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

Abstract

Today, food industries as well as the consumer are in search of nutritious food options which also sustainable. Also, it is important to provide low-cost functional food keeping in mind each group of society suffering from various deficiencies like anemia. Studies highlight spirulina's diverse health benefits, spanning antioxidants, immune support, anti-inflammatory effects, and potential aid against malnutrition, obesity, diabetes and anemia. 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. The study involved the use of spirulina for preparing pancake premix by incorporating with other super flours such as quinoa, soy flour and amaranth. This would require no expert skills and is less time consuming. After conducting the nutritional and microbial analysis, it was concluded that the prepared pancakes were nutritionally rich and were able to understand the shelf-life study of the product when stored for longer period of time. The final product was packaged well in the silver bags. The packaging looked neat and clean and was capable of preserving the product's shelf life. The objective of the present research was intended to develop Spirulina pancake premix which could be utilized as nutritional supplement for anaemic adolescent girls.

Keywords:

~~Anemia~~, ~~Spirulina~~, ~~Premix~~, ~~Nutritional analysis~~, ~~Microbial~~ analysis

1. Introduction

Foods which are nutritionally dense and sustainable are high in demand for the food industries. It gets more serious to provide low-cost functional food when it comes to cope up with the deficiencies like anemia so to provide the needful 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).* There is extensive documentation on indigenous populations across various regions using S. platensis as a food source *(Sarra Bensehaila et.al, 2015).* The concentrated nutrition of spirulina makes it an excellent food supplement for people of all ages and lifestyles *(A M Sharoba et. 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 this spirulina 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

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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).*

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 premix by 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, A et.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 Flour 20g	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 155.5g per 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 to avoid 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-brown color.

- **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.

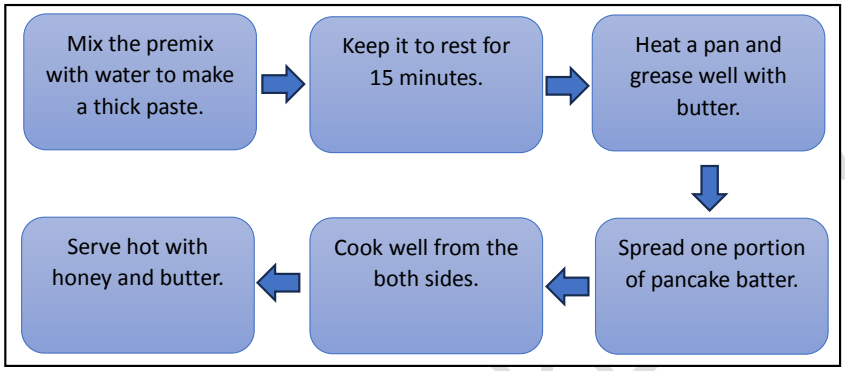

Figure 1. Flow diagram of pancake preparation

2.4 Nutritional analysis

Moisture

As per AOAC 2020, a petri plate was dried in an oven at 105 degrees Celsius for 20 minutes to 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:

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

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 the process.

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.

Total Carbohydrate = 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 powder was packed with no preservatives in silver disposable bags which 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.

