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RESEARCH PAPER

Quality characteristics of bioactive iron and its supplementation in females of childbearing age

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Key Message: Blackstrap molasses as waste material of the sugar industry has high bioactive iron which possesses better combating ability against anemia in reproductive females and can be successfully utilized in food industries for value addition to overcome the nutrient deficiencies in populations.

Abstract: An experiment was conducted using molasses (abundant waste material of the sugar industry) and synthetic sources of iron to evaluate storage stability, nutritional quality and sensory acceptability of different molasses based products. The blood of 787 pregnant, 862 non-pregnant and 862 lactating volunteers were examined for hemoglobin levels, where the frequency of anemic (Hb level less than 11 g/dL) was 38.00, 36.00 and 37.00 %, respectively. The blood samples investigated for other biological indicators marked them iron deficiency anemia (IDA). It was exhibited that 70.29 % pregnant, 65.92 % non-pregnant and 67.71 % lactating females were found to have IDA. During supplementation study, the significant effect of bioactive and synthetic iron against IDA among women of reproductive age group was noted. Stability study of different treatments at the beginning and after 60 days of storage showed a variation in different parameters. Regarding inorganic micro-elements, iron (Fe) was high in T_3 (treatment containing ferrous sulphate) followed by T_0 (control) and T_2 (treatment consisting of molasses with other food items) after the prescribed storage interval. During acceptability studies, the treatment T_2 fetched the highest scores that varied non-significantly during storage. Treatment containing a mixture of molasses along with other food items revealed greater improvement in Hb regeneration and total iron-binding capacity (TIBC) of all groups of women. Hence, it has been concluded that inorganic residues and acceptability of products comprising of a mixture of bioactive iron foods was greater as well as it has been found safe and effective in reducing IDA among females of reproductive age.

Keywords: Anemic women, Blackstrap molasses, Iron deficiency anemic, Natural foods, Value addition

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Introduction

Iron is a mineral matter which is indispensable for the growth of living things. The deficiency of iron has a negative effect on the metabolism of humans and animals (Zargar et al., 2015; Ogłuszka et al., 2020). Various findings revealed that anemia is closely linked to iron, vitamin A, B group vitamins and folate (Mahmood, 2014). Food components especially ascorbate, folate and B group vitamins have a significant role in hemoglobin regeneration and their inadequate dietary intake can lead to severe hemoglobin deficiency (Andres, 2018). Hence, these food nutrients should be taken in a balanced proportion to overcome iron deficiency. The iron binds with a blood protein (Transferrin) which absorbs and carries iron with the help of its exterior receptors. During imbalanced food intake, transferrin cannot bind extra iron particularly under excess dietary intake, because under these circumstances further iron is not taken up by circulating blood protein and is termed as non-bound iron. This excessive non bound iron may be deposited in the liver, heart and kidneys and can cause severe toxicity in the vital organs of the human body (Wessling-Resnick, 2017). Iron absorption in the body is largely influenced by various food components and phytonutrients of innate origin which are categorized into iron inhibiting or iron enhancing substances (Cepeda-Lopez et al., 2015; Zhang et al., 2021). Sugar industries in Pakistan annually produce more than 30 million tons of blackstrap molasses (B. molasses). Fumaric acid present in B. molasses has a good role in iron-chelating and resultantly iron absorption and gastrointestinal discomfort are improved in the patients. Hence, B. molasses can be widely used in subjects suffering from iron deficiency anemia due to its higher acceptability level among them (Tunuguntla & Sullivan, 2004). B. molasses is the by-product that is obtained as black concentrated syrup during sugarcane processing in the sugar industry and is a good bioavailable iron source that contains more than 250 mg iron per Kg. It is a rich source of microelements that supply greater content of highly bioavailable iron as compared to meat, besides that it provides less energy and is free of fat too (Jain & Venkatasubramanian, 2017). Synthetic iron preparations are in common use to enhance iron status which leads to abdominal problems but molasses relieve gastrointestinal tract (GIT) problems, promote iron reserves and improve body energy levels.

Molasses is beneficial in the menstrual period, lactation, expecting phase and also in nourishing kids. The experiments conducted on molasses for control of iron deficiency anemia by raising serum iron levels and low efficacy of common iron supplements designates molasses a good option and safe substitute (Tunuguntla & Sullivan, 2004). B. molasses is free of poisonous elements and contains maximum quantity of bioavailable iron. Moreover, it is economical, a balanced treasure of minerals and a waste material of the sugarcane industry which also boost up the immune system (Rahiman & Pool, 2016; Jain & Venkatasubramanian, 2017).

Dates fruit are consumed all over the world especially in the developed countries as it has several advantages such as appealing taste, tang, aroma, consistency and can be added in munchies, bakery products, fermented foods, juices and syrups. These are also a concentrated source of microelements that retain supreme position in enzymatic actions, metabolic activity, chromosome making, hemoglobin regeneration, bone composition and strengthening. Dates fruit is an abundant source of minerals such as iron (Fe), potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P). Dates consist of 0.58 mg/100 g iron and its role in red blood cell production is well known. Apricot fruit originates from the *Rosaceae* family that is very useful for human health and is widely grown in Pakistan, USA, Iran, Russian and Mediterranean territories. It has good therapeutic potential and can be used in the treatment of many diseases like iron deficiency anemia, asthma and hemorrhagic toxemia (Yilmaz et al., 2012). Apricots contain 400 mg/kg iron content and 3.0 mg dry ferrous sulphate powder contributes one mg iron (Baird-Gunning & Bromley, 2016). Considering this information regarding blackstrap molasses rapeseed, dates and apricot useful against IDA, an experiment was conducted using food-based iron sources and standard iron preparation to explore storage stability, nutritional quality, acceptability of developed product and its impact on nutritional status of anemic childbearing women (15-50 years).

