Original Article

Ready-to-Use Therapeutic Food (RUTF) Formulations with Functional Food and Nutrient Density for the Treatment of Malnutrition in Crisis

Abstract

Background: Ready-to-use therapeutic food (RUTF) spread has been highly effective in the treatment of malnutrition in those affected by disasters since it does not require preparation and baking and has a long shelf life and sufficient energy to improve growth and weight loss. Such features may be crucial during crises such as wars and natural disasters. The present study aimed to design a high-energy and nutrient-dense RUTF formulation. **Methods:** Soybean flour and milk protein concentrate were used as protein sources, corn flour, and sugar were employed as carbohydrate sources, cacao butter substitute was used as the lipid source, and vitamin/mineral, beta-alanine, arginine, *Nigella sativa*, and sesame seeds were used as a functional food. The study was performed in accordance with the guidelines of the Institute of Medicine (IOM) and criteria for food products. **Results:** To design the formulation, we used carbohydrates (45% of total energy), protein (13% of total energy), fat (42% of total energy), vitamins, minerals, beta-alanine, and arginine, and 100 grams of the diet was considered to release 525 kilocalories of energy. The experimental results of food safety at the determined intervals (at the beginning and 45 and 90 days after the production) showed acceptable values. **Conclusions:** Since nutritional requirements are among essential human needs (especially in the management of malnutrition in crisis), it is of utmost importance to prepare RUTF products in order to meet all human nutritional needs by facilitating the easy use of these products, particularly for the prevention of malnutrition and diseases.

Keywords: *Crisis, emergency food products, functional ingredients, nutrition*

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Introduction

Natural disasters and other complex humanitarian emergencies (e.g., international or internal conflicts) continue to displace hundreds of thousands of people across the world. The individuals requiring food and humanitarian assistance have tripled since the mid-1980s. According to the World Health Organization (WHO), malnutrition is the most common cause of death in disasters. Malnutrition has been reported to contribute to more than one-third of child deaths. Lack of access to highly nutritious foods (especially in the context of rising food prices) is a common cause of malnutrition. In 2015, 76.7 million people in the world received the World Food Program (WFP) food assistance in emergencies (~3.2 million tons of foods), including 62.6 million women and children, as well as 6.1 million refugees.[1] At the early stages of an emergency, it is essential to ensure that foods with acceptable quality and sufficient quantities are available to the affected individuals. Refugees often have inappropriate food preparation utensils and facilities and must rely on ready-to-use therapeutic food (RUTF) for the management of malnutrition and diseases.[2-4]

A report detailing RUTF has been published regarding food relief efforts by a committee appointed by the Institute of Medicine (IOM) in response to the escalation of natural and manmade disasters and complex humanitarian emergencies.[5,6] RUTF is used for therapeutic purposes in malnourished individuals[7] and patients with acquired immunodeficiency syndrome (AIDS).[5,7] The key advantage of RUTF is that it is a ready-to-use paste, which does not need to be mixed with water, and the risk of bacterial proliferation is avoided in case of accidental contamination. The product could be consumed directly by children and provides sufficient nutrient intake for complete recovery. In addition, it could be stored for 3-4 months without refrigeration, even at tropical temperatures.[8]

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RUTF has been used in emergencies and crises (e.g., natural disasters and warfare) in order to promote crisis management. Some of the important properties of RUTF include high nutritional value, high nutrient content, high energy density, no need for preparation, readiness to use, long-lasting preservation, low spoilage owing to the low moisture content, low volume and weight, and ease of transportation and distribution. The energy level, type, and quantity of fat, protein, carbohydrates, fiber, and micronutrients should be considered in the formulation of RUTF.[4,7] RUTF should also provide all the required nutrients in a crisis for the prevention of malnutrition and diseases. Trial and error is a primary approach to the formulation of RUTF, and researchers initially employ candidate compounds and subsequently assess their concentrations repeatedly to achieve the essential and desirable profiles.[8]

The present study aimed to develop a novel RUTF with functional food and nutrient density in accordance with the WHO and IOM instructions. The use of local products in the formulation was essential, and the product had to have acceptable sensory features.

