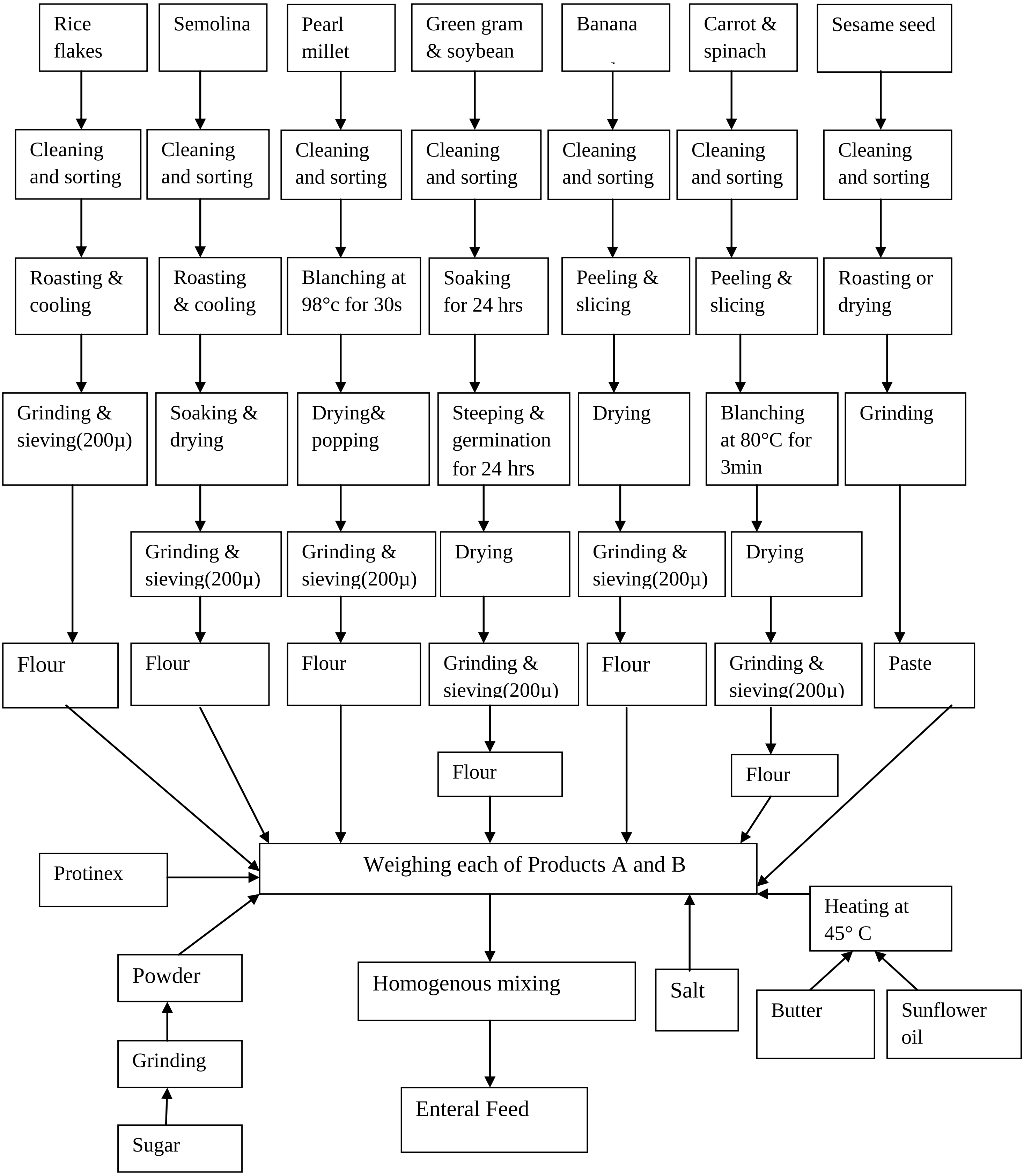

Fig 3.1 Outline for the preparation of Enteral Feed (Pilot plant scale)

3.2.3 Product preparation

The calculated amounts of ingredients for two different formulas (protein formula and standard formula) were calculated on dry basis. Flow chart diagram of different ingredients used for the preparation of enteral formula feed is shown in Fig 3.1

A Grinding and milling

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

B Sieving of the ground powder product

All the ground flour was sieved by using 200µ sieve available in the laboratory.

C Mixing

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

C 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.

3.2.4 Evaluation of prepared Enteral Feeds

A Physicochemical analysis of product

A I Moisture content

Moisture content was determined by using hot air oven (ambassador, working temperature 0 to 300^{o} C) as per Rangana, 2001.