Materials and Methods

Procurement of raw materials and preparation of treatments

The B. molasses was purchased from Madina Sugar Mill, Chiniot (Punjab, Pakistan). Chemicals were purchased from scientific stores while dried fruits and the other material was bought from the common market. Treatments of T_0 (Placebo in capsule), T_1 (blackstrap molasses alone in sachet), T_2 (a mixture of blackstrap molasses and other iron-rich foods in sachet) and T_3 (dried ferrous sulphate powder in capsule) were prepared by using automatic capsule filling and sachet packing machine according to the formulation given in Table 1.

Table 1 Various treatments of iron intervention

Treatments	B. molasses (g)	Dried dates (g)	Dried apricots (g)	Rape seeds (g)	Ferrous sulphate (g)	Lactose/filler (g)	Available iron (mg)
T_0	-	-	-	-	-	0.30	0.00
T_1	24.00	-	-	-	-	-	04.5
T_2	17.20	3.0	3.0	3.0	-	-	04.5
T_3	-	-	-	-	0.02	0.24	04.5

T_0 = Control i.e. 0.30 g lactose as filler in amber color capsule; T_1= 24.00 g B. molasses supplied 4.5 mg iron (Fe) packed in red sachet; T_2 = 17.20 g B. molasses plus 3.0 g dates powder, 3.0 g apricots powder and 3.0 g rape seeds powder supplied 4.5 mg Fe packed in green sachet; T_3= 0.02 g $FeSO_4$ powder plus 0.24 g lactose filler supplied 4.5 mg Fe in green color capsule; B. molasses = Blackstrap molasses.

Proximate analysis, minerals profile, calorific value, sensory evaluation and storage stability of treatments

Proximate composition of all treatments was done at baseline and after every 15 days for a total of 60 days according to the methods given in Association of Official Analytical Chemists [AOAC], (2000). Similarly, microelements such as iron, calcium, potassium, magnesium and copper in all treatments were determined through Atomic Absorption Spectrophotometric technique (Unicam, UK) after digestion of samples according to the method of Richard (1969) while the caloric content of each treatment was assessed by using a combustion calorimeter (CAL 3K, DDS, South Africa) and organoleptic acceptability of treatments was tested using 9-point hedonic scale by qualified judges by adopting the method of Larmond (1977).

Efficacy study

The current research experiment including human volunteers was carried out following international guidelines and its approval was issued by the Ethical Committee of Home and Food Sciences Department, GC University, Faisalabad. Written consent from the agreed volunteers and approval of the concerned authorities for conducting the research study on human subjects was taken. The pregnant, non-pregnant and lactation phase women of 15-50 years age group whose blood and serum analysis revealed Hemoglobin, Serum ferritin level, serum iron (Fe) level, transferrin saturation (TS) and mean corpuscular volume (MCV) less than11.0 g/dL, 12 μg/L, 60 μg/dL, 15% and 80 femtoliter/fL (volume of red blood cell), respectively while serum total iron-binding capacity (STIBC) levels more than 350 mg/dL (Anand et al., 2018; Restrepo-Gallego et al., 2020) were chosen to be the volunteers for the present research trial. The female volunteers in this study were selected from the 15-50 years age group because iron deficiency is more prevalent in childbearing age that ranged from 15-49 years (Kubuga et al., 2019). However, subjects suffering from hepatitis B, hepatitis C, abnormal liver function test, abnormal renal function test, social constraints, hemorrhages, abnormal menstrual cycle and bleeding were discarded from the participant of the current trial. Deworming of the recruited participants of the study was done by the administration of standard and additional doses of Praziquantel (40 mg/kg body weight) so that effectiveness of supplementation may not be compromised. The location of the research trial was finalized after the permission of relevant higher-ups, consisting of twenty Government centers of the Population Welfare Department, Faisalabad, Punjab, Pakistan. A two-stage sampling technique was adopted for the volunteer's assortment.

The sample size for pregnant, non-pregnant and lactating women was calculated by the following formula:

$$n = t^2 X p (1 - p) X / m^2$$

In this formula, n denotes requisite sample size and m is error margin while t is confidence interval and p is reported incidence of IDA that was 27 % & 20 % among pregnant and non-pregnant females in Punjab-Pakistan and 22.5 % for lactating women in Pakistan (Al-Buhairan & Oluboyede, 2001). By applying said equation, the requisite sample was 7531 (Pregnant = 2359, Non pregnant = 2585 and Lactating = 2587) women.