Methods

Ingredients

In this study, the ingredients used for the production of RUTF included corn flour (Ilia Factories, Kermanshah, Iran) as a source of carbohydrates, whole soybean flour (Toos Soyan, Mashhad, Iran) and milk protein concentrate (MPC) (Golshad, Mashhad, Iran) as protein sources, cocoa butter substitute (CBS) (Cargill, Kuala Lumpur, Malaysia) as the lipid source, granulated sugar (Iran Sugar Co., Tehran, Iran) as the source of simple sugars, lecithin and polyglycerol polyricinoleate (PGPR) (Nestlé Iran Co., Tehran, Iran), , cocoa powder (Delfi Cocoa, Johor Darul Takzim, Malaysia), vitamin/mineral premix (Osve Iran Pharm Inc., Tehran, Iran),[5,7] and beta-alanine and arginine (Karen Iran Pharma Inc., Tehran, Iran). The vitamin/mineral premix was formulated by Osve Iran Pharma to meet the IOM requirements for the estimate of food preferences (EFPs) [Table 1].

RUTF production

After the adjustment of 16 experimental formulations, the optimal formulation was produced in terms of the material type, consumption, rheology, and organoleptic properties [Table 2]. Pilot tests were performed on one kilogram of the prepared RUTF using a ball mill refiner (Arman Kherad Toos Inc., Mashhad, Iran) with stainless steel balls (diameter: 9.5 mm). The mixing process was performed at 100 rpm, and the recycling mass was carried out through the ball bed at the medium flow of 2-3 kg/min-1 using a recycling pump. In order to prepare 1,000 grams of the RUTF, we considered the weight distribution of the corn flour (400 g), soy flour (180.6 g), MPC (116.3 g), CBS (210.3 g), powdered sugar (149.7 g), cocoa powder (30 g), lecithin (12.5 g), PGPR (32.5 g), and multi-vitamin and minerals [Table 1]. Initially, the RUTF was melted in an oven at the temperature of 60°C, and the cocoa powder,

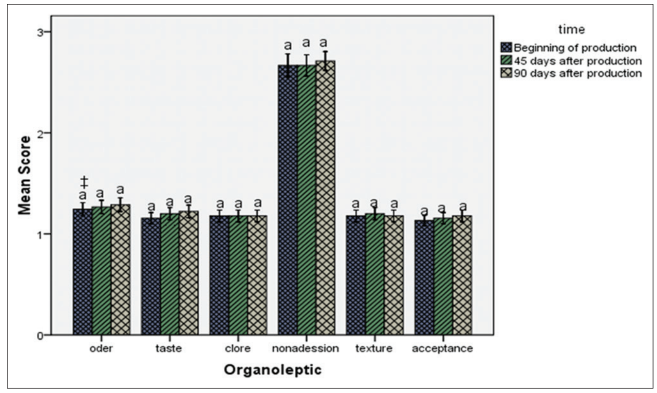

Figure 1: Sensory evaluation of optimal formulation at the beginning of production, 45 days and 90 days after production at a temperature of 38 ° C. ‡Different lowercase letters mean a statistically significant difference in each category (Similar letters mean no statistically significant difference)

Table 1: The amounts of energy, macronutrients and micronutrients used for RUTF is based on RDA

Nutrient	RDA
Energy (kcal. d-1)	2100
CHO (g. d-1)	230 g
Protein (g. d-1)	70 g
Fat (g. d-1)	100 g
Vitamin A (IU. d-1)	900 IU
Vitamin C (mg. d-1)	60 mg
Vitamin D (IU. d-1)	200 IU
Vitamin E (IU. d-1)	15 IU
Vitamin K (µg. d-1)	120 µg
Thiamine (mg. d-1)	1.2 mg
Riboflavin (mg. d-1)	1.3 mg
Niacin (mg. d-1)	16 mg
Vitamin B6 (mg. d-1)	1.7 mg
Folic acid (µg. d-1)	400 µg
Vitamin B12 (µg. d-1)	2.4 µg
Biotin (µg. d-1)	30 µg
Pantothenic acid (mg. d-1)	5 mg
Calcium (mg. d-1)	1200 mg
Iron (mg. d-1)	8 mg
Phosphorus (mg. d-1)	700 mg
Iodine (µg. d-1)	150 µg
Magnesium (mg. d-1)	400 mg
Zinc (mg. d-1)	11 mg
Selenium (µg. d-1)	50 µg
Copper (µg. d-1)	50 µg
Manganese (mg. d-1)	2.3 mg
Chromium (µg. d-1)	30 µg
Molybdenum (µg. d-1)	45 µg
Potassium (mg. d-1)	4700 mg
Sodium (mg. d-1)	1500 mg
Beta-alanine (mg. d-1)	1000 mg
Arginine (mg. d-1)	2000 mg