A II Reconstitution behaviour

The developed enteral feeds were reconstituted by mixing the known quantity of dry powder in different volumes of warm water with constant stirring and then cooling it to acceptable temperature. Reconstitution quality was be judged on the basis of flow behavior through standard ryle’s tube of, 12 pt and 14 pt sizes. Reconstitution time and flow rate of the reconstituted sample was recorded.

A III Crude fat

Fat content was determined by using solvent extraction method using the petroleum ether as a solvent, according to Rangana, 2000.

A IV Crude protein

Crude protein of all product was determined by kjeldhal method following the procedure described by Rangana,2000. Percentage of crude protein was calculated as Crude protein = % nitrogen × Factor (F). Factor used were 6.25 for all products.

A V Crude fiber

Crude fiber was determined as per Rangana, 2001.

A VI Total ash

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

A VII Total carbohydrate

Total carbohydrate was determined by difference method i.e. total carbohydrate=100- (sum of the content of moisture, protein, fat, ash, and crude fiber), following the procedure of Rangana, 2000.

A VIII Calcium

The calcium content of the product was determined according to Titrimetric Method (NIN, 2003). Calcium is precipitated as oxalate when titrated with standard potassium permanganate solution. 2 ml of sample was taken into a 15ml centrifuge tube with 2ml of distilled water and 1ml of 4% ammonium oxalate solution and mixed them thoroughly and left the mixture as such overnight. The contents were mixed again and centrifuged for 5min at 1500 rpm. The supernatant liquid was poured off and the centrifuge tube was drained by inverting the tube for 5min on a rack (care should be taken not to disturb the precipitate). The mouth of the centrifuge tube was wiped with a piece of filter paper. The precipitate was stirred and the sides of the tubes were washed with 3ml of dilute ammonia. It was centrifuge again and drained as before. The precipitate was washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate. The precipitate was then dissolved in

2ml of 1 N H_2SO_4. The tube was heated in water bath for 1 min and titrated against 0.01N $KMnO_4$ solution to a definite pink color persisting for at least 1 min.

A IX Iron

Iron content was determined by using spectrophotometry as per Rangana, 2001.

A X Vitamin C

Vitamin C content in the product was determined according to Rangana, 2000 by the reduction of 2,6-dichlorophenol endol dye.

B 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

C 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).

C I Total plate count (TPC)

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

C II Yeast and mold

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

D Cost calculation

The cost of the two enteral formula feed from two different formulations were calculated including a profit of 10% (Appendix C).

Part IV

Results and discussion

Two enternal feeds were prepared by following the formulation procedure in material and methods. We labeled Sample A for the protein rich feed which was named Protein plus and Sample B for normal standard feed which was named Standard. After formulation we carried out the physiochemical analysis, reconstitution behavior analysis, microbiological assay and cost evaluation of the products.

4.1 Analysis of products

The Chemical analysis of the products A and B were carried out. The result is tabulated in table 4.2 and table 4.3

Table 4.1 Nutritional composition of the developed protein rich RTR enteral formula feed

Parameters	***Guidelines for Nutritional composition**	**Amount in product A(protein plus)**
Energy(kcal)	2000	2051
Protein (gm)	100	101.12 (1.23**)
Fat (gm)	33 gm-66	48.68 (0.73**)
Carbohydrate	320	302.10 (1.02**)
Crude fiber (gm)	8.5	15.38 (0.5**)
Total ash(%db)	-	1.6 (1.12**)
Moisture(%db)	-	7.1 (1.2**)
Calcium(mg)	Above 600	850 (1.8**)
Vitamin C(mg)	56-317	99 (0.85**)
Iron(mg)	4.5-24	42.23 (0.52**)

*Modifications in the RDA is on the basis of the literatures provided by Joshi and Sharma (2014), Srilakshmi (2014) and ICMR (2010)

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

Table 4.2 Nutritional composition of the developed standard RTR enteral formula feed

Parameters	***Guidelines for Nutritional composition**	**Amount in product B (Standard)**
Energy(kcal)	2000	2041
Protein (gm)	75	74.45 (1.43**)
Fat (gm)	33 -66	44.69 (0.23**)
Carbohydrates (gm)	320	335.29 (2.1**)
Crude fiber (gm)	8.5	16.69 (0.61**)
Total ash(%db)	-	1.61 (2.1**)
Moisture (%)	-	7.8 (1.31**)
Calcium(mg)	Above 600	650 (1.67**)
Vitamin C(mg)	56-317	61.2 (1.1**)
Iron(mg)	4.5-24	30.13 (0.28**)

*Modifications in the RDA is on the basis of the literatures provided by Joshi and Sharma (2014), Srilakshmi (2014) and ICMR (2010)

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

The chemical analysis of product A (Protein plus) and product B (Standard) were carried out. The comparative view of both the developed feed showed equal nutrient sufficiency. The difference in protein and carbohydrate was justifiable as the protein feed was intended to provide more amount of protein than standard feed.