Taking into account the financial resources and expediency, 2511 volunteers (one-third of the calculated sample) arbitrarily chosen for the ongoing research activities (Pregnant = 787, Non-pregnant = 862 and Lactating = 862). Hence, the blood of pregnant women (787), non-pregnant women (862) and lactating women (862) were tested to cull them for cutoff Hemoglobin concentration using Hemocue Hb Photometer, Sweden. Resultantly, 38.50 % of Pregnant women (n=303), 36.43 % non-pregnant women (n=314) and 37.00 % lactating women (n=319) were found to have anemia with a hemoglobin level less than 11 g/dL and the above-mentioned samples were additionally evaluated for complete blood count, serum ferritin, serum iron, TIBC, TS and MCV to establish IDA prevalence. Moreover, 213 (70.29 %) pregnant, 207 (65.92 %) non-pregnant and 216 (67.71 %) lactating women had IDA and, 12 (5.62 %) pregnant, 4 (1.93 %) non-pregnant and 9 (4.17 %) lactating women were excluded from the study due to social constraints, HbV +, HcV +, abnormal LFT, abnormal RFT and Hemorrhoids. Finally, 200 subjects from each group were randomly selected for uniformity of sample and assorted into 4 sub-groups as per treatments.

Blood sample of each volunteer was taken in 2 labeled blood vials which were lined with Al film, packed in an ice container and sent to Nutritional Analysis Lab, Government College University, Faisalabad-Pakistan for determination of blood group (prevalence of IDA in blood type), complete blood count, serum iron, serum ferritin and TS following standard techniques as reported by Sharami et al. (2017).

Impact of various treatments on anemia indices

Pregnant, non-pregnant and lactating women were screened regarding IDA according to the standard criteria given in Fig. 1(a-c). Treatments were provided to selected women of the childbearing group for three months so that 50% of requirements of Recommended Dietary Allowance regarding Fe can be fulfilled i.e. daily three capsules/sachets (pregnant women), two capsules/sachets (Non-pregnant women) and one capsule/sachet (lactating women) except placebo (Table 1).

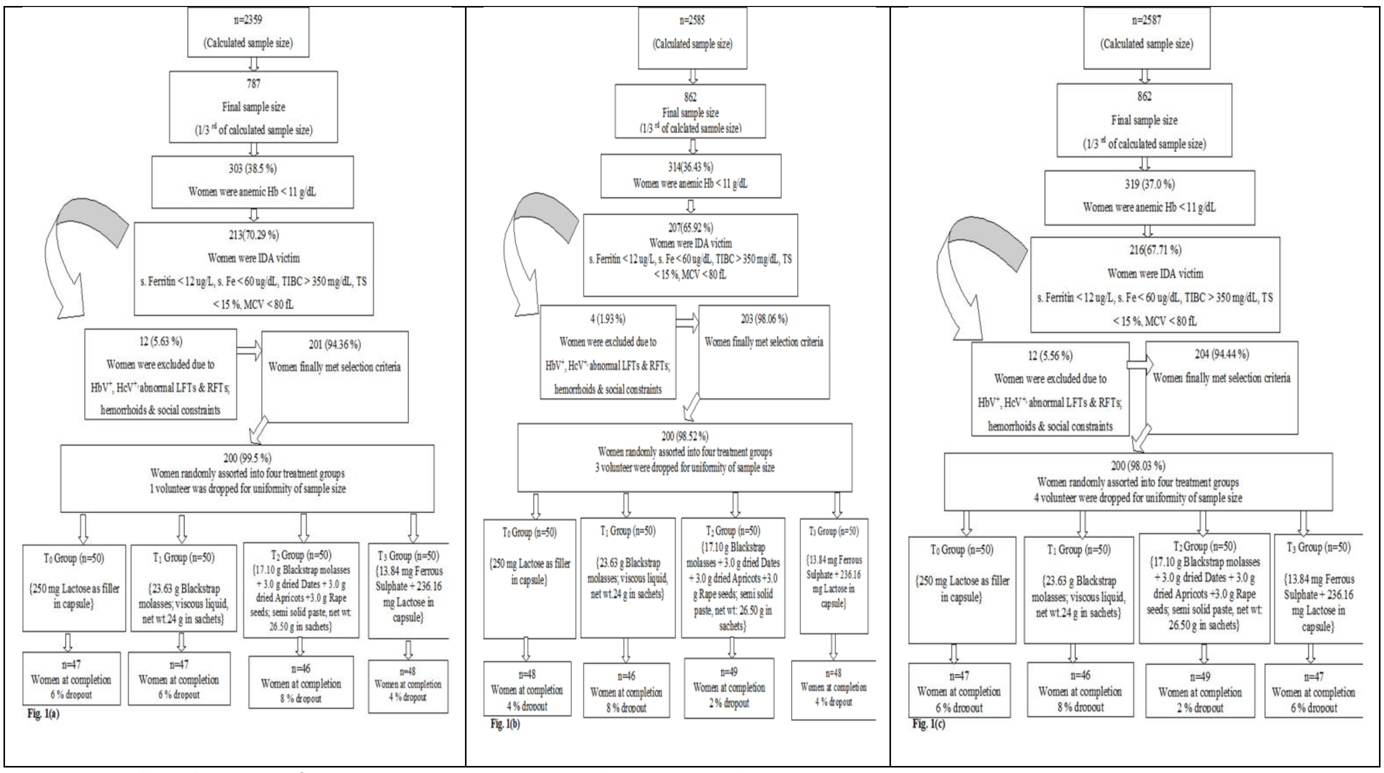

Fig.1 (a-c) Flow diagram of pregnant, non-pregnant and lactating volunteer' screening and assortment

