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Fortification of calcium and iron with *Phoenix dactylifera* (Palm Date) and *Euryale ferox* (Makhana) in Wheat and Ragi cookies: Formulation, Physiochemical and Nutritional analysis

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Abstract
The preparation of the calcium and iron rich Wheat-Ragi cookies fortified with date palm and makhana were done with an aim of improving women and child health. The formulated cookies were prepared with varying concentrations of the date syrup, makhana powder, ragi flour and wheat flour. The samples which gave the highest sensory score in terms of taste, flavor, and texture were selected for further analysis, It was observed that the test cookies were rich in crude fiber (6.12%), phenolics (876µg/mg GAE) and antioxidants (78% DPPH radical; scavenging activity), while low in fat (10.08%) when compared to the control cookies (wheat-ragi cookies) which contained 4.85 % fiber, 54 µg/mg GAE phenolics, and 20.68 % fat. The test cookies (P) were also high in calcium (92 ppm) and iron (2.82 mg/ g) content compared to the control cookies, thereby, making them highly nutritious.
Furthermore, the cutting strength of the test cookies was better than the controls due to the presence of date syrup in the test samples, making them softer. The ICP-MS analysis of makhana powder did not detect any metal except nickel, which was within the permissible limit for human intake.

Keywords: formulated cookies, energy content, palm-date syrup, makhana-powder, carbohydrate content, metal detection, cutting strength

1. Introduction
There are many different types of the diseases which are evolving just from our food habits and leads to the deleterious health effect. The majorly affected population is toddlers and adults which have a fast metabolism and needs a healthy diet. In the daily routine of fast –forward life, there is the need of the diet or food products which should be available to fulfill the body energy level from a small diet and can also be helpful in removal of diseases like cancer, obesity, cardiovascular diseases, stress, and breathing problems.
These different types of health related issues are leading to a low effective outcome which can be prevented from trying to have some healthy eating behavior which should be established in the childhood and maintain till adolescence and till middle age. Many young generations like to have the food which is full of fat, added sugar, carbohydrate and lower intake of vitamins, minerals, and fiber.
A cookie is a type of confectionery which is made by mixing sugar, butter**,** and milk, baking at 170 °C for 10 -12 minutes, and then let it allow to cool and settle down at room temperature and kept it in an airtight container. As moisture plays an important role in deteriorating the food texture and shelf life of the product. Fruits, nuts, caramel, candies, and other flavors are sometimes added either inside or on top of the cookies. A cookie is the confectionery item which is consumed on a very large scale. Previously studies were done the textural and sensory properties of the cookies by the incorporation of the guar gum with different water level and baking temp [1].
The process of Food fortification and enrichment includes the addition of micronutrients (essential rare elements and vitamins) to the food. It may be a purely commercial choice to provide extra nutrients in a food, while other times it is a public health policy which aims to reduce the number of the people with dietary deficiencies within a population [2].
Here, in the Palm-Date –Makhana cookies, the basic focus was to incorporate the calcium content of the makhana into the product which would benefit the bone health of an individual. Palm-Dates were used to enrich the cookies with the iron content and to make the product more fibrous. Higher the iron content would help in preventing the causes of anemia and blood-related problems, fiber contains would help in maintaining the good gut health of an individual ion, so while having snacks, grab a bite which is enriched in the calcium and iron content so that your body need of microelements should also be fulfilled. The product was formulated to basically focus on the health of the women and the young kids, as during and after pregnancy there is a drastic change in the bone calcium level of the women, so, to enhance the level of calcium and iron in the body, this product was designed.
Palm-Dates are the good source of the iron content, phenolic content, rich in antioxidants, anti-inflammatory, natural radioactivity and also rich in the dietary fiber content along with less amount of the fat [3, 4, 5]. Makhana is the very best source of the calcium and iron bath, rich in the amino acid index, rich in vitamin C, help in maintaining good bone health, and less in fat profile [6]. Food fortification with dates has been used in previously developed products like muffin, biscuits, puddings, cakes, and in candies also. Fortification with the makhana powder is also a new kind of work in the field of the food. As earlier work on makhana had been done in the form of the: formation of the resistant starch, analysis

of the physical and the hygroscopic properties of the makhana [7]. Makhana is the rich source of antioxidant and is proven to provide the health benefits. Makhana may also contain some heavy metals which came through the water in which they are grown and may also cause severe damage to health [8].