4.1.1 Carbohydrate

The Table 4.1 shows that the total carbohydrate content of the developed protein enteral feed was calculated to be 59.11g per 100g of the feed. The total carbohydrate content of the developed protein plus feed was accounted to provide 59 per cent of the total calorie content (2051 kcal/511g).

The Table 4.2 shows that the total carbohydrate content of the developed standard enteral feed was calculated to be 64.46g per 100g of the feed. The total carbohydrate content of the developed standard feed was accounted to provide 65.69 per cent of the total calorie content (2041 kcal/520g).

Parallel to these observations, the compositional data of a standard enteral formula feed reported by Nilesh (2011) and Shike (1994) exhibits that a major source of energy in the feed should come from carbohydrates (45 to 65 percent), as it known to be the essential fuel for the cells of body and brain(Nilesh *et al.,* 2011).Various studies have shown the results, which were found to be in accordance with the present findings and prove the sufficiency of carbohydrate content in the developed standard enteral feed. Verma (2006) have documented different forms and concentrations of carbohydrate amongst various formulas. The forms may be starch, glucose polymers, disaccharides and monosaccharide in their polymeric form with 50 to 60 per cent of total calories(Verma, 2006).

The prepared protein feed and standard feed has 59% and 64% of carbohydrate respectively which is in accordance with the studies done and shows similar result as the feed prepared by Joshi and Sharma(2014) who had 49% in protein feed named Nutriagent Protein plus and 57% in standard feed named Nutriagent Standard (Joshi and Sharma, 2014).

4.1.2 Protein

The protein content of the developed protein rich formula was of main concern in terms of the sufficiency of this nutrient for patients with higher protein needs. The crude protein content of the feed was estimated to be 19.7g per 100 g and 101.12g protein from the total weight of the formula feed (511g) (Table 4.1). The protein content was calculated to be 19.72 per cent of the total energy of the developed feed.The composition data of high protein enteral feeds suggested by DAA (Dietetic Association of Australia) (2011), has also depicted that a feed is called protein rich only when it possesses 20 per cent of its calorie content from protein. The results is same as the enteral feed of high protein prepared by Joshi and Sharma (2014) which has 20% of the energy by protein(Joshi and Sharma, 2014).

The patients who typically do not require fluid restriction and have higher protein requirements should consume the feed with 1.2 to 2.0 g protein/kg/day (Srilakshmi, 2014).

This is in accordance with the developed protein rich feed, under the present study, as it delivers 1.6g protein/kg/day (total 101g protein) for a reference man of 60kg weight.

Studies done by various researchers like Ritz (2001); Kowanko (2001) and Schofield (1985) have provided the guidelines for nitrogen and protein requirements under various physiological stages. According to them, under the normal condition with no catabolic illness, 0.8 to 1.0g protein/kg/ day are sufficient whereas, under post-operative and septic complication, this demand increases to 1.2 to 1.4g/kg/day. Sever sepsis and multiple organ failure or burns can enhancethe protein requirements to 1.4 to 2.0g/kg/day. The comparison of these findings with the protein rich feed developed under the present study demonstrates the suitability of this feed even for severe diseased condition, with 1.68 g protein per kg per day (for a reference man) (Joshi and Sharma, 2014).

The data presented in the table 4.2, portray that the standard RTR enteral feed contained 14.32g protein per 100g of the feed weight. The total formula feed provided 74.45g of protein per 520g of formula powder, which accounts for the 14.59 per cent of the total calorie content of the developed standard enteral formula feed. The standard feed has same amount of protein as prepared by Joshi and Sharma (2014) whose standard feed had 75g protein (Joshi and Sharma, 2014).

According to Sharma (1999) criteria for selection of standard enteral formula is that it should provide 9% to 24% of total calories as protein or more, depending upon the physiological stage of the patient (Joshi and Sharma, 2014).The guidelines given by DAA (2011) have also pictured the compositional details of various enteral formula feeds. According to it, a standard formula must deliver protein in the proportion of 15 per cent of its total energy content.