Questionnaires like food frequency and daily intake diary were the instruments for assessing and calculating the dietary intake of volunteers before and throughout the study period. Various important parameters like disease etiology, manifestation, BP, volunteer's temperature and respiratory rate of the subjects were recorded during the trial (Herranz-Lopez et al., 2019). All the volunteers were divided into four different treatment groups following random distribution. Every subject ingested her specified treatment as capsule/sachet so that daily 50% Recommended Dietary Allowance of all volunteers for iron was obtained. Each subject regularly went to the Population Welfare Center after a week and received a pack of her treatment that was sufficient for seven days and returned her daily intake diary. After the administration of intervention for 90 days, every subject visited again Population Welfare Centers and their anthropometric measurements, caloric intake and vital signs and symptoms were recorded. Blood samples of the volunteers were retrospected and evaluated as done previously.

Statistical analysis

To analyze and evaluate the present trial recorded data, SPSS-20 software was used so that data significance can be assessed. Data distribution and frequency were statistically checked by using descriptive statistics. Figures and tables were made from the statistically analyzed data and results were interpreted so that conclusions can be made with solid logic. Data were analytically tested by the technique of one-way ANOVA and with the help of least significance difference examination; group significance was identified and formulated. Pearson's correlation was used to correlate the various variables amongst the studied groups (Steel & Torrie, 1997).

Results and Discussion

Effect of treatments on proximate composition

The proximate composition of various iron treatments was tested for 60 days at every fortnight interval. All proximate variables were significantly affected by treatment but the storage span did not behave so. After two months of storage, treatment T_1 revealed the highest moisture, while placebo treatment had the least moisture as shown in Table 2. Treatment T_1 exhibited high moisture because it was composed of blackstrap molasses which contain high moisture content (17.0 %). T_0 and T_3 were packed in capsules that were stored in an airtight and absorbent containing jar due to which variations in the moisture content of such treatments were found to be non-significant. Two other treatments of T_1 and T_2 behaved similarly because both were packed in sachets of polypropylene film that restricted moisture incorporation from the outer environment (Vartiainen et al., 2016).

The protein content of treatment T_2 was greater while it was least in treatment T_1 at the end of 60 days storage as given in Table 2. Treatment T_2 contained greater protein than any other treatment because it was a mixture of molasses, dried fruits (dates and apricots) and rapeseed which are rich in protein. An author reported 32.0-43.0% protein in dates while in apricots it ranged from 3.0-4.5% (Fatima et al., 2019) and rapeseed contains 1.00 % protein (Balalic et al., 2017). Protein content was found to be low in Treatment T_1 because it was composed of only molasses that is not its good source. Both treatments of T_1 and T_2 were filled in sachets of bi-oriented polypropylene film due to which reason their protein content did not vary significantly after storage span.

Table 2 Effect of treatment on proximate composition (%)

Proximate variables R=3	Treatments	Storage (days) 0	15	30	45	60
Moisture content	T_0	10.11±0.11^c	10.11±0.11^c	10.12±0.20^c	10.13±0.18^c	10.15±0.06^c
	T_1	22.12±0.33^a	22.14±0.46^a	22.11±0.42^a	22.19±0.20^a	22.20±0.29^a
	T_2	15.31±0.21^b	15.31±0.33^b	15.30±0.09^b	15.39±0.23^b	15.35±0.36^b
	T_3	10.31±0.10^c	10.30±0.26^c	10.33±0.10^c	10.27±0.20^c	10.40±0.00^c
Crude protein	T_0	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c
	T_1	1.51±0.01^b	1.51±0.03^b	1.50±0.03^b	1.51±0.02^b	1.52±0.01^b
	T_2	3.07±0.06^a	3.08±0.02^a	3.07±0.05^a	3.07±0.03^a	3.08±0.08^a
	T_3	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c
Crude fat	T_0	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c
	T_1	0.10±0.00^b	0.11±0.00^b	0.10±0.00^b	0.11±0.00^b	0.10±0.00^b
	T_2	2.17±0.02^a	2.16±0.05^a	2.17±0.01^a	2.17±0.05^a	2.16±0.03^a
	T_3	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c
Crude fiber	T_0	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c
	T_1	0.15±0.00^b	0.16±0.00^b	0.14±0.00^b	0.15±0.01^b	0.16±0.00^b
	T_2	2.80±0.02^a	2.87±0.07^a	2.83±0.07^a	2.84±0.02^a	2.84±0.04^a
	T_3	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c	0.00±0.00^c
Ash content	T_0	0.00±0.00^d	0.00±0.00^d	0.00±0.00^d	0.00±0.00^d	0.00±0.00^d
	T_1	10.45±0.20^b	10.50±0.24^b	10.46±0.15^b	10.44±0.17^b	10.46±0.06^b
	T_2	10.64±0.11^a	10.70±0.14^a	10.63±0.09^a	10.65±0.19^a	10.64±0.11^a
	T_3	1.79±0.01^c	1.80±0.01^c	1.82±0.01^c	1.78±0.01^c	1.82±0.01^c
Nitrogen free extract	T_0	89.89±0.91^a	90.10±1.10^a	89.88±1.87^a	89.87±2.08^a	89.95±1.81^a
	T_1	65.67±1.28^c	65.64±1.02^c	65.65±0.82^c	65.64±0.38^c	65.56±0.51^c
	T_2	65.98±1.06^c	66.05±0.98^c	65.98±1.28^c	65.93±1.41^c	65.93±1.59^c
	T_3	88.51±0.72^b	87.84±0.34^b	87.67±0.39^b	87.97±0.40^b	87.55±0.31^b