RDA: Recommended Dietary Allowance; RUTF: Ready-to-use therapeutic food

Table 2: Components used in RUTF final formulations (%based on weight)

Ingredient	RUTF
Corn flour	40
Soybean flour	18.06
MPC	11.63
CBS	21.03
Powdered sugar	14.97
Cocoa powder	3
Lecithin	1.25
PGPR	3.25
vitamin and mineral	According to Table 1

RUTF: Compact food Bars, MPC: Milk Protein Concentrate, CBS: Cocoa butter substitute, PGPR: Polyglycerol polyricinoleate

refined sugar, lecithin, and PGPR were added to the molten CBS. The blended material was transferred to a ball mill refiner at the temperature of 45°C, and the soy flour was appended. Following that, the steel balls that crushed the particles were added to the mill. The ball mill refiner was equipped with a pump for the circulation of the compounds, which helped stir the samples. At the next stage, the MPC and corn flour were added. The milling operation continued for four hours at the temperature of 45°C, and the functional foods (vitamin/minerals, beta-alanine, and arginine) were added to the mix at the final stage. After removing the samples from the ball mill, the molding process was performed using special chocolate molds, followed by cooling at the temperature of 4ºC for 30 minutes and the storage steps. In addition, the evaluation of macronutrients, micronutrients, and food safety were carried out on the RUTF.

Chemical composition

After each formulation, the levels of simple sugars, complex carbohydrates, lipids (saturated and unsaturated fatty acids), protein, moisture, acidity, and ash of the RUTF were measured in accordance with methods 933.04, 945.34, 939.02, 931.04, 970.21, and 923.03 of the Association of Official Analytical Chemists (AOAC), respectively.[9] Furthermore, the measurements of food safety parameters were performed by microbial, chemical, and stability tests, including lipid peroxide index, fat oxidation analysis (thiobarbituric acid reaction), and level of vitamins. Food safety tests and measurement of thiamine and ascorbic vitamin levels were also carried out, and the obtained data were compared three times on days zero, 45, and 90 at the temperature of 38°C. Notably, the level of vitamins was measured by high-performance liquid chromatography.[10,11]

Consumer sensory testing

The nine-point hedonic scale (1 = Very Nice, 9 = Very Unpleasant) was used to evaluate the sensory properties of the rations. The sensory evaluation of the samples was performed based on six factors, including odor, taste, color, non-adhesion (mouth coating), texture, and general acceptance, zero (immediately after production), 45, and 90 days after the production. At the end of the questionnaires, open questions were suggested for comments to improve the RUTF. The participants received snacks and drinks at the end of the tests to appreciate their participation.

Statistical analysis

Data analysis was performed in SPSS version 16.0 (SPSS Inc., Chicago, IL). The data were obtained from the sensory analysis and food safety experiments (microbial and chemical analysis). The possible differences between the mean values were compared using a general linear model, univariate ANOVA, and the least significant difference (LSD) test at the significance level of 5% ($P < 0.05$).