4.1.3 Fats

According to Shike (1994), fat content of enteral formula feeds should provide 15 to 30 per cent of the total calories. Similarly, Sharma (1999) has also reported that fat should provide 1 to 47 per cent of total calories, depending upon the type and severity of the disease.(Joshi and Sharma, 2014)Corresponding to the above stated findings, the protein formula feed developed under the present study contained 9.52g fat from the 100g of the feed, eventually 48.68g fat from the total amount of the feed (511g) and the standard formula feed contains 8.59g fat from 100g and eventually 44.69g from the total amount of feed (520g)

The fat of the protein feed and standard feed provide 21.36% and 19.7% of total calories which shows that the present product gives less calorie by fat as the product prepared by Joshi and Sharma (2014) which gives 30% calories by fat in both standard and protein rich feed. But the result is in accordance to Jain and Joshi (2005) where they have studied the fat composition of the standard enteral formula feed i.e. 17.56 per cent of the total calories.In an another high protein diet developed by the Department of Dietetic, PGI, Chandigarh has delivered fat as 18 per cent (4.3g fat) of the total calories i.e. 215kcal (Jain and Joshi, 2005; Joshi and Sharma, 2014).

4.1.4 Fiber

The crude fiber content of the developed protein rich feed was obtained to be 3.01g per 100g and 15.38g from the total weight of the developed feed (511g) (Table 4.1). and that of standard feed was obtained to be 3.2g per 100 g and 16.69g from the total weight of the developed feed (520g) (Table 4.2).According to Nilesh (2011), fiber is added to enteral feeds to improve stool consistency. The most commonly added fiber is soy polysaccharides or whole soybean. This helps in maintaining the normal bowel movements. Recommended intake of dietary fibre is approximately 20 to 30g/day. Fiber intake of less than 30g/day did not seem to impair Vitamins, minerals or drug bioavailability in the gut (Nilesh *et al.*, 2011).

Commercial protein rich formula Newtrition Isofiber (O' Berin KMI) shows fiber content to be 14.3g from the total volume of the feed, which is similar, to the fiber content of the developed formula. The fiber content in the protein feed prepared by Joshi and Sharma (2014) shows 13g of protein which is similar to the protein feed produced which has 15.38 g of fiber.

Various earlier studies have reported the fiber content of standard enteral formula feed. McClave (2007) and Claustre (2002) have reported the fiber content of a standard feed to range from 4.24g to 7.0g /L, which was found to be similar of present study (8.34g/Litre or 16.69g/2L) to meet the daily requirements and to maintain on the safe level according to Nilesh et al (Nilesh *et al.*, 2011). Fredstroh (1991) has reported the fiber content of enteral feeding solution to be 1.9 to 3.3g/250 ml, which is in conformity with the fiber content of the present developed formula feed (2.08g/250ml or 16.69g per 2000ml). The standard feed prepared by Sharma and Joshi (2014) has 12 g of protein which is in the safe range of fiber intake of enteral feeding as the standard feed that was produced which has 16.69g (Joshi and Sharma, 2014).

4.1.5 Vitamins

The presently developed protein rich formula feed and standard feed was estimated for its Vitamin C content. The 100g protein feed was found to contain 19.37mg of vitamin C and that in standard feed was11.76. The total weight of the protein feed and standard feed i.e. 511g and 520g, contains 99g and 61.2 of Vitamin C respectively.

Berner (1989) has stated that various enteral feeding solutions which are designed to provide 1500 to 2000 kcal, are intended to suffice the recommended dietary allowances (RDAs) for vitamins and minerals in the patient which is found to be 56-317 mg for Vitamin C. Nilesh (2011) has shown the micronutrient content of various enteral formula preparations. He has suggested a range of nutrients to be present in any enteral preparation. According to him, the range for vitamin Cshould be 56 to 317mg. The values estimated in the presently developed feeds for these nutrients were falling within the given range (Nilesh *et al.*, 2011). The Vitamin C content in the products is similar with the product prepared by Joshi and Sharma (2014) who has 101 gm in protein feed and 98 gm in standard feed (Joshi and Sharma, 2014).

4.1.6 Minerals

The presently developed protein rich RTR enteral feed was calculated to provide 8.26mg of iron and 166.34mg of calcium content from 100g feed, whereas, the total amount of the feed i.e. 511g, delivered 42.23mg iron and 850mg calcium per day (table 4.1).

The estimated mineral composition of the presently developed standard RTR enteral formula feed is demonstrated in table 4.2. The data exhibits the presence of 30.13 mg of iron and 650 mg of sodium from the total amount (520g) of the developed standard formula feed.