Data indicate mean values and standard error of means. Similar lettering of mean values indicates non-significant results. R donates replications

Fat content was recorded as highest in T_2 and lowest in T_1 after prescribed storage interval with a gradually decreasing trend as shown in Table 2. Treatment T_2 depicted the highest fat content because the food mixture has contributed high fat to this treatment. T_1 and T_2 were crammed in sachets of bi-oriented polypropylene film and therefore fat content did not show significant variation after the designated storage interval (Lorini et al., 2018).
The crude fiber was found to be highest in T_2 and lowest in T_1while all treatments revealed a rolling pattern regarding crude fiber throughout storage (Table 2). Treatment of T_2 ranked top with regards to crude fiber because different food "blend" has sizeable fiber concentration that is in accordance with the results of Alghamdi et al. (2018). Crude fiber did not vary significantly in T_1 and T_2 due to the packaging of both treatments in a sachet of bi-oriented polypropylene film that might be the reason for this stagnant pattern. An author conducted a study on fortified flour and noted a non-significant variation in crude fiber. It was also noticed that the packaging type and storage interval had not exerted any significant effect on fiber concentration. Ash content was graded to be high in T_2 and least in T_3. Moreover, an undulated variation was recorded in ash percentage throughout the storage interval as shown in Table 2.

Treatment of T_2 represented the highest ash because molasses and dried fruits are reported to be composed of inorganic material, micro minerals along with starch, fats and protein. However, T_3 depicted the least ash content that might be due to the meek quantity of lactose in the dry ferrous sulphate powder in the said treatment. Flour fortification was done using iron salts and it was found that fortification significantly affected the ash contents.

Treatment T_0 had the highest percentage of nitrogen-free extract and it was lowest in treatment T_1whie it varied non-significantly throughout the storage period as found in Table 2. It was high in T_0 than other treatments that may be the presence of only filler in this treatment. Blackstrap molasses were physicochemically analyzed in Cuba and were found to contain 88.0% nitrogen-free extract. In another study (fortification of flour with iron salt) it has been exhibited that packaging type and storage interval had no significant effect on nitrogen-free extract (Huma et al., 2007).

Effect of treatments on minerals and caloric content

The effect of treatments on micro minerals was found to be significant while storage did not behave so. After 60 days of storage, iron was maximum in treatment T_3 and less in T_2 as found in Table 3. The treatment T_3 had the highest iron content due to the reason that it had dry ferrous sulphate powder which is an affluent source of iron (Baird-Gunning & Bromley, 2016). During the entire course of storage, iron content did not change considerably. It might be the positive effect of packaging. Brazilian nuts were kept in either Aluminium film or polypropylene packaging and stored for 270 days. Moisture, protein content and mineral matter showed non-significant changes in polypropylene packaging throughout storage interval and nuts also showed superior dietetic quality at the end of the study (Lorini et al., 2018).

Micro minerals like Ca, Mg and K showed non-significant variations after the end of two months of storage and were present in the highest concentration in T_1 while lowest in treatment T_2 (Table 3). But Cu concentration was highest in the case of T_2 and its lowest value was noted in T_1 after the completion of 60 days of storage. Ca, Mg and K content was higher in the treatment of T_1 that contained only blackstrap molasses while Cu was high in T_2 treatment which was a mixture of molasses along with dried fruit and the presence of these minerals in both treatments (T_1 and T_2) might be from blackstrap molasses that is said to be the treasure of minerals along with their enhancers (Jain & Venkatasubramanian, 2017). However, the storage interval did not exhibit any significant variation on these minerals that may be by the proper packing of different treatments in capsule and BOPP. Dehydrated mushrooms were packed in four types of packaging materials like polypropylene, polyethylene without vacuum, polyethylene with vacuum and aluminum film that were stored for 3 months. Mineral matter depicted non-significant changes in all types of packaging materials after 12 weeks and subsequent packaging increased the shelf life of mushrooms (Ajayi et al., 2015).

Calorie content was significantly affected by treatment type while storage span behaved differently against this variable. At the end of storage, the calorie value of treatment T_0 was high and it was low in T_1 as given in Table 3. T_0 had high-calorie content due to the reason that it was filled with filler (lactose) that is a good source of calories. However, the calorie value of different treatments after 60 days of storage varied non-significantly that may be by the proper packing of different treatments in capsule and sachets. The calorie content of sugarcane concentrated juice products (similar to molasses) was reported to be more than 290 Kcal /100 g (Cakmakci & Tosun, 2010). An international publication reported the calorie content of molasses as 292, dates fruit as 277, dry apricot powder as 241 and rapeseed as 28 Kcal/100 g, respectively that corroborates the present results.