Palm-Date - Makhana Formulated Cookies: The idea of formulating this product was to enhancing the woman health before & after pregnancy as there is a drastic change in the bone calcium concentration of the person. So, as to maintain good bone health and iron content in the body, this idea was incorporated in the cookies. This would help in maintaining the good bone and blood health of the person as makhana and dates are rich in calcium and iron content.

There were at least 6-7 different formulation was tried with varying concentration of the dates and makhana powder to make the product more acceptable and desirable, but the final formulation was (P) which was most acceptable. All the previous formulations didn't work out due to the reason of hardness in the baked product and that was rectified by the replacement of the dates with date syrup and with the ingredient amount which was finally used in the product.

Here, after trying 6 different formulations of date and makhana, the final product (try 6) was finalized for making the cookies because they were best in both textural and sensory parameters and were taken for further analysis. The previous trials that contain an increase in makhana made the cookies very granular leaving a granular mouth feel, which is not acceptable. On the other hand, the increase in palm-date made them very hard and not chewable. Similarly, an increase in ragi flour also made the cookies harder.While, a decrease in wheat flour resulted in a flour with poor elasticity, which was difficult to roll out into cookies.

2. Material and Methods

2.1 Materials

Makhana, dates (for preparation of date syrup), ragi flour, baking powder, wheat flour, condensed milk, Elachi (Cardamom) and butter all the material procured from a local vendor.

The makhana powder was produced by grinding the makhana with the help of the heavy cruiser. The date syrup was made by soaking the dates in the lukewarm water (4-6 hours), messenarated and then heated to achieve honey like consistency.

The makhana and date extracts were prepared by dissolving the substrate into the solvent (petroleum ether: methanol (1:1)). Extraction was carried out on an orbital shaker for 24hours at room temperature. Solvents were evaporated under vacuum and resulting extracts were stored at 4ºC.

Preparation of cookie extracts: Cookies were broken into small pieces, crushed, weighed and dissolved in ethanol. The samples were then filtered through the muslin cloth and the filtrate was used for nutritional and phytochemical analysis.

The chemicals and solvents used in the estimation of the Physio-chemical, nutritional and phytochemical analysis were obtained from HiMedia Laboratories, Mumbai, India.

2.2 Methods

2.2.1 Phytochemical Analysis

Preparation of cookie extracts: Cookie was broken into small pieces and then crushed and weighed. Add ethanol and vortex the sample, filter through the muslin cloth and the filtrate was used for estimating the %DPPH scavenging antioxidant property and TPC.

DPPH scavenging radical activity: The activity of the different fractions for the free radical scavenging activity was measured by the DPPH radical scavenging method. DPPH solution of 0.1mM concentration in ethanol was added to 0.5ml of properly diluted phenolic extracts. The change in absorbance at 515 nm was measured after 30 minutes incubation time. The DPPH radical- scavenging activity of the phenolic extract was calculated according to the following equation.

$$\text{Radical Scavenging Activity (\%)} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Blank}} * 100$$

2.2.2 Nutritional Analysis

i) Moisture Content: A firm amount of the crushed formulated cookie sample was kept in a pre-weighed glass petri-dish and dried in hot air oven at 130ºC for 4 hours. The loss in weight was calculated as the percentage of moisture content (MC) of a sample [9].

$$\text{MC (\%)} = \frac{w2 - w1}{2w} * 100$$

Where, W= weight of the sample, a W_1= weight of sample along with dish after heating and W_2= weight of sample along with dish before heating.

ii) Ash Content: the samples were weighed before and after burning at 600º for 4-6 hours and the loss in weight were calculated as a percentage of the ash content of sample [10].

$$\text{Ash (\%)} = x = \frac{\text{weight of ash}}{\text{weight of sample}} * 100$$

iii) Protein Estimation: The Serial dilution of the samples was prepared and then the addition of biuret reagent was done. Samples were incubated for 20-30 minutes and finally, the absorbance was taken at 550nm.