According to Fuller et al. (2001), the iron content of the enteral feed is crucial, as it helps in replenishing the blood loss in case of various medical conditions. The iron content of the standard formula feed should be maintained in the range of 10 to 40 mg per day. Shenkin (2008) has documented the safe levels of iron to be 40mg from the standard enteral feed (Shenkin, 2008). The calcium content of both the feeds also meets the daily requirements of calcium which is 400 mg and also said to be above 600 mg for proper fulfillment of requirements as stated by ICMR (2010). So the prepared feeds meet the requirements for iron and calcium.

4.1.7 Energy density

The presently developed protein rich RTR enteral formula feed was analysed for its nutrient content. The data obtained are illustrated in Table 4.1. As indicated in the tables the total energy content of the developed feed was found to be 401.37kcal per 100g and 2051kcal from the total weight (511g) of enteral feed. The energy density of the developed feed was 1 kcal/ml, after reconstitution of the feed to a volume of 2000ml. The total energy content reveals that it provides approximately 34kcal of energy per kg of the body weight of a reference man of 60 kg.

The total weight of the developed standard RTR enteral formula feed (520g) provided 2041 kcal or 392.5 kcal/100g (Table 4.2). The feed was designed in such a way, that after reconstitution with water, to a total volume of about 2000 ml, it provides the energy density of 1 kcal per ml per day.This energy density is similar to the density of the developed protein rich enteral formula feed (1kcal/ml).

According to the DAA (2011), the total calorie density of protein rich or high nitrogen enteral formula feed should range within 1.0 to 1.2 kcal per ml of the feed.Parallel to this study, Murrey and Hellersted (1990) has documented that using actual direct measurements of energy expenditures, it has been determined that the average sized patients lying comfortably in bed require about 23 kcal/kg body weight. The energy requirement of the hypermetabolic (those patients with major sepsis or significant thermal burn) person increases to about 40 to 50 kcal/kg/day. The energy density of the presently developed formula feed is quite close to the reported range i.e. 34 kcal/kg/day for a reference man. As the currently developed enteral formula is protein rich sufficient amount of energy is also crucial to maintain protein sparing action. The best nutritional goal remains the provision of enough calories along with the increased proteins to meet the energy demands as well as to reduce the nitrogen losses. This saved allowance of protein would be helpful in healing wound and regenerations of the damaged tissues(Nilesh *et al.*, 2011).Although, the energy density (1.0 kcal/ml) of the protein rich formula feed was similar to the standard formula feed, but the distribution of energy constituents was different.

The nutritional composition of the standard enteral feed, formulated under various research studies, were referred during the present investigation. The major findings of these studies indicated that the calorie density the feed has tremendous implication in terms of health management of the patient. Most of the standard enteral formula provides 1.0 kcal/ml and the major contributors for energy density are carbohydrate followed by fat and protein (Nilesh *et al.*, 2011; Verma, 2006). The study is in accordance with the product prepared by Joshi and Sharma (2014) where protein rich and standard feed provides 407 kcal/100 gm and 378 kcal/100gm (Joshi and Sharma, 2014).

4.2 Reconstitution behaviour

Both the developed RTR enteral formula feeds were reconstituted in the ratios 1:3 feed and water, for the assessment of their reconstitution behaviour (flow rate, reconstitution time).

Table 4.3 Reconstitution behaviour of the developed RTR enteral formula feeds

Enteral feed	**Ratio of feed :wateradded**	**Reconstitute time (mins)**	**Flow rate in 12 pt ryle tube (ml/min)**	**Flow rate in 14 pt ryle tube (ml/min)**	**Total volume of feed**
Protein Plus	1:3(75% 0f water with 25% of enteral protein feed)	8.3±1	29±2.5	36±1.0	2000ml or 2L
Standard	1:3(75% 0f water with 25% of enteral standard feed)	9.5±1	31±2.5	37±1.0	2000ml or 2L

According to the guidelines given by ASPEN (2005) there are various feeding methods for providing enteral nutrition to the patients. In Bolus feedings method the formula feed is administered with a flow rate of 300 to 400ml, in a duration of 5 to 20 minutes, after every 4 to 6 hours, whereas, in intermittent feeding method the enteral formula feed is administered at a rate of 200 to 350ml, in a duration of 10 to 30 minute, after every 4 to 6 hours. On the other hand, in continuous feeding method, the flow rate of feed varies depending on the caloric density of the formula, ranging from minimum of 10 to 40 ml per hour to maximum of 50 to 100ml per hour.

Table 4.4 The average flow rate of enteral formula feed under various administration methods suggested by ASPEN

Administration method	**Range of flow rate (ml/min)**	**Average flow rate (ml/min)**
Bolus feeding	300 – 400 ml per 5 to 20 minutes	30- 40 ml/ minutes

So, the flow rate of the feeds on the reconstitution in ratio 1:3 feed to water is in accordance to the guideline in both 12 pt and 14 pt ryle tube shown in table 4.4.

