Development Of Ready-To-Eat (Spirulina Pancake Premix) Nutritional Supplement forAnemic Adolescent Girls: Nutritional and Microbial Analysis

Abstract

This study aimed to create an iron-rich dietary supplement utilizing spirulina and other nutritionally dense ingredients—quinoa, soybean, and amaranth—in the form of a pancake premix. The pancake premix was formulated, prepared, and analysed for its nutritional composition.

The premix was meticulously developed by combining ingredients from various food groups, incorporating vanilla powder for flavour. The preparation involved simple steps to produce the final pancake product. Following the preparation, a thorough nutritional analysis was performed, covering moisture, ash, fat, protein, iron, crude fibre, and total carbohydrate content, adhering to established protocols such as AOAC methods.

The results revealed that the spirulina pancake premix contained 4.5% moisture, 12% ash, 4.6% fat, 11.7% protein, 67.8% carbohydrates, 5.7% iron, and 0.83% crude fibre. Furthermore, based on the Food Composition Table, the premix provided 460 kcal of energy, 16g of protein, 87g of carbohydrates, 3g of fat, and 8.5g of iron per serving, meeting substantial nutritional requirements.

Microbial analysis indicated limited microbial growth, attributed to the airtight sealing of the premix in silver plastic bags, which effectively prevented contamination and extended the product's shelf life.

The pancake premix demonstrated promising nutritional content and microbial control, suggesting its potential as an iron-rich dietary supplement for addressing nutritional deficiencies, particularly among anemic adolescent girls.

Keywords:

Anemia, Spirulina, Premix, Nutritional analysis, Microbial analysis

1.Introduction

Foods which are nutritionally dense and sustainableare high in demand for the food industries.It gets more serious to provide low-costfunctional food when it comes to cope up with the deficiencies like anemia so to provide the needfuls to all the groups of the society. One of the low costs and readily available food is spirulina. Spirulina is a cyanobacterium that occurs in the form of either blue-green bacteria or blue-green algae which is available only in lakes with high alkalinity*(D.J. Kumari et.al, 2011; AlFadhly et.al, 2022).*There is extensive documentation on indigenous populations across various regions using S. platensis as a food source*(Sarra Bensehailaet.al, 2015).* The concentrated nutrition of spirulina makes

it an excellent food supplement for people of all ages and lifestyles *(A M Sharobaet. al.,2014).*Spirulina is one of the natural sources containing the highest amount of protein, essential and nonessential amino acids, beta-carotene, a precursor of vitamin A, vitamin B12, and essential fatty acid γ-linolenic acid *(Arpita Mohan et.al., 2014).* In addition to thisspirulina also contains a host of other beneficial nutrients including; carotenoids, vitamin E, copper, manganese, magnesium, iron, selenium, and zinc*(J.C Dhillon et. al., 1995).*Numerous research investigations demonstrate the wide array of health advantages offered by Spirulina, encompassing antioxidant, immune-modulating, anti-inflammatory, anticancer, antiviral, and antibacterial properties. Additionally, it exhibits positive effects against conditions such as hyperlipidemia, malnutrition, obesity, diabetes, toxicity induced by heavy metals and chemicals, as well as anemia*(Lee et al. 1998; Lorenz 1999; Hoseini et al. 2013; Kulshreshtha et al. 2008).*Additionally, growing spirulina is better for the environment than making animal protein or plant alternatives. It's grown in controlled settings with low pollution and produces a lot in a small space*(Tommaso Fantechi et al. 2023).*

Ready-to-eat foods require no additional preparation and are typically kept refrigerated or at room temperature and offers the benefits of convenience, health and variety *(Daman Preet Kour et.al 2022;Muktawat and Varma, 2013).*Hence,spirulina can be used preparing pancake premixby incorporating with other super flours such as quinoa, soy flour and amaranth. This would require no expert skills and is less time consuming.

