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ACCEPTABILITY AND EVALUATION OF IRON RICH CHOCOLATES DEVELOPED USING LOTUS STEM (NELUMBONUCIFERA) AND DATES (PHOENIX DACTYLIFERA) FOR ADOLESCENT GIRLS

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ABSTRACT

According to the World Health Organization (WHO), anemia is defined as hemoglobin levels <12.0 g/dL in women and <13.0 g/dL in men. The frequency and severity of iron deficiency anemia signifies a major health problem. The adolescent girls are the worst hit by this kind of anemia mainly due to their inability to take an iron rich diet and also to compensate the losses incurred due to menstruation. This study was done keeping the high demand of chocolates among adolescents which encourages development of an iron rich product made using iron rich ingredients while taking base as chocolate. The study on acceptability and evaluation of iron rich chocolates using lotus stem and dates for adolescent girls was done in four stages. The first stage involved procurement of iron rich raw materials (lotus stem and dates). In the second stage, the product was developed using lotus stem powder, dates and edible chocolate. The third stage involved gauging its acceptability using composite score card by trained panelists. Proximate analysis was done to calculate protein, carbohydrate, fat and iron content in the product. Three samples were made by varying the quantities of the ingredients. The second sample which was Sample B had a mean overall acceptability score of 15.7±2.26 and showed an increase in the iron content as compared to the standard chocolate which had an overall acceptability score of 14.7±4.44. This product hence could be given to girls to counter the effects of iron deficiency anemia and increase their nutritional status.

KEYWORDS: Iron deficiency anaemia, adolescent, menstruation, iron rich chocolates.

INTRODUCTION

Iron had early medicinal uses by Egyptians, Hindus, Greeks, and Romans. During the 17th century, iron was used to treat chlorosis, a condition often resulting due to iron deficiency.In the human body, iron mainly exists in complex forms bound to protein (hemoprotein) as heme compounds (hemoglobin or myoglobin), heme enzymes, or non-heme compounds (flavin-iron enzymes, transferring, and ferritin). The body requires iron for the synthesis of its oxygen transport proteinsand for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reduction reactions.

Dietary iron occurs in two forms: heme and nonheme. The primary sources of heme iron are hemoglobin and myoglobin from consumption of meat, poultry, and fish which ishighly bioavailable (15%-35%) and dietary factors have little effect on its absorption, whereas non-heme iron is obtained from cereals, pulses, fruits, and vegetables and iron absorption is much lower (2%-20%) and is strongly influenced by the presence of other food components.

Iron requires an acidic environment to help aid its absorption into the body. Thus, ascorbic acid overcomes the negative effect on iron absorption of all inhibitors and increases the absorption of both native and fortification iron. The enhancing effect of ascorbate is largely due to its ability to reduce ferric to ferrous iron. Phytate is the main inhibitor of iron absorption. The negative effect of phytate on iron absorption is dose dependent. Polyphenols occur in various amounts in vegetables, fruit, some cereals and legumes, tea, coffee, and wine. In cereals and legumes, polyphenols add to the inhibitory effect of phytate. Calcium showns negative effects on nonheme and heme iron absorption, which makes it different from other inhibitors that affect nonheme iron absorption only. Dose-dependent

inhibitory effects are seen when 75-300 mg calcium is present.

The most significant and common cause of anemia is iron deficiency. If iron intake is limited or inadequate, anemia may occur. This is called iron deficiency anemia. Iron deficiency anemia can also occur when there are stomach ulcers or other sources of slow, chronic bleeding like colon cancer, uterine cancer, intestinal polyps, hemorrhoids, etc, low intake of bioavailable iron, increased iron requirements as a result of rapid growth, pregnancy, menstruation, and excess blood loss caused by pathologic infections, such as hook worm and whipworm causing gastrointestinal blood loss and impaired absorption of iron.

Typical features are lowered oxygen delivery to the tissues, and include pallor, fatigue, apathy, fainting, and breathlessness. Hence, it becomes important that care is taken to provide a diet that is rich in iron or to prepare iron rich foods or snacks that are easily available and can be consumed at anytime of the day like a chocolate which has been used to treat a wide variety of ailments, and in recent years, multiple studies have found that chocolate can have positive health effects, providing evidence to centuries-long established use.