Results and Discussion

Chemical composition

Macronutrient content and energy

To design the RUTF formulation, the ingredients had to contain the essential nutrients recommended by the WHO and IOM.[7] The IOM recommends that 450 grams of RUTF per day is required to release the energy level of 2,100-2,200 kcal/day.[6,7] The carbohydrates of RUTF should also provide 40-50% of energy, which is equal to 25-100 g/kcal.[6,12] The starch obtained from legumes and cereals (e.g., corn and wheat) could mainly provide the carbohydrate source and also partially supply proteins. A specific quantity of simple sugars should be added to RUTF to elevate the calorie density and eliminate the undesirable taste of vitamins and minerals, which also constitutes 34% of the energy released by the total carbohydrates.[6,7] Dairy sources should be added cautiously to RUTF due to high levels of lactose. The lactose level of the product should not exceed 12 g/day due to lactose intolerance in many individuals. Legumes and grains also provide certain amounts of fiber. However, the absence of fiber in RUTF is non-problematic considering the short-term use of the diet.[6,7,13]

As for the lipid content of RUTF, the permissible range is 35-45% calories, which is equivalent to 39-50 grams per 1,000 calories. In this context, it is important to define the type and quantity of the added lipids to RUTF.[7] These lipids should include stable and resistant fats with the least unsaturated bands. Therefore, only the addition of hydrogenated lipids has been recommended, and no animal fats are allowed in the production of RUTF.[5,13] In tropical areas, the oils with higher melting points could also be used in RUTF formulations.[8,13] The main lipids used in the texture of RUTF should meet several criteria, such as the acceptable flavor of food, meeting the energy requirements, anti-oxidation stability, containing essential fatty acids, low weight, no degradation during transportation and storage, and sufficient fat-soluble vitamin absorption.[6,8] Omega 6 and 3 fatty acids are needed to provide 3.0-10.0% and 0.3-2.5% of the total energy, respectively.

Protein sources should provide up to 10-15% of the total dietary energy (34 g/kcal) to prevent kidney stress and thirst.[5,6] The sensory profile of foods (e.g., color, odor, appearance, and flavor) could be influenced by the protein content. In a study in this regard, Yang *et al*. reported that high protein levels adversely affect the edibility of food products. Free amino acids also have negative effects on food taste and increase the costs of food preparation; therefore, only the addition of essential amino acids has been recommended to provide for the required protein sources in foods. On the other hand, meat has not been recommended as a protein source in RUTF, and studies have emphasized on the addition of high-quality plant and milk proteins instead, especially plant-derived proteins. Notably, the absence of some amino acids in plant protein could be compensated by using legumes and cereals to enhance protein quality.[5,8,13]

In the present study, 100 grams of the final formulation contained 525 kilocalories, which included 58.59 grams of total carbohydrates, 25.02 grams of lipids, and 16.275 grams of protein, which was in total compliance with the IOM guidelines [Table 3]. In the current research, the total carbohydrate proportion of the total energy was 45%, and the level of the simple carbohydrates in100 grams of the product was 17.77 grams, which included 2.91 grams of lactose in milk and 14.97 grams of glucose in sugar beet. The share of energy from simple carbohydrates was 30% of the total carbohydrate energy, which was lower than the recommended values by the IOM; the maximal simple carbohydrate is up to 34% of the total carbohydrate energy share.[5,6] The amount of the complex carbohydrates in 100 grams of the final formulation was 40.71 grams, while 70% of the total energy from the carbohydrate contribution was complex carbohydrates, which are nutritionally essential to physiological processes such as blood sugar homeostasis, high fiber provision, and feeling of satiety.[5]

Table 3: value and energy contributions of macronutrients per 100 grams of RUTF final formulations

Macronutrients	Final value (g)	Energy contributions (%)
carbohydrate total	58.59	45%
complex carbohydrate	40.71	70% of total carbohydrate energy
corn flour	34.9	-
soybean flour	5.81	-
simple carbohydrate	17.88	30% of total carbohydrate energy
powdered sugar	14.97	-
MPC	2.91	-
Fat	24.97	42%
CBS	21.03	-
corn and soybean flour	3.86	-
Protein	16.275	13%
MPC	8.14	-
soybean flour	8.13	-

RUTF: Ready-to-use therapeutic food, MPC: Milk Protein Concentrate, CBS: Cocoa butter substitute