Quinoa (Chenopodium quinoa Willd.)is a plant species in the Chenopodiaceae family that was first appeared in the Andes and can adapt regardless of soil and environmental circumstances.*(Ehab Th. El-Said. Et.al., 2021).* It is a gluten-free pseudo cereal accompanied by great nutritional advantages because of its high protein, fat, fibre, essential fatty acids, vitamin, and mineral content *(Navruz-Varli et.al. 2016; Stikic R et.al. 2012).*Quinoa has a significant impact on the rheological, technical, and characteristics perceived by the senses in baked goods and could be used to manufacture bread products *(Atef, Aet.al 2014).*

Soybean has been able to provide high-quality proteins at low prices*(Amit Arjun Kulthe et. al 2016).*Soy proteins are distinct among plant proteins due to their high biological value and the inclusion of necessary lysine, which is a limiting amino acid in most cereals.*(Riaz 1999; Kaur et al., 2005).*Defatted soy flour (DSF) is a less expensive, more convenient, traditional, and high-protein source for the world's rapidly growing population. *(Tripathi and Mishra 2005).*

Amaranth on the other hand, is a saviour in terms of nutrition for the malnourished world. Amaranth is a plant that exists in various variations, typically characterized by its wide leaves and purplish-green color, capable of growing up to a height of eight feet*(Tucker, J. B. 1986).* In India, amaranth is used widely in fasting rituals as it is considered as a“pseudocereal”. It holds a wealth of nutrients like protein, lysine, carotenoids, dietary fibre, iron, vitamin C, vitamin A, riboflavin, thiamine, folic acid, calcium, among others. Moreover, it boasts a significant quantity of bioactive elements such as tocopherol (α, β, and γ), DPPH (2,2-diphenyl-1-picrylhydrazyl), anthocyanins, lutein, and various phenolic compounds*(Bhattarai et.al 2018).*Apart from nutritional richness, it is also high in satiety value.

In this study, the aim was to prepare an iron rich dietary supplement for anemic adolescent girls and do nutritional analysis.

2. Materials and Methods

2.1 Materials

The research was conducted at Babasaheb Bhimrao Ambedkar University's Food Science and Technology Laboratory (FSTL). The ingredients were procured from the local vendors of Lucknow and online retailer (Amazon & blink it) after designing a realistic meal that precisely reflects the desired proportion and nutrition.

2.2 Preparation of premix powder.

The pancake premix powder was prepared by choosing at least one ingredient from each food groups given by Indian Council of Medical Research which are listed below.

Table 1.Ingredients of spirulina pancake premix

Food Groups	**1. Milk and Meat Products**	**2. Pulses and Legumes**	**3. Cereals, Grains and Products**	**4. Fruits and Vegetables**	**5. Fats and Sugar**
Ingredients	Milk 30 g	Soya Flour20g	Refined wheat flour 25 g Quinoa 20g Amaranth 5g	Date Powder 30g	Butter 15g Sugar 18g

Apart from this, flavouring was achieved by adding vanilla powder into the mixture.The combined weight of all the components used to prepare the pancake premix was 155.5gper serving.

2.3 Preparation of pancakes

The premix was prepared by combining all the ingredient in one bowl. After that, it was used to prepare the final product.

- **Prepare the Batter:** In a mixing bowl, pour the pancake premix according to the requirement. Add the specified amount of water and mix until the batter is smooth. Keep at rest for 10-15 minutes to set. Do not over mix the batter.
- **Preheat the Pan:** Heat a non-stick frying pan or griddle over medium heat. Optionally, add a small amount of butter or oil toavoid adherence.
- **Pour the Batter:** Once the pan is heated, pour a small amount of batter onto the cooking surface. Use a ladle or measuring cup to maintain consistent pancake sizes. Leave space between each pancake to allow for spreading.