Certain components of foods or foods in combination can be used that help in increasing the nutritional value of the product. Lotus stem and dates have been known to be rich in bioavailable iron along with certain other essential minerals that are required by the human body but still they are not popular enough to be incorporated in the diet daily so as to overcome the effects of iron deficiency anemia.Hence, a radical change of strategy is required to help the adolescent girls present in the population to combat iron deficiency anemia. Using a popular food item like chocolate and incorporating the highly bioavailable ingredients could help in mitigating the problem of iron deficiency anemia highly prevalent in the developed as well as the developing world.

METHODOLOGY

The present study was aimed at developing an iron rich product while taking chocolate as the base ingredient. The study is divided into different phases for the ease of performing.

Phase 1: Formulation of the lotus stem powder.
The first phase of the study aimed at the formulation of lotus stem powder. The lotus stem was washed with boiling water to remove all the mud and other impurities. It was left to drip for a while. It was cut into thin slices, and put into the microwave to remove all the moisture content from it. It was then ground to form a powder which would be used later.

Phase 2: Development of chocolates using lotus stem and dates and its samples using their different proportions.

The second phase involved the formation of iron rich chocolates, using food chocolate as a base. Three samples were prepared along with the standard version of chocolate by varying the quantities of the lotus stem and dates (Table 1). The amounts used were such that it met at least one fourth of the RDA. The food chocolate was first melted to a proper consistency for the ease of the incorporation of lotus stem powder and dates. After the incorporation, the chocolate was mixed and put into moulds to freeze for about an hour. It was broken down into further pieces to move along to the next phase of the experiment.

Table 1: Sample ingredients variations.

Ingredients	Standard	Sample A	Sample B	Sample C
Chocolate	62.5gm	37.5gm	37.5gm	37.5gm
Lotus Stem	-	20gm	15gm	25gm
Dates	-	5gm	10gm	-

Phase 3: Sensory evaluation by using Composite score card test.
The third phase involved gauging the acceptability of the chocolates that were formed. Sensory tests were important to conduct, so as to see if the product so formed would be actually accepted in the real world or not. Composite score card test was used to measure the acceptability of the chocolates so formed. Trained (10) panelists were chosen.

Phase 4: Determination of the nutritive value of the product using proximate analysis.
The product so formed was analyzed using proximate analysis to see the amount of carbohydrate, fat, protein and iron so obtained.Samples that were taken were kept in a protected place and care was taken to not expose it to damp air, dust or soot. The sampling instruments were clean dry and dust free when they were used. The samples were placed in air tight containers and were kept in such a way that the temperature of the material did not change in accordance with the normal temperature.

Determination of Fat
Roese-Gottlieb Method was used, wherein ammonia was used to dissolve the milk protein before fat extraction. Roese-Gottlieb Method includes the following steps:

- Introduce 4 g sample into a Mojonnier fatextraction tube or similar apparatus which is diluted to 10 rnl with water.
- 1.2 ml ammonia solution is added. Alcohol is added and mixed along with 25 cui ether and shook vigorously for about 30 seconds
- 25 ml petroleum ether was added and shook again.
- After this, ether-fat solution is drawn off. Extraction must be repeated for accuracy.

Purity of fat in sample can be tested by dissolving in a little petroleum ether. If residue remains, wash out

fatcompletely with petroleum ether, dry, weigh and calculate the mass of the fat.