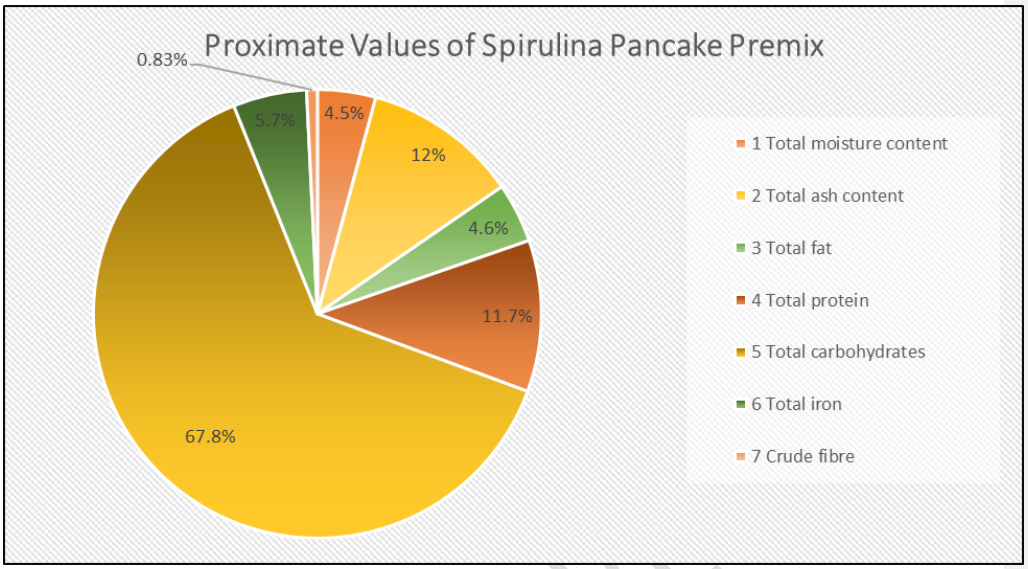

Figure 2. Diagram showing Proximate values of Spirulina Pancake Premix

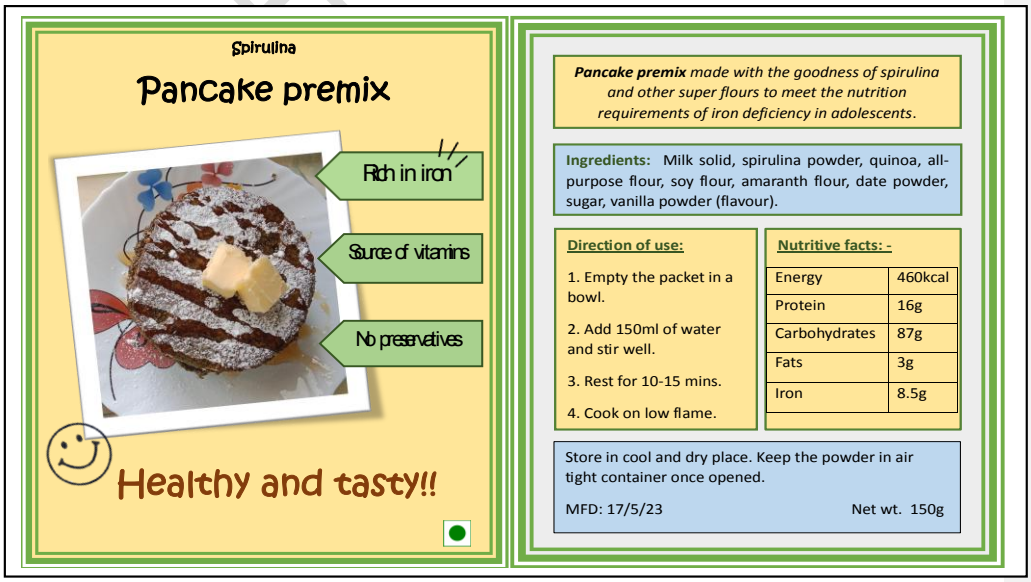

Figure 3. Label for packaging (Front and Back)

5. Conclusion

After doing the proximate analysis, it can be concluded that the prepared pancakes are nutritionally rich and are able to achieve their purpose i.e., to manage anemia in adolescent girls. Moreover, since the pancakes include a variety of food groups, it is a complete meal.

The pancakes also have high satiety value with acceptable sensory characteristics i.e., taste, colour, odour, texture etc. One can consume these pancakes on a regular basis without worrying about the side effects as the premix powder is made with all the natural and high-quality ingredients. Not just adolescents, these pancakes are beneficial to anyone of any age suffering with iron deficiency. One can also have them as evening snack or for school going adolescents and children, it is a great way to pack all the essential nutrients their lunch box.

Moreover, less microbial growth shows that the product is safe to consume and can be stored for a longer duration in an appropriate atmosphere. The final product was package well in the silver bags. The packaging looked neat and clean and was able to maintain the shelf life of the product.

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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