- **Cook the Pancakes:** Cook the pancakes until bubbles form on the surface. This usually takes around 2-3 minutes. Check the edges; they should start to look set.
- **Flip the Pancakes:** Once bubbles have formed and the edges look set, gently flip the pancakes using a spatula.Continue cooking for another 1-2 minutes on the opposite side until it turns a golden-browncolor.
- **Repeat:** Continue making pancakes with the remaining batter, adding more oil or butter to the pan as needed to prevent sticking.
- **Serve:** Once all pancakes are cooked, stack them on a plate. Add your favourite toppings, such as syrup, honey, fresh fruit, nuts, or whipped cream.

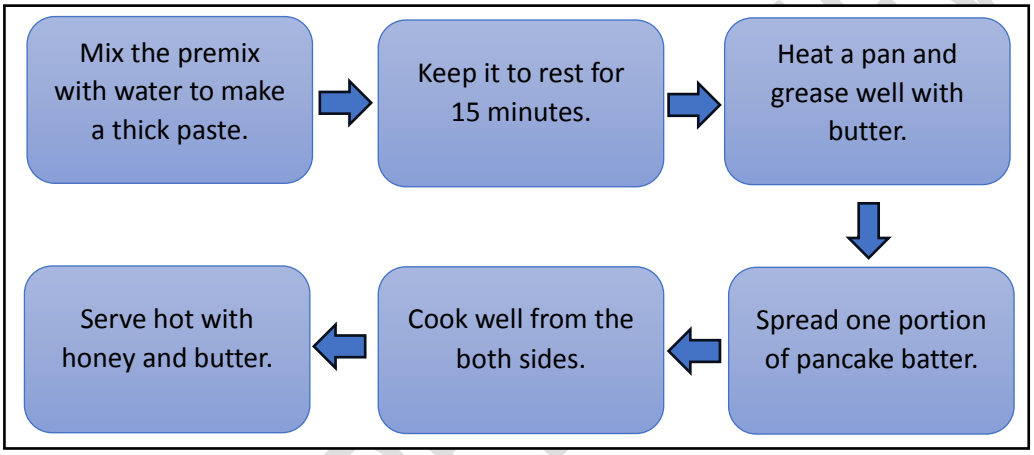

Figure 1.Flow diagram of pancake preparation

2.4 Nutritional analysis

Moisture

As per AOAC 2020, apetri plate was dried in an oven at 105 degrees Celsius for 20 minutesto remove the any moisture content.The petri dish was weighed without and then with the sample.5 grammes of sample was maintained at 135 degrees for 2 to 3 hours.The sample was cooled, weighed, and the results were recorded.Finally, the calculations were completed by putting all of the values into the formula, which is:

$$\text{Moisture } \% = \frac{Ws - (W2 - W1)}{Ws} X\ 100$$

Where, W_1, weight of dish

W_2, weight of dish after drying

W_s, weight of sample

Ash

Ash estimation was done on the basis of AOAC 2020 where a clean porcelain crucible was taken and dried properly to remove the moisture. The crucible was placed in a hot air for drying at 105 degrees Celsius for 20 minutes. The crucible was placed in the desiccator for cooling and was weighed empty. Then, a marginalized portion of the sample was kept in the pre weighed crucible and was weighed. At 700 degrees Celsius, the sample was maintained for two to three hours in the muffle furnace. Then, the sample was taken out and was kept in the desiccator for cooling. At last, the sample was weighed and calculation was done by putting the values into the formula:

$$\text{Ash\%} = \frac{W2 - W1}{Ws} X\ 100$$

Where, W_1, weight of crucible

W_2, weight of crucible with ash

W_s, weight of sample

Fat Estimation

As per AOAC 2020, the Soxhlet method was used to calculate the fat content of the product. With a filter paper, a thimble was made and weight on the prepare thimble was noted. 3 grams of the spirulina premix sample was taken into the thimble. Then after putting a cotton inside, thimble was folded to enclose the sample. the Soxhlet extraction unit was set by placing the sample in it. About 200ml of di-ethyl ether was added into the Soxhlet flask for the process of fat extraction.The sample was kept for 6 hours of continuous cycle for the completion of theprocess.