Determination of Protein

The full assembly consisted of a round bottom flask of 1000 ml capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube. The other end of the bulbis connected to the condenser which is attached, by the means of a rubber tube, to a dip tube which dips into a known quantity of standard sulphuric acid contained in a beaker of 250 ml capacity. The amount of protein was determined by:

- Two grams of the material was accurately weighed, and transferred to the Kjeldahl flask, taking precaution to see that particles of the material do not stick to the neck of the flask.
- Add about 10 g of anhydrous sodium sulphate, about 0.2 to 0.3 g of copper sulphate and 20 mI of concentrated sulphuric acid.
- Heat just below the boiling point of the acid until frothing eases. Increase heat until the acid boils vigorously and digests for 30 minutes after the mixture becomes clear and pale green or colorless.
- Cool the contents and transfer quantitatively to the round bottom flask with water, the total quantity of water used being about 200 ml. Add with shaking a few pieces of pumice stone to prevent bumping.
- Add about 50 ml of the sodium hydroxide solution carefully through the side of the flask so that it does not mix at once with the acid solution but forms a layer below the acid layer.
- Mix the contents of the flask and shut off the burner and detach the flask from the condenser.
- Rinse the condenser thoroughly with water into the beaker. Wash the clip tube carefully so that all traces of the condensate are transferred to the beaker.
- When all the washings have been drained into the beaker, add two or three drops of methyl red indicator solution and titrate with the standard sodium hydroxide solution.

CALCULATION

Total protein percent by mass= 875(B-A)N/M
Where,
B=volume in ml of the standard sodium hydroxide solution used to neutralize solution in the blank determination.

A= volume in ml of the standard sodium hydroxide solution used to neutralize the excess of acid in the test with the material,
N= normality of the standard sodium hydroxide solution, and
M=mass in g of the material taken for the test.

Determination of Iron

Determination of iron content was done using atomic absorption spectroscopy. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration. The electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. Each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity.

Determination of carbohydrate (By Calculation)

Carbohydrate content of the sample was estimated by subtracting from 100 the sum of volume(per 100gm) of the moisture, crude protein, crude fat, ash and crude fiber. Calculation content= 100-[moisture+ crude protein +crude fat +ash (per 100gm)]

RESULTS AND DISCUSSION

Sensory Analysis of the product formed was done using composite score card rating test to measure the acceptability of the chocolates so formed, by trained panelists (10 in number) showed a higher overall acceptance of Sample B as compared to Standard Chocolate exhibiting a higher mean rating of 15.7±2.26 (Table 2) as compared to 14.7±4.44of standard sample in case of untrained panelists along with a lower standard deviation of 2.26 points, implying a lower dispersion and higher level of confidence of a greater overall acceptability of Sample B as compared to the Standard Sample. The chocolates that were formed were also statistically analysed using anova, which showed the higher acceptability of the Sample B with it also being statistically significant at $p<0.05$.

Table 2: Composite Score Card Test Scores.

Parameters	Standard	Sample A	Sample B	Sample C	p-value	F-value
	M±SD	M±SD	M±SD	M±SD		
Appearance	16.1±3.28	15.2±1.93	16.2±3.35	13.6±3.09	0.198	1.638
Texture	7.7±1.82	7.5±1.50	7.4±1.17	7.2±1.61	0.909	0.18
Colour	8.5±1.17	7.7±1.56	7.5±1.26	8.1±1.37	0.373	1.073
Taste	16±4.08	16.2±2.04	16.4±2.06	15.1±3.84	0.804	0.33
Aroma	7.7±1.88	6.8±1.22	7.25±1.58	7.1±1.19	0.605	0.623
Mouthfeel	7.8±1.93	7.2±1.54	7.8±1.75	7.5±1.50	0.834	0.288
Overall Acceptability	14.7±4.44	15.3±2.05	15.7±2.26	14.5±2.87	0.808	0.324