Table 3 Effect of retention period on micro-elements concentration (mg/100 g) and caloric content (Kcal/100 g)

Parameters R=3	Treatments	Retention period (days) 0	15	30	45	60
Fe	T_0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	T_1	23.00 ± 0.28^{b}	22.96 ± 0.02^{b}	23.11 ± 0.17^{b}	23.01 ± 0.39^{b}	23.01 ± 0.21^{b}
	T_2	21.56 ± 0.16^{b}	21.52 ± 0.40^{b}	21.45 ± 0.20^{b}	21.50 ± 0.46^{b}	21.53 ± 0.26^{b}
	T_3	1801.83 ± 9.23^{a}	1799.87 ± 4.62^{a}	1798.98 ± 4.62^{a}	1801.12 ± 8.97^{a}	1803.32 ± 8.81^{a}
Ca	T_0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	T_1	600.00 ± 9.35^{a}	602.04 ± 6.88^{a}	600.01 ± 3.46^{a}	600.02 ± 10.57^{a}	598.03 ± 14.43^{a}
	T_2	441.92 ± 8.38^{b}	443.90 ± 7.58^{b}	444.92 ± 10.79^{b}	443.91 ± 4.97^{b}	444.92 ± 7.19^{b}
	T_3	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Mg	T_0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	T_1	96.02 ± 2.00^{a}	95.50 ± 1.43^{a}	96.20 ± 1.44^{a}	96.03 ± 1.38^{a}	96.01 ± 1.20^{a}
	T_2	80.19 ± 0.92^{b}	80.57 ± 0.84^{b}	81.00 ± 0.67^{b}	80.58 ± 1.77^{b}	80.60 ± 0.93^{b}
	T_3	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
K	T_0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	T_1	1264.0 ± 29.70^{a}	1263.0 ± 9.25^{a}	1264.9 ± 27.38^{a}	1264.0 ± 28.02^{a}	1263.8 ± 28.53^{a}
	T_2	1052.7 ± 12.16^{b}	1055.9 ± 14.45^{b}	1054.9 ± 12.97^{b}	1055.4 ± 22.36^{b}	1054.9 ± 16.39^{b}
	T_3	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Cu	T_0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	T_1	0.92 ± 0.01^{b}	0.91 ± 0.01^{b}	0.93 ± 0.01^{b}	0.92 ± 0.01^{b}	0.91 ± 0.02^{b}
	T_2	0.95 ± 0.02^{a}	0.94 ± 0.02^{a}	0.95 ± 0.01^{a}	0.92 ± 0.02^{a}	0.95 ± 0.02^{a}
	T_3	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Calories	T_0	385.90 ± 0.96^{a}	385.67 ± 0.94^{a}	385.84 ± 1.79^{a}	385.81 ± 1.53^{a}	385.80 ± 1.17^{a}
	T_1	289.58 ± 4.80^{d}	289.78 ± 4.38^{d}	282.73 ± 4.90^{d}	289.41 ± 6.03^{d}	289.56 ± 5.10^{d}
	T_2	317.86 ± 4.01^{c}	317.50 ± 7.33^{c}	317.50 ± 3.82^{c}	317.53 ± 6.06^{c}	317.51 ± 8.51^{c}
	T_3	377.90 ± 6.02^{b}	377.59 ± 6.20^{b}	377.55 ± 5.46^{b}	377.24 ± 5.49^{b}	377.23 ± 4.12^{b}

Data indicate mean values and standard error of means. Similar lettering of mean values indicates non-significant results. R donates replications; Kcal= Kilocalorie.

Effect of treatments on sensory attributes

Organoleptic suitability scores of treatment T_2 were high as compared to others which may be due to its packing in sachets of bi-oriented polypropylene film (Table 4). Guava leather (a fruit product) produced according to the standard procedure was packed in punnet & BOPP packaging and stored at a temperature of 25±5 °C for 120 days. BOPP packaging depicted better nutritional quality and storage stability as compared to punnets packaging. Microbiology study showed product fitness for human consumption until after 120 days in both types of packaging. However, the sensory quality score was recorded highest under BOPP packaging even after a storage interval of 120 days (Singh et al., 2019). Kunda was packed in low-density polyethylene, plastic and metallic material and its storage stability and sensory quality were tested at 30 and 5 °C. Kunda remained fit for human consumption after 42 days of storage at 30 °C and it maintained its acceptability even after three months at 5 °C under all types of packaging material. However, deterioration in nutritional quality in metallic and plastic packaging was the least. Therefore, it was suggested that Kunda may be packed in metallic and plastic sachets for enhanced shelf-life. Quality characteristics of moisture, microbial load, hardness, nutrient loss and tinge of processed nutraceutical pasta were tested after a 30 days interval stored for 120 days. Hardness and nutrient losses showed linear variations. On the whole, the sensory acceptability of the developed pasta in BOPP film at the end of the storage study ranked as excellent keeping in view the decisive quality characteristics (Gull et al., 2017). Similarly, Salami was packed in high barrier packaging material (containing air or gaseous nitrogen or vacuum), BOPP and polyethylene for 48 days at 19-20 °C while another similar sample was kept unpacked. The organoleptic, nutritional and microbial evaluation was done at the end of the study. Salami stored in polyethylene and BOPP film revealed the most adequate sensory quality and acceptability attributes along with less stiffness and chewability scores after storage while the other packaging type could not preserve organoleptic acceptability to such an extent (Canel et al., 2019).

Impact of treatments on anemia indices

The majority of Pakistan's population is suffering from several nutritional problems particularly due to the financial instability of under developing countries, high inflation rate, underfeeding practices and a poor literacy rate of the mass population. Due to this, iron deficiency anemia has a high prevalence in a large segment of females and children. Blackstrap molasses: as cheap, waste material and huge derivatives of sugar mills may be in the easy approach to the mass susceptible segment of the population in under-developing nations.

Safety and efficacy aspects of synthetic supplements like polysaccharide iron indicate that they are clinically less effective than common ferrous products (Kinlin et al., 2020) while there is a greater risk of hypersensitivity reactions from ferric carboxy maltose and iron sucrose than ferric isomaltose (Pollock & Biggar, 2020). Therefore, food products urbanized using edible food iron are quite effective in enhancing hemoglobin regeneration (Man et al., 2021) as well as provide a high content of iron compared to the animal source. Moreover, these improve the digestive system in a better way and are more useful in course of the menstrual cycle, breastfeeding and pregnancy (Jain & Venkatasubramanian, 2017; Prieto-Patron et al., 2020).

Table 4 Effect of treatments on sensory attributes

Parameters R=3	Treatments	Retention period (Days)				
		0	15	30	45	60
Color	T_0	5.12±0.09^{c}	5.13±0.03^{c}	5.14±0.03^{c}	5.12±0.06^{c}	5.11±0.09^{c}
	T_1	6.95±0.14^{b}	6.95±0.16^{b}	6.96±0.14^{b}	6.97±0.11^{b}	6.94±0.06^{b}
	T_2	7.59±0.08^{a}	7.60±0.10^{a}	7.59±0.02^{a}	7.58±0.13^{a}	7.57±0.08^{a}
	T_3	5.18±0.10^{c}	5.10±0.13^{c}	5.12±0.05^{c}	5.13±0.08^{c}	5.11±0.00^{c}
Odor	T_0	5.42±0.09^{c}	5.45±0.07^{c}	5.44±0.12^{c}	5.48±0.10^{c}	5.43±0.11^{c}
	T_1	6.69±0.13^{b}	6.70±0.10^{b}	6.69±0.09^{b}	6.68±0.12^{b}	6.69±0.05^{b}
	T_2	7.41±0.12^{a}	7.40±0.11^{a}	7.41±0.14^{a}	7.40±0.16^{a}	7.40±0.06^{a}
	T_3	5.22±0.08^{d}	5.21±0.11^{d}	5.18±0.03^{d}	5.21±0.05^{d}	5.15±0.02^{d}
Texture	T_0	5.13±0.07^{c}	5.18±0.01^{c}	5.17±0.06^{c}	5.15±0.11^{c}	5.14±0.06^{c}
	T_1	6.88±0.14^{b}	6.89±0.14^{b}	6.88±0.10^{b}	6.89±0.06^{b}	6.87±0.11^{b}
	T_2	7.78±0.03^{a}	7.79±0.14^{a}	7.78±0.16^{a}	7.77±0.17^{a}	7.76±0.08^{a}
	T_3	5.11±0.10^{c}	5.10±0.09^{c}	5.07±0.11^{c}	5.10±0.02^{c}	5.09±0.00^{c}
Taste	T_0	5.19±0.08^{c}	5.17±0.03^{c}	5.22±0.12^{c}	5.18±0.12^{c}	5.17±0.06^{c}
	T_1	6.53±0.04^{b}	6.50±0.13^{b}	6.44±0.04^{b}	6.52±0.12^{b}	6.51±0.10^{b}
	T_2	7.38±0.14^{a}	7.35±0.14^{a}	7.40±0.18^{a}	7.37±0.16^{a}	7.35±0.05^{a}
	T_3	5.07±0.10^{c}	5.10±0.09^{c}	5.07±0.11^{c}	5.06±0.08^{c}	5.05±0.00^{c}
Overall acceptability	T_0	5.08±0.05^{c}	5.08±0.12^{c}	5.09±0.04^{c}	5.00±0.01^{c}	5.10±0.06^{c}
	T_1	6.85±0.16^{b}	6.85±0.10^{b}	6.82±0.10^{b}	6.79±0.04^{b}	6.83±0.16^{b}
	T_2	7.71±0.16^{a}	7.65±0.11^{a}	7.71±0.06^{a}	7.74±0.17^{a}	7.69±0.01^{a}
	T_3	5.05±0.11^{c}	5.06±0.06^{c}	5.00±0.10^{c}	5.10±0.08^{c}	5.04±0.00^{c}

Data indicate mean values and standard error of means. Similar lettering of mean values indicates non-significant results. R donates replications

Hemoglobin levels

Hemoglobin concentration in all three groups of women (pregnant, non-pregnant and women passing through lactation) was significantly affected by different treatments and study periods. Moreover, the interactive effects of study duration and different treatments on hemoglobin concentration among three women groups responded in the same manner. Hemoglobin level of control groups decreased in a non-significant manner irrespective of pregnant women which showed a significant decrease that might be because during pregnancy iron needs are more and intake is less. Treatment T_2 depicted greater enhancement in Hemoglobin concentration in all-women groups after the end of three months study interval (Table 5). This highest increase in Hemoglobin levels of women by treatment T_2 may be because the stated treatment contained a blend of iron-rich foods especially blackstrap molasses which is known as the treasure of minerals. In a study, blackstrap molasses (250mg iron /Kg) was recurrently given to a patient of IDA for 90 days and it was observed that the hemoglobin level of that person significantly improved to 12 g/dL (Tunuguntla & Sullivan, 2004) although previously he was treated and administered with several iron supplements and had not any relief in this regard. A research trial was conducted on women of the reproductive age group which was daily fed with a 2.0 Kg supplementary meal of Hibiscus sabdariffa leaves (iron-rich food source in Ghana) and each100 g meal had 1.71 mg Fe (Kubuga et al., 2019). The hemoglobin status of anemic women showed significant improvement at the end of the study.

Another author observed that the use of natural iron-rich edibles on mass levels following a fortified food strategy for the management of iron deficiency anemia is gaining more popularity and practicability (Shubham et al., 2020). Chronic iron deficiency is widely spread and has prevalence in more than 1/5th females of Pakistan. Considering this issue, milk was supplemented with iron to produce fortified milk that intervened in rats and its efficacy against iron deficiency anemia was evaluated (Akhter et al., 2014). Fortified buffalo milk was intervened for 60 days in the Dawley rats. Hemoglobin levels of the rats fed on fortified milk showed momentous improvement than the control group.

Serum total iron-binding capacity (TIBC)

Treatments, study interval and their interaction posed a highly noteworthy effect on TIBC in all three groups of women. In female groups (pregnant, non-pregnant and lactating), women fed on treatment T_2 experienced a greater decline in serum total iron-binding capacity at the end of 90 days that is evident from Table 5 & Fig. 2 but this decline in TIBC was less in volunteers fed on treatment T_3.

Table 5 Hb (g/dL) and serum TIBC (ug/dL) of volunteers

Women	Treatments	Days		P. value	Days		P. value
		Hb at 0	Hb at 90		TIBC at 0	TIBC at 90	
Pregnant (no. of samples = 184)	T_0	9.41±0.136^{c}	8.67±0.102^{e}	0.005	387.87±0.892^{c}	402.50±0.821^{a}	0.005
	T_1	9.27±0.135^{cd}	11.00±0.036^{a}	0.002	389.84±0.895^{bc}	376.58±0.970^{d}	0.004
	T_2	9.09±0.175^{d}	11.01±0.039^{a}	0.000	392.22±1.076^{b}	373.42±1.154^{e}	0.002
	T_3	9.86±0.123^{b}	11.07±0.043^{a}	0.004	387.90±0.843^{c}	374.21±0.916^{de}	0.004
Non-Pregnant (no. of samples = 184)	T_0	9.67±0.149^{b}	9.37±0.149^{bc}	0.006	403.10±1.120^{c}	416.49±0.968^{a}	0.004
	T_1	9.08±0.109^{c}	11.74±0.045^{a}	0.005	407.34±0.809^{b}	389.34±0.808^{e}	0.003
	T_2	9.08±0.148^{c}	11.92±0.073^{a}	0.000	405.460.771^{bc}	385.46±1.404^{f}	0.001
	T_3	9.51±0.064^{b}	11.71±0.077^{a}	0.005	407.69±0.740^{b}	392.68±1.026^{d}	0.004
Lactating (no. of samples = 184)	T_0	10.03±0.111^{c}	9.70±0.097^{cd}	0.007	381.56±1.041^{b}	394.29±0.662^{a}	0.007
	T_1	9.47±0.200^{e}	12.11±0.076^{a}	0.006	382.04±0.831^{b}	364.06±0.710^{d}	0.005
	T_2	9.29±0.158^{e}	12.33±0.069^{a}	0.001	383.3±0.682^{b}	363.39±0.692^{d}	0.002
	T_3	9.60±0.132^{de}	11.76±0.057^{b}	0.007	381.85±0.645^{b}	366.63±1.114^{c}	0.004

Data indicate mean values and standard error of means. Similar lettering of mean values indicates non-significant results.

TIBC has an unequivocal relationship with the left ventricular mass index in patients of IDA (Chen et al., 2020). A study was conducted about the nutritional status of 500 adolescent college girls (Hb level of 7–9 g/dL) whose diet was supplemented with seaweed consolidated chocolate. Supplement developed from standardized recipes consisted of 58 mg Fe and 12.0 mg of bioactive iron per 100 g. The study of natural food iron sources showed promising, positive, encouraging and improving effects on all anemia indicators especially HB, TIBC, MCV, serum iron and ferritin levels. Although seaweeds are natural foods that have low consumption but are abundant in seaside places that may serve as a nutrient-dense source with valuable remedial activity. In another study, lactoferrin bovine fortified with iron dispensed to 68 anemic women for one month and significant improvement in patients Hb, serum Fe and TIBC were recorded after the study (Ramakrishnan et al., 