According to the findings of the current research, the designed formulation contained 24.97% fat, and the fat proportion of the total energy was 42%. In addition, the fat used in the formulation was 21.11% CBS, and 3.86% was constituted by the materials used in the product (e.g., corn flour and soybean flour); this is consistent with the obtained experimental results. CBS contains saturated fatty acids during the hydrogenation process, which contributes to reducing the risk of oxidation and results in high stability. Furthermore, the use of CBS was observed to increase the shelf life of the product.[5,6]

In the present study, the percentage of the trans fatty acids in the samples based on the 2,100-kilocalorie diet was estimated at 0.41 gram. According to the American Heart Association, a 2,000-kilocalorie diet should be approximately 2.5 grams. In addition, the amount of saturated fatty acids in the mentioned combination based on the 2,100-kilocalorie diet is estimated at 80 grams, which should be 24 grams per 2,000 kilocalories based on nutritional standards, so that it would be higher than the global standards. The amount of unsaturated fatty acids is recommended in accordance with the nutritional standards of 44 grams per 2,000 kilocalories, which is approximately 16 grams in the mentioned combination and less than the recommended standards. As saturated fats must be used to increase the shelf life of such rations, these outcomes are inevitable. Considering that the duration of adherence to such diets is three days and their maximum use time is 15 days, it is unlikely that the consumers would experience any health complications.[6,13]

In the final formulation of the present study, the level of total protein was estimated at 13%, which reduced the risk of dehydration in case of the lack of water supplies. The consumed proteins contained 50% MPC (less than 3% of lactose) as an animal protein and 50% soybean as a plant protein, which provided all the essential amino acids to the human body.

Micronutrient content

In the current research, vitamin and mineral micronutrients were added to the formulation in the form of premix at the final stage of production. Vitamins such as ascorbic acid, thiamine, folic acid, and retinol were evaluated due to instability during food processing. These vitamins were also evaluated in the samples during storage (three months), and their stability was recorded. Table 4 shows the results of the vitamin analysis based on each 100-gram bar.

Minerals are not often affected by heat processes. According to the current research, the levels of ascorbic acid, folic acid, and retinol declined within 90 days, while this amount was noticeably high for vitamin C compared to folic acid and retinol. Vitamin C is considered to be the most

Table 4: Vitamin quantities per 100-g CFB according to chemical analysis

Fatty acids	Vitamin Premix	CFB after production	CFB after 45 day	CFB after 90 day
Ascorbic acid (mg/bar)	15	15.2±1.8•	13.2±4.9	12.62± 4.9
Thiamin (μg/bar)	400	512.2±51.6	516.3±55.0	514.7±49.0
Folic acid (μg/bar)	100	110.7±11.1	99.16±7.0	96.6±5.0
Vitamin A (IU/bar)	225	232.7±51.5	211.1±77.0	209.0±16.0

RUTF: Ready-to-use therapeutic food, •mean±SD (3 repetitions) are presented for the measures

sensitive water-soluble vitamin, which is oxidized easily in the presence of moisture, heat, and light.[6,14] Vitamin C plays a pivotal role in protection against oxidative stress, wound healing, and normal immune function, which makes it an essential element in emergency food recipients. Furthermore, vitamin C increases iron absorption, which is more important in the individuals with iron deficiency, especially women and children.[14,15] A key approach to preventing the reduction of vitamin C in EFPs is to encapsulate vitamins with ethyl cellulose, which minimizes the vitamin loss. Accordingly, sufficient vitamin C is given to the consumer after a period of maintenance.

In the current research, the amount of thiamine in the RUTF was higher than the amount of this compound in the premixes during the 90 days. Although some of the thiamine was lost during the process, a significant portion of the other main components of the RUTF (e.g., corn and soybean meal) than the vitamin premix were added to the diet, so that high levels of thiamine could be interpreted in the diet.