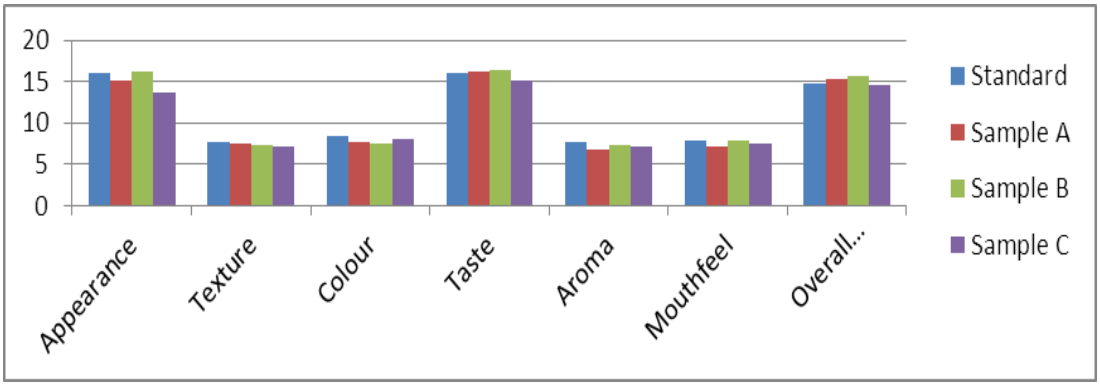

Fig 1: Mean Acceptability Scores of Chocolates.

In the proximate Analysis conducted in laboratory, the sample B showed an increase in the Iron content. The iron content came out to be of 11.61 mg in a sample of 62.5gms as compared to a standard sample of Chocolate containing just 2.46mg of Iron. Thus, Sample B showed almost a 7.3 times increase in iron content than a standard chocolate sample. Even though Sample B had an iron content lower when compared to Sample A, the Sample B proved to be better in terms of its acceptability and showed statistically significant results with $p<0.05$.

Table 3: Proximate Analysis Scores of Chocolates.

Parameters	Standard	Sample A	Sample B	Sample C	p-value	F-value
	M±SD	M±SD	M±SD	M±SD		
Energy	147.46±0.57	146.26±0.57	139.33±1.15	146.26±0.57	<0.01	70.789
Carbohydrate	36.51±0.75	36.31±0.57	34.66±0.57	36.31±0.57	0.022	5.723
Protein	2.13±0.01	3.53±0.05	3.41±0.11	3.58±0.11	<0.01	190.122
Fat	19.08±0.57	11.48±0.25	11.33±0.06	11.44±0.11	<0.01	424.637
Iron	2.46±0.05	14.27±0.57	11.61±0.57	16.27±0.57	<0.01	445.358

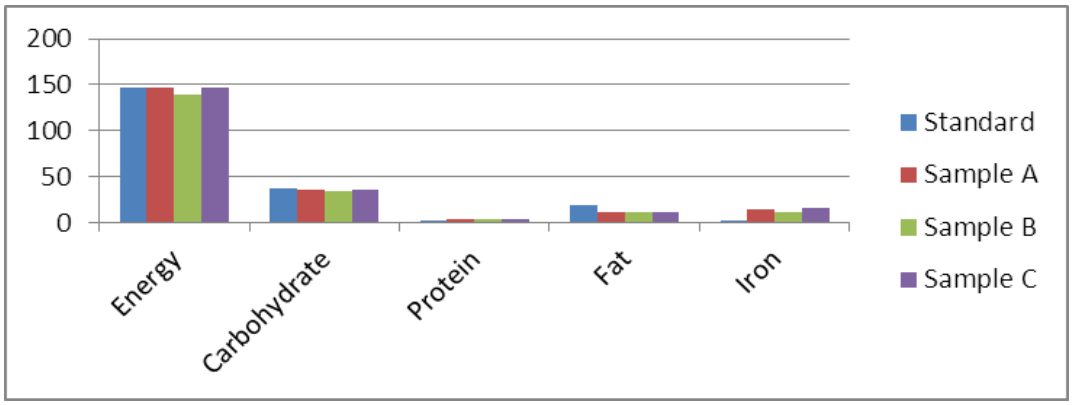

Fig 2: Proximate Analysis Scores of Chocolates.

CONCLUSION

Thus, among the three samples made, Sample B showed a better overall acceptability as compared to other samples as well as with the Standard Chocolate sample in the composite score card test. Also, the proximate Analysis showed iron content in Sample B to be around 7.3 times (18.052mg in 100 gms) the iron content in a standard chocolate sample (3.9mg in 100gms). Thus, Sample B product could be given to adolescent girls to counter the effects of iron deficiency anemia and increase their nutritional status.

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