After cooling, the thimble was removed followed by collecting the remaining diethyl ether with extracted fat into a pre weighed beaker. The beaker was then put on a hot plate to evaporate the excess solution until the extracted fat was obtained in dried form.

The beaker with extracted fat was finally weighed and the crude fat was calculated by putting the obtained values in the formula

$$\text{Crude Fibre \%} = \frac{W2 - W1}{Ws} X\ 100$$

Where, W_1, weight of flask

W_2, weight of flask with fat

W_s, weight of sample

Protein Estimation

The protein content of samples was estimated by the Kjeldahl method. 5gm of samples was introduced in digestion flask and to that 10 ml of concentrated H_2SO_4 and 5gm of digestion

mixture of K2SO4:CuSO4: Na2SO4 (equal ratio) was added. The flask was stirred to fully combine the ingredients before being placed on a heater to begin digestion until the mixture became transparent (blue green in colour). The entire procedure took three hours to complete.

The resulting solution was brought to room temperature and put to a volumetric flask of 100 ml. Distilled water was used to top up the volume.Thereafter, ten millilitres of digest were gently added to the distillation tube, along with 10 ml of 0.5 N NaOH.

Due to the presence of NH4OH, a yellowish tint emerged during distillation.Next, the distilled liquid was subjected to titration with a 0.25mol/L standard HCL solution until a pink colour was obtained. In order to determine how much titrant was utilised, the initial and final readings were recorded at this phase and marked as Vs. The nitrogen content of acetanilide or tryptophan after addition of 1 g of saccharose was determined at the titration stage for the blank, and the volume of titrant utilised was indicated as Vb. The %N in samples was calculated via the given formula followed by the calculation of % P by multiplying the %N with the protein factor (PF) that is 6.38*(Kirk et. al. 1950).*

$$\text{Protein \%} = Vs - Vb \times F \times C \times f \times M(N)\, m \times 1000 \times 100$$

Where,

Vs- volume of titrant used for sample

V_b- volume of titrant used for blank

F- molar reaction factor of titrant (HCl-1 and H2SO4- 2)

C- concentration of titrant (mol/L)= 0.25mol/L

f- Factor of titrant = 1

M(N)- Molecular weight of Nitrogen = 14.007g/mol

m- sample weight

1000- conversion factor(ml into L)

%N- % weight of N

Iron Estimation

The concentration of metals in the samples was determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) with a Perkin Elmer instrument. Before the analysis, 100 µL of the extracted samples was combined with 100 µL of an internal standard (IS). This mixture was then adjusted to a total volume of 5 mL by adding a diluent comprising a solution of 15 mL methanol + 0.005% v/v Triton X + 10 mL nitric acid, topped up to 1000 mL with double distilled water. The metal concentrations were assessed by comparing them against a standard through a linearity curve.*(Şahan et.al. 2007; Chan et.al. 2006)*

Total Carbohydrate

Measurement of total carbohydrate content in a sample is calculated based on calculations.

TotalCarbohydrate = 100% - % (protein + fat + ash + water).

Microbial Analysis

The pour plate method was employed to analyze the microbial content in Spirulina pancake premix powder. The process began with the preparation of nutrient agar, where the agar powder was mixed with distilled water, sterilized, and cooled to maintain its liquid state. Subsequently, a series of dilutions were made for the premix powder to facilitate the enumeration of viable microbial colonies. These dilutions were then aseptically transferred onto nutrient agar plates and evenly distributed. Around 15-20 ml of the cooled nutrient agar was carefully poured onto each plate to cover the surface uniformly. Following this, the prepared plates were incubated at an appropriate temperature for microbial growth, typically ranging from 30-37°C. After 24-48 hours of incubation, colonies that developed on the plates were manually counted to estimate the microbial load in the original Spirulina pancake premix powder.

$$\text{CFU} = \frac{\text{Number of Colonies Counted} \times \text{Dilution Factor}}{\text{Volume Plated}}$$

Where,

Number of Colonies Counted: The count of visible colonies on the agar plate.