Functional food

Functional foods are the foods that promote health and prevent diseases in addition to meeting normal nutritional needs.[16] Some of the most nutritionally practical and beneficial compounds include arginine, beta-alanine, *Nigella sativa*, and sesame seeds, which could reduce fatigue and oxidative stress, enhance performance and the immune function, and decrease the production of pre-inflammatory interleukins.[14,17]

Arginine is an essential amino acid, which is involved in the synthesis of creatinine (an endothelial nitric oxide). The need for arginine increases in case of stress, trauma, and infections. Furthermore, this compound has anti-stress, antioxidant, and anti-inflammatory properties, delays fatigue, and improves performance by reducing the storage of the metabolites that cause fatigue, accelerating the healing of alkalosis, and eliminating ammonium from the muscles. This supplement also boosts the immune system and significantly reduces the risk of infection.[18,19]

Beta-alanine is involved in the formation of a peptide known as carnosine, which acts as a buffer peptide and reduces fatigue by neutralizing hydrogen ions. Even after a single session of beta-alanine use, muscle carnosine and athletic performance could continue to enhance.[20] Studies have indicated that decreased fatigue by beta-alanine use could be achieved through mechanisms such as the reduction of fatigue metabolites (adenosine, diphosphate, lactate, and hydrogen ions), preventing the inhibition of energy production enzymes, increasing calcium sensitivity in muscle tissues, and increasing the release and reabsorption of calcium in the sarcoplasmic reticulum.[21,22]

Sesame seeds are considered to be valuable oilseeds. Sesame contains high levels of oil (~50%), protein (~25%), essential fatty acids, antioxidants, vitamins B, D, E, and F1, and lecithin.[23,24] These components have remarkable antioxidant properties, which promote physical health and prevent diseases. Moreover, sesame is an abundant source of manganese, potassium, iron, zinc, and calcium.[24]

The chemical composition of *Nigella sativa* is oil (~36-38%), protein (~26%), carbohydrates (~25%), volatile oil (0.4-0.5%), various amino acids, vitamins, and mineral substances.[24,25] Black seed is a prolific source of essential and unsaturated fatty acids. Linoleic acid is involved in weight gain, and the incorporation of black seed into RUTF formulation could be highly beneficial in the treatment of malnutrition. Notably, black seed oil has remarkable antioxidant and antibacterial properties.[26]

Chemical analysis

Water activity (a_w)

In the present study, moisture could control and lowers the water activity value of the RUTF, which is essential to achieving the requirements for a three-year shelf life. Dried foods had a moisture content of 2-20%, which is equivalent to the a_w value of 0.2-0.6 as an acceptable range.[13,14] The final RUTF formulated with the vitamin/mineral premix at the early stage of production had a moisture content of 6.06±0.13% in the present study, which is within the acceptable range based on the IOM guidelines. After 45-90 days of production, the moisture content of the samples was 5.29±0.16% and 5.17±0.16%, respectively, which was not considered significant.

Our RUTF formulation was designed in a chocolate structure, which resulted in the minimal water activity of the product and high durability over time. No protocols are available regarding the microbial growth inhibition of food. However, the water activity of 0.61 is associated with the lowest possible microbial growth, while microbial growth becomes unlikely with the water activity of 0.6.[7,27]

Peroxide value

Lipid oxidation is considered to be a severe deterioration in various food products, which limits their shelf life and adversely affects consumers. The nutrients containing fat, even in small amounts, are susceptible to oxidation and may eventually oxidize. The detrimental effects of oxidation on food are not only limited to malnutrition and the production of odorous substances; among the other disadvantages of this phenomenon are the loss of color and nutrients and accumulation of health-threatening substances.[28,29]

The rate and degree of lipid oxidation depend on factors such as unsaturated fatty acids, temperature, time, oxygen exposure, oxygen removal, and added antioxidants or reductive agents.[30] In the present study, the peroxide value of the samples was evaluated zero, 45, and 90 days after the production, indicating no significant difference in this regard. On day 14 of the production, the peroxide value of the samples was 0.5%, and on days 45 and 90, the peroxide value of the samples increased to 1.19 and 1.28, respectively. However, the peroxide values in all the experiments during storage were lower than the standard limits (2 mEq of active oxygen per 100 g of sample). Low moisture content and water activity and the presence of antioxidant compounds in the formulation were probably some of the factors limiting the progression of lipid oxidation. The packaging method is of utmost importance given the effect of oxygen concentration on the rate of the lipid oxidation reaction.[31] In this technique, the concentration of oxygen in the enclosure should reduce as far as possible. In the studies conducted by Natick Research Center on the EFP, the effect of using oxygen sorbents was reported to decrease the peroxide value compared to conventional packaging samples.[15] Another study in this regard aimed to investigate the influential factors in the shelf life of energy diets (including peroxide value), and the results indicated that the peroxide values were lower than the standard limits after six months of storage at the temperature of 38°C, which could be attributed to antioxidant effects. In the current research, the peroxide value increased from 0.41 on day zero to 0.67 on day 90.[32]