The mean reconstitution time taken by the developed protein formula feed and standard formula feed are 8.3 and 9.5 respectively.Emmanuel and Okorie (2002) mentioned the formulation of an enteral feed based on malted maize and malted ground nut. The reported mean reconstitution time of the enteral mix was 473 seconds or 8 minutes. Similarly, Jain and Joshi (2005) reported the reconstitution time of the developed enteral formula feed to be 242 seconds (4 minutes). The variations in the mean reconstitution time could be assigned to the difference in the composition of the enteral formula feeds. Also, Joshi and Sharma (2014) reported the reconstitution time of developed protein RTR feed and standard RTR feed to be 8 and 7 min respectively.

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 fig 4.1 and fig 4.2

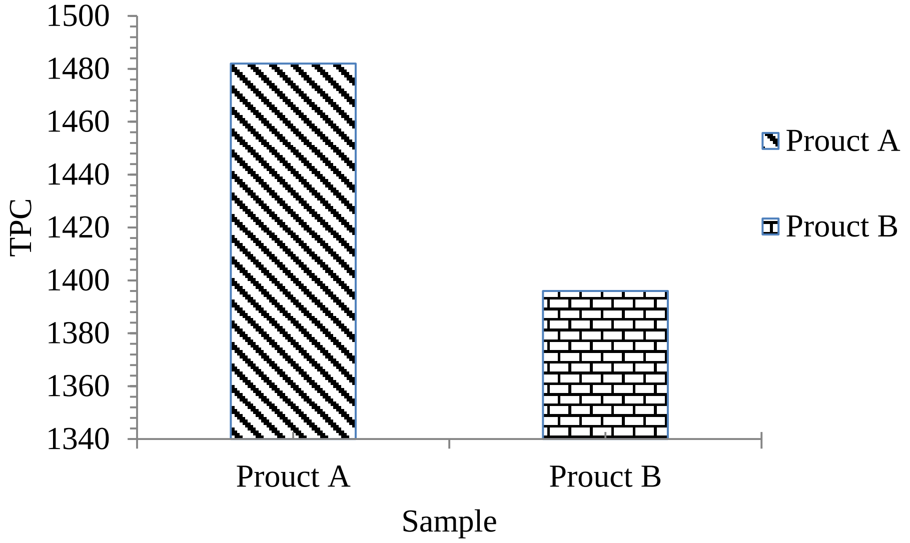

Fig. 4.1 Total Plate Count (TPC) of formulated samples

The total plate count ot the products were found to be1482 Cfu/g and 1396 Cfu/g for the product A and B,which was within the acceptable limit as specified by 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).

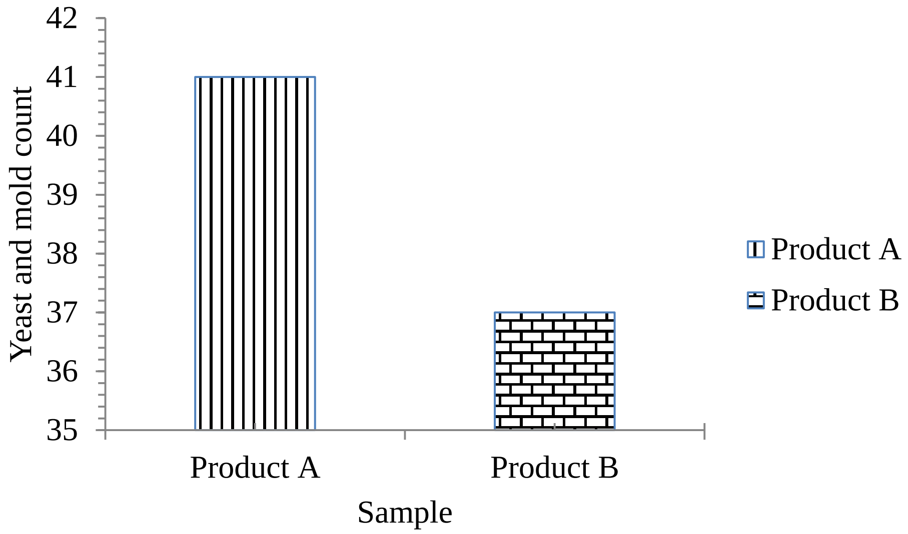

Fig. 4.2 Yeast and mold count of formulated samples

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 products A and B were found to be 41/g and 37/g which were within the acceptable limit of 50/g in food (UNICEF, 2007).