iv) Crude fiber estimation: Digest the fat-free sample in the H2SO4 and NaOH by 30 minutes washing each and allow the residue to dry at 105ºC overnight and final residue was burnt at 600ºC for 4 hours, The amount of fiber can be calculated as [11]**:**

$$\text{Crude fiber} = \frac{(w2 - w1) - (w3 - w1)}{w} * 100$$

where, W**=** weight of the sample, the W_1= weight of empty crucible, W_2= weight of sample + weight of empty crucible and W_3= weight of the sample after ignition+ weight of empty crucible.

v) Determination of fat content: Dissolve sample in an organic solvent (petroleum ether) and make the double extraction so that maximum amount of fat can be extracted by removal of organic solvent at 60ºC in the oven. The final calculation of fat was done by the help of a formula as:

FAT (%) = weight of extracted fat * 100/ weight of sample

vi) Carbohydrate Estimation: Sample was prepared by serial dilution and addition of phenol and H_2SO_4 was done. The sample was incubated for 20 minutes in dark and the absorbance was taken at 490nm.The carbohydrate was calculated by following formula.

vii) Total phenolic content: Cookies were treated with the Follin Ciocalteu Regent method for the estimation of the phenolics, and expressed in terms of µg Gallic acid equivalents (GAE)/mg of dry extract.

Preparing the Food, Sample: Sample was dissolved in methanol and 1N of Follin reagent, 20% of sodium carbonate was added. Samples were incubated at room temperature and for 15-20 minutes and finally, the absorbance was taken at 725nm.

viii) Energy value: The calorific value per 200gm was estimated by the system of Atwater (kcal) using the formula:

Energy (Kcal) = (4.0 * %carbohydrate) + (4.0 * %protein) + (9.0*%fat)

2.2.3 Mineral analysis

i) Iron Analysis: Sample was weighed and turned into ash. 2.0M HCL, 0.1M KSCN was added and sample ash was washed and mixed well. Absorbance was taken at 458nm [12].

ii) ICP: Inductively coupled plasma: Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry, which has the ability to detect metals and several non-metals at concentrations as low as one part in 10 [15] (part per million, ppm) on a non-interfered low-background isotopes. Makhana sample was analyzed for the impurities using ICP from Footwear Design and Institute (FDDI), NOIDA.

iii) Calcium analysis: Preparation of the standard solution (EDTA, sodium hydroxide, dilute HCL solution and dilute NaOH solution) and reagents (buffer solution, standard calcium solution) were prepared in the fixed proportions.

Sample titration: Sample was taken in the diluted form and add a buffer to maintain pH (approx 10). A pinch of erichrome black T (till red color appeared) and titrated with EDTA (till blue color appeared)

iv) Cutting Strength: The sample was tested for Cutting strength at IARI, NEW DELHI and the amount of force (N) required to cut the cookies and the distance traveled by the blade/cutter/probe (mm) infers the cutting strength of the product.

2.2.4 Formulation of cookies

Here (table 1), after trying 6 different formulations of date and makhana, the final product (try 6) was finalized for further analysis as they were best in both textural and sensory parameters.

3. Results and Discussion

3.1 Formulation of cookies

Several different formulations were prepared with varying concentrations of the dates and makhana powder to make the cookies desirable on sensory aspects (Table 1). Many formulations were excluded due to their hardness of the final baked product because of the fiber content of dates and rectified by the replacement of the dates with date syrup and with the ingredient amount which was finally used in the product. An increase in the amount of ragi flour also made the cookies harder, while a decrease in wheat flour resulted in a batter with poor elasticity, which caused difficulty in rolling out the cookies. Some trials that contained high amounts of makhana powder made the cookies very granular leaving a granular mouth feel and hence, not acceptable. The formulation (Try 6) was finalized (Table 2) for making the cookies was mediated on the basis of its textural and sensory parameters and thereafter, taken for further analysis. Control cookies (C) were also formulated with wheat and ragi flours and did not contain any date and makhna.

Table1: Trial for selection of ingredients for cookie formulation with best sensory characteristics.