Dilution Factor: The factor used for dilution of the original sample.

Volume Plated: The volume of sample plated onto the agar plate.

3.Packaging of product

The premix powderwaspacked with no preservatives in silver disposable bagswhich were bought from a nearby market and were sealed to air tight to prevent spoilage from microbial growth.

Package labelling was done in this study as per Food Safety and Standard (Packaging & Labelling) regulation, 2011. The label was prepared with all the necessary details on Microsoft word and the printout was then pasted on the packets as finishing.

4. Results and discussions

The obtained results of the proximate analyse reveals that spirula pancake premix contains 4.5% of the total moisture content,12% ash, 4.6 % fat, 11.7 % protein, 67.8% carbohydrates, 5.7 % iron and 0.83% crude fibre.

According to the Food Composition Table, the premix contains 460kcal of energy, 16g of protein, 87g of carbohydrates, 3g of fat, and 8.5 g of iron which is good enough to fulfil the requirements of nutrition in one meal.

It was found that there was less microbial growth which was 110 cfu/ml .Air tight sealing in silver plastic bags provided the same atmosphere to the food, hence prevent microbial contamination and increase the shelf life of the product.

Also, the study did not include all the labelling parameters as the product was produced on experimental basis and not for commercial purpose.

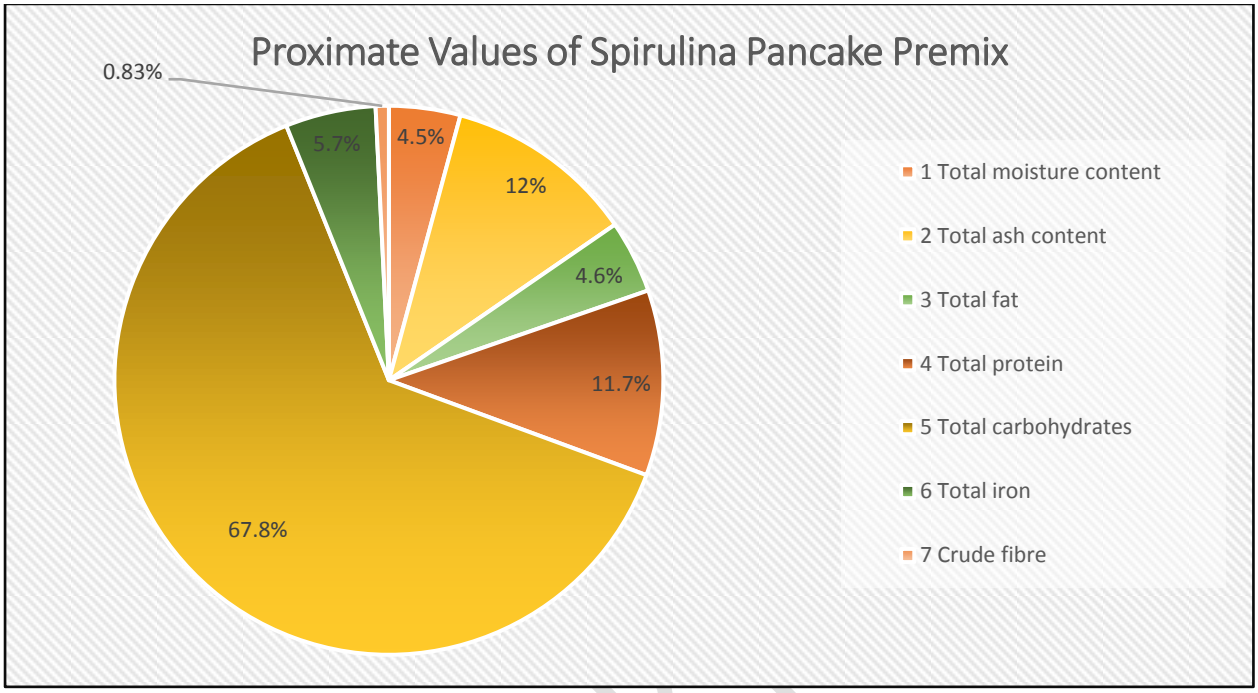

Figure 2.Diagram showing Proximate values of Spirulina Pancake Premix

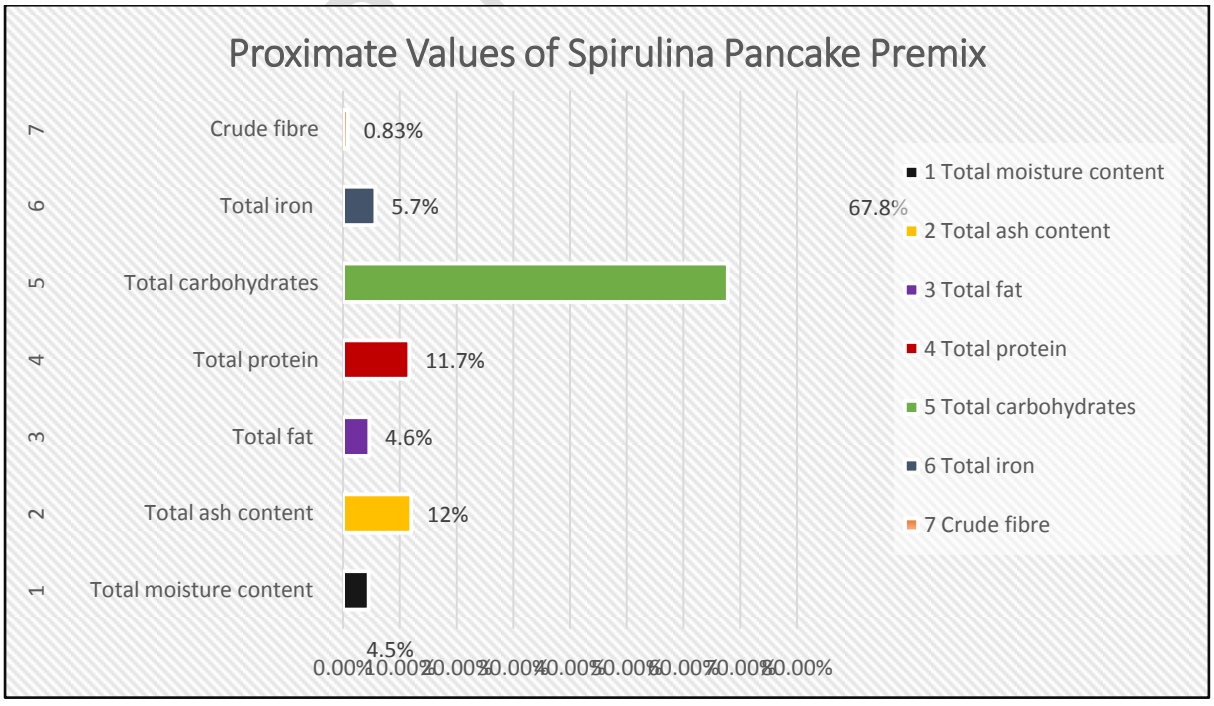

Figure 3. Bar graph showing Proximate values of Spirulina Pancake Premix

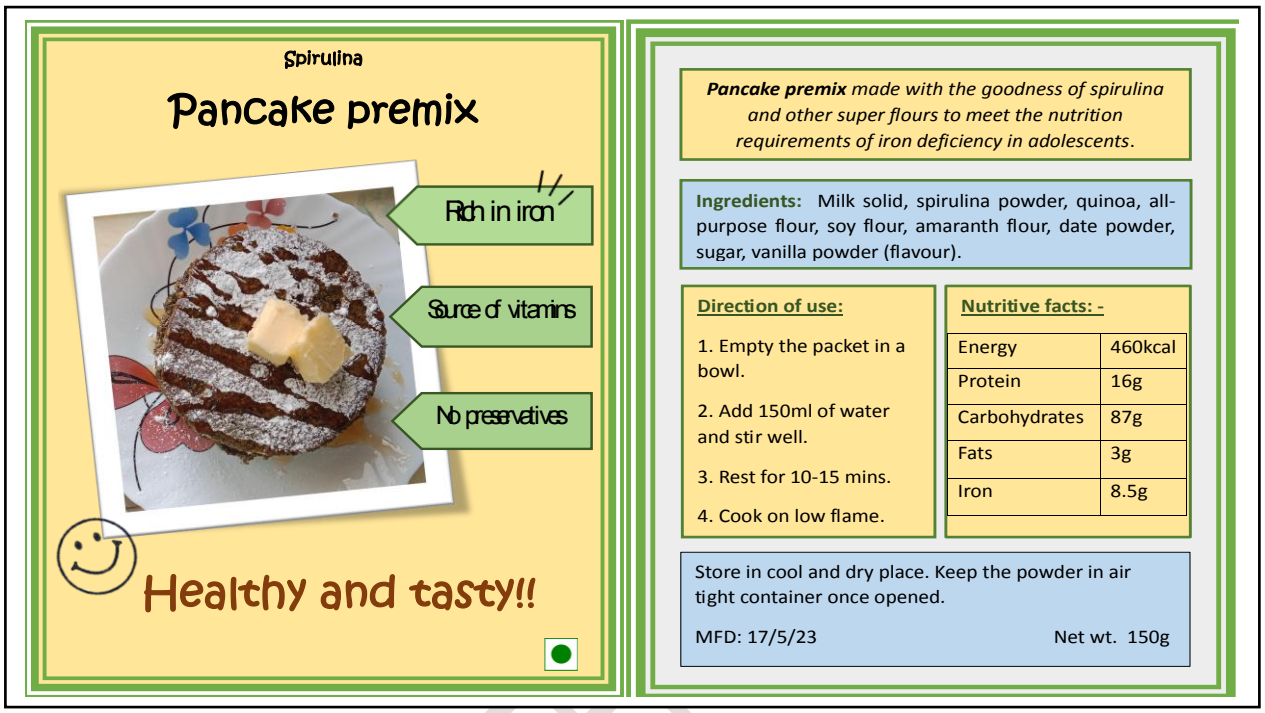

Figure 4.Label forpackaging (Front and Back)

5. Conclusion

The development of an iron-rich dietary supplement in the form of a spirulina-based pancake premix, incorporating quinoa, soybean, and amaranth, presents promising prospects for combating nutritional deficiencies, especially anemia among adolescent girls. The comprehensive nutritional analysis revealed substantial protein, iron, and other vital nutrients within the premix, showcasing its potential to fulfill significant dietary requirements in a single serving. The limited microbial growth observed, owing to the effective airtight packaging, suggests an extended shelf life and enhanced safety of the product. These findings underscore the feasibility and convenience of utilizing this premix as a convenient and nutritionally dense supplement, highlighting its potential significance in addressing specific dietary deficiencies, particularly in vulnerable demographic groups. Further studies focusing on broader applicability, taste optimization, and clinical trials would reinforce its viability as an accessible solution for nutritional support.

6. Disclaimer

The goods employed in this study are widely and mostly used in our research area and country. There is no conflict of interest between the writers and manufacturers of the items because we want to use these products for the advancement of knowledge rather than for litigation. Furthermore, the research was not supported by the production firm, but rather by the authors' own personal efforts.

9. References

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