4.5 Cost of the product

The cost calculation of the product A and B were carried out and found out to be Nrs 84.69 and 45.62 per 100 gm respectively (calculation given in Appendix). The cost of the product may vary as there may be increase or decrease in the cost of raw materials as per the season. These cost of the products however are substancially lower than that of comercially available enteral feeding in South East Asian Countries. Table 4.4 shows cost comparison of the developed enteral formula feeds with the commercially available enteral formula feeds.

Table 4.5 Cost comparison of developed enteral feed with commercial feed

Product	**Quantity (gm)**	**Cost(Nrs)**
Product A(Protein Plus)	1000	846.9
Product B(Standard)	1000	456.2
*Ensure	400	960
*Jevity 1.5	1000	9000
*Iso source 1.5	8.4 oz(238)	1000

*These are the commercially available enteral feed in South East Asia and price is converted in Nrs from Indian currency.

The cost comparison of the developed enteral formula feeds with other commercially available formula proves the cost effectiveness of the developed feeds. They deliver wholesome nutrition with economic price.The cost effective of the product can also be done by substituting the protinex by the protein dense skim milk powder.

Part V

Conclusions and recommendations

5.1 Conclusions

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

a) By using easily and locally available raw materials two products as enteral feed was developed. One of them is used for post-operative patient which is calorie and protein dense and other is used for normal patients which meets the normal RDA of reference man.
b) From the analysis of product A(protein plus), it was concluded that it provides 19.7gm protein, 9.53gm fat, 59.11 gm carbohydrate, 166.34 mg calcium, 19.37 mg of ascorbic acid, 8.26 mg of iron and 401.37 Kcal per 100 gm of the products. The moisture, ash and crude fiber content of the product were found to be 7.1%, 1.6% and 3.01% respectively.
c) From the analysis of product B(standard), it was concluded that it provides 14.32 gm protein, 8.59gm fat, 64.46 gm carbohydrate, 125mg calcium, 11.77mg of ascorbic acid, 5.79 mg of iron and 392.5 Kcal per 100 gm of the products. The moisture, ash and crude fiber content of the product were found to be 7.8%, 1.61% and 3.2% resp.
d) After reconstitute of the feed in the ratio1:3 feed and water the reconstitution behavior (flow rate and reconstitute time) was observed. The flow rate of protein feed in 12 and 14 pt ryle tube to be 29 and 36 ml/min respectively and that of standard feed in 12 and 14 pr ryle tube to be 31 and 37 ml/min respectively. The reconstitution time for 1:3 ratio of feed to water was found to be 8.3 and 9.5 for protein and standard feed.
e) The total plate count, and yeast and mold count in product A (protein plus) was 1482Cfu/g and 41 Cfu/gm respectively whereas the total plate count, and yeast and mold count in product B (standard) was found to be 1396Cfu /g and 37 Cfu/gm respectively. The product was safe from microbiological point of view.

5.2 Recommendations

This study can be further continued with the following recommendations.

- Clinical trials in patients can be done to check the efficiency of the product.
- Further study of the trace elements of the products could be carried out.

Part VI

Summary

Malnutrition is a major cause of mortality and morbidity in hospitalized patients. Studies have reported 40%-50% of surgical patients to be malnourished on admission to hospital. There is also a evidence that about 40% of patients who were malnourished at the time of their admission and the majority of these patients continued to be nutritionally depleted throughout their hospital course. So to minimize this condition proper feeding at hospital should be carried out taking consideration of their disease condition. Special care should be given to the patients who cannot be feed orally and should be provided with enteral feeding. So, management of those patients like post-operative patients can be done with specially formulated high protein and high calorie, energy dense food and to normal hospitalized patients who need enteral feeding can be done with formulated high calorie and normal protein food.This work describes how best to manage cases of malnutrition in hospitalized patients using ready to reconstitute enteral formula prepared from locally available foods.

Ready to reconstitute enteral feeding is a powder based feed which should be mixed in water to make a water based food that can be used in tube feeding for the patients who cannot take their food orally. This type of formula helps to reduce the malnutrition of the patients who cannot take their food orally.

Two different formulations were made from locally available raw materials based on the nutrient requirement of post-operative patients and RDA of the reference man. The raw materials were processed and theanalysis 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 for post-operative patients(protein feed) were found to be 19.37 %, 9.53%, 59.11%, 3.01%, 1.6%, 19.37mg/100gm, 8.26mg/100gm and 166.34 mg/100gm respectively. The second product which is used for normal patients (standard feed),it was concluded that it provides 14.32 % protein, 8.59% fat, 64.46 % carbohydrate,1.61% of ash,3.2%of fiber 11.77 mg/100gm of ascorbic acid, 5.79 mg/100gm of iron and 125 mg/100gm calcium. The protein feed and standard feed provide 401.37Kcal and392.5Kcal per 100 gm. One of the product provides nutrients necessary for post-operative patients and other provides nutrients necessary for normal hospitalized patients.