Component	Try 1	Try 2	Try 3	Try 4	Try 5	Try 6 (p)
Makhana	30gm	40gm	30gm	30gm	20gm	20gm
Date-Palm	30gm	30gm	20gm	20gm	20gm	20gm
Wheat	20	20	40	40	40	40
Ragi	20	10	20	10	20	10
Physical and sensory properties of cookies	Granular and very hard	Granular, hard and not chewable	Granular, hard and not chewable	Granular and soft	Not granular but hard and not chewable	Not granular, chewable and crunchy

Ingredients	Product (P)	Control (C)
Makhana	20gm	Absent
Date syrup	20ml	Absent
Wheat flour	20gm	60gm
Ragi flour	10gm	10gm
Condensed milk	30gm	30gm
Butter	20gm	20gm
Elachi	1 pinch	1 pinch
Baking powder	1 tb sp	1 tb sp
Castor sugar	5gm	5gm

3.2 Nutritional analysis of formulated cookies: The formulated cookies (P and C) were subjected to analysis of various nutritional parameters, as shown in Table 3. Table3: Nutritional profile of the formulated cookies

Table 2: Ingredients in finalized cookies

Nutritional content of cookies	Product(P)	Control
Moisture %	0.502±0.4	0.329±.3
Ash %	3.269±0.1	1.519±0.1
Fat %	10.08±0.07	20.68±0.07
Carbohydrate %	79.92±0.65	70.56±0.65
Total protein %	4.88±0.15	8.91±0.75
Crude fiber %	6.12±0.53	4.85±0.40
Energy (kcal)	429.92±0.87	459±0.73
DPPH %	78.356±0.49	27.756±0.51
Iron (mg/g)	2.82	0.524
Calcium(ppm)	92	54
Phenolics content (µg/ml)	92	54

As can be seen from the Table 3, the fortified cookies are a rich in calcium, iron, ash content, dietary fiber, carbohydrates, phenolics, and antioxidants compared to the control cookies.

The reduction in protein in the formulated product and its controls was may be due to a combination of makhana and date syrup and their structural properties which cause the loss of protein. Proteins are also used to make various bio-molecules like enzymes, hormones, neurotransmitter, and many other tiny molecules that serve different important functions [13].

The iron content of cookies would help in the metabolizing proteins and plays an important role in the production of hemoglobin and red blood cells. The deficiency of the iron can give birth to different types of the diseases like anemia, chronic anemia, cough and pre-dialysis anemia. The major health benefits of iron include the eradication of different causes of fatigue and helps in maintaining the good blood health [14].

The daily intake of calcium and iron for healthy adults and children above 4 years is 1000 mg and 10 mg, respectively. Therefore, by just munching few of the formulated cookies can enhance the calcium and iron intake of the population along with providing other health benefits like high fiber and ash.

3.3 Cutting strength of cookies

Table 4: Cutting strength of the cookies.

Sample	Cutting strength (N)
Product (P)	41.19
Control	79.25

As can be seen from the Table 4, the force applied for cutting the cookies was more in the control than in the test samples. This is due to the hardness of the control cookies containing only wheat and ragi. While, the presence of date syrup in the fortified cookies made them softer, and hence required lesser force for cutting.

The cutting strength of cookies showed a similar trend for hardness and breaking strength of cookies. The increase in cutting strength of cookies may be due to the relatively higher water content in the dough [15].

3.4 ICP: Inductively coupled plasma

Table 5: ICP-MS Analysis of heavy metal present in makhana

Test name	Result (ppm)
Chromium	Not detected
Cobalt	Not detected
Nickel	5.22
Arsenic	Not detected
Cadmium	Not detected
Antimony	Not detected
Lead	Not detected
Mercury	Not detected

As can be seen from Table 5, nickel present in the sampled makhana was under the permissible limit and as per the daily intake of Ni is ranged from 0.0001-0.0024 mg/kg/day can be estimated using a reference body weight of 70 kg. The source of Nickel may the water in which it was grown and according to the table the presence of nickel was 5.22ppm, hence, it does not pose threat to human health.

4. Conclusion

Nutritional analysis of formulated wheat-ragi cookies with palm-date syrup and makhana powder showed that the cookies are rich in fiber, ash, calcium, iron, carbohydrates, phenolics, and antioxidants, while low in fat. Being rich in the dietary fiber and carbohydrates, it shall provide adequate energy after physical workouts. Diets rich in carbohydrates and low in fat can be helpful in reducing weight by keeping the cholesterol under control. The high iron content in the cookies will help combat iron deficient anemia to some extent, especially in young children and women. The high calcium of the cookies will help build stronger bones while increasing % of phytochemicals will provide anti-oxidant, anti-aging, anti-inflammatory properties. Henceforth, the formulated cookie shall prove to benefit a wider range of people ranging from children to working adults.

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