Thiobarbituric acid reaction

The measurement of thiobarbituric acid reactive substances (TBARs) is a common approach to assessing the oxidation status of foods.[33] TBAR compounds are naturally found in numerous biomaterials (e.g., aldehydes and lipid peroxides) and increase in response to oxidative stress.[34] TBAR assays are often reported as an equivalent of malondialdehyde content of the sample; this compound is resulted from the degradation of unsaturated fatty acids. TBAR assays are primarily used for research purposes, and no standards have been defined for foods in relation to this index. Our findings indicated that the status of the rations was acceptable in terms of the progression of oxidation and absence of significant differences in the results of various diets.

Microbiological analysis

The most important microbial determinants of the quality and health of food are the total counts of aerobic mesophilic bacteria, coliforms, *Escherichia coli*, Salmonella, *Staphylococcus aureus*, molds, and yeasts, which were evaluated in the designed RUTF in the present study on days zero, 45, and 90 of the production. According to the obtained results, the total counts of the microorganisms were within the standard ranges [Table 5].

In general, the use of compounds with low water activity, presence of antimicrobial agents, food production process, and storage temperature are among the causes of the non-growth of the microorganisms that were measured in our research. In another study, Brisske *et al*. (2014) reported that the low water activity of most of the compounds used for the production of compact food led to the absence of optimal growth conditions for most of the spoilage and foodborne pathogens, which is also the case in similar products.[14,33] On the same note, Farajzadeh *et al*. (2011) concluded that the ration produced after six months at the temperature of 38°C was within the standard limits microbiologically, which could be attributed to the use of compounds with low water activity and water unavailability for the growth and activity of microorganisms, as well as the use of antimicrobial compounds such as vanilla essential oil.[32]

Conclusions

According to the results of the experiments on the nutrient-dense, high-energy RUTF, compliance with the IOM guidelines regarding the compositional specifications may result in obtaining an acceptable bar. The protein requirement of an individual could be met by specific amounts of soy flour and MPC, totaling approximately 16 grams of protein per 100 grams of ration. The IOM has recommended the use of a vitamin/mineral premix for the formulation of RUTF. None of the vitamins in this study showed a significant reduction in the mixing approach, while the amount of the vitamins decreased during storage. To prevent or delay degradation, heat-sensitive vitamins should be encapsulated appropriately. According to our findings, microbial growth was inhibited due to the low levels of moisture and water activity. In addition, this ration had no significant difference in terms of the sensory profile, microbial and chemical characteristics, and peroxide value with the freshly produced samples after 90 days of storage at the temperature of 38°C (equivalent to 18 months at 27°C). The qualitative features and product durability were within the standard ranges. As a result, the designed formulations in this study have the potential for the effective treatment of malnutrition in the victims of various disasters.

Table 5: The results of microbiological tests of final formulation at different times and temperatures of 38

final formulation at different times	Sample at the beginning of production	45 days after production	90 days after production	Standard range
Total bacterial count (mesophilic count) (cfu/g)	1.8×10^{2}‡	2.1×10^{2}	2.3×10^{2}	10^{3}
Total and fecal coliforms	<10	<10	<10	10
Staphylococcus aureus	Negative	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative	Negative
Yeasts and moulds	<10	<10	<10	10^{2}

‡cfu/g: Colony-forming unit/gram

Authors' contributions

S. H., R. A., M. M., and G. A. designed the research; S. H., V. H., and G. A. drafted the manuscript; S. H. designed the tables and figures; G. H. and G. R. participated in the drafting and editing of the manuscript. All the authors read and approved the final manuscript.

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Conflicts of interest

There are no conflicts of interest.

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