After reconstitute of the feed in the ratio1:3 feed and water the reconstitution behavior (flow rate and reconstitute time) was observed The flow rate of protein feed in 12 and 14 pt ryle tube to be 29 and 36 ml/min respectively and that of standard feed in 12 and 14 pr ryle tube to be 31 and 37 ml/min respectively. The reconstitution time for 1:3 ratio of feed to water was found to be 8.3 and 9.5 for protein and standard feed.

The microbiological analysis of the products were carried out where TPC and yeast and mold count were tested. The TPC and yeast and mold count in the protein feed was 1482Cfu/g and 41 Cfu/gm respectively and that of standard feed was 1396Cfu /g and 37 Cfu/gm respectively. However due to limitation in time shelf life could not be studied.

This study where ready to reconstitute enteral formulas have been prepared from locally available foods which contain all the essential nutrients required for the post-operative patients and the normal patients that can be provided by enteral feeding. to overcome the malnutrition in hospitalized patients. If further researched the industrial production of R.T.R enteral formula using different locally available foods in Nepal could be possible.

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Appendices

Appendix-A

Table A.1.1 Cost calculation of the product A (protein feed)

Particular	Cost (NRs/kg)	Weight of product (g)	Cost (NRs)
Semolina	113	80	9.04
Rice flakes	70	60	4.2
Pearl millet	50	25	1.25
Whole green gram	220	50	11
Soybean	120	60	7.2
Sesame seed	350	50	17.5
Carrot	20	10	0.2
Banana	-	10	0.8
Spinach	-	10	-
Protinex	2000	135	270
Oil	150	10	1.5
Butter	800	5	4
Sugar	80	30	2.4
Total raw material		335	
Total raw material cost			329.09
Processing and labor cost (10% of raw material cost)			32.9
Packaging cost			50
Profit 10 %			41.1
Total cost for feed			453.09
Total cost for 1000 gm			846.89

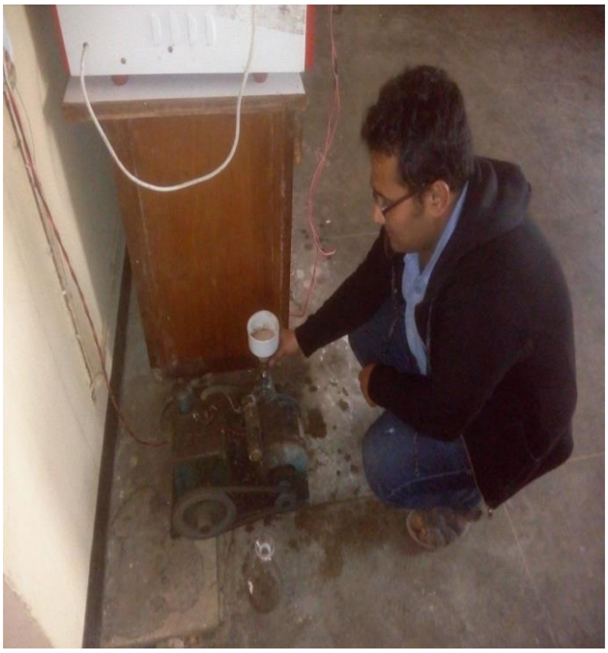
Table B.1.2 Cost calculation of the product B (standard feed)

Particular	Cost (NRs/kg)	Weight of product	Cost (NRs)
Semolina	113	110 gm	12.43
Rice flakes	70	100 gm	7
Pearl millet	50	60 gm	3
Whole green gram	220	50 gm	11
Soybean	120	40 gm	4.8
Sesame seed	350	530 gm	10.5
Carrot	20	10 gm	0.2
Banana	-	10 gm	0.8
Spinach	-	10 gm	-
Protinex	2000	50 gm	100
Oil	150	10 gm	1.5
Butter	800	5 gm	4
Sugar	80	60 gm	4.8
Total raw material		345	
Total raw material cost			160.03
Processing and labor cost (10% of raw material cost)			16
Packaging cost			50
Profit 10 %			22.6
Total cost for feed			248.63
Total cost for 1000 gm			456.20

Color plates

P1 Formulated Samples A(protein plus) and Sample B(standard)

P2 Determination of crude protein



P3 Determination of Crude fiber

P4 Determination of Flow rate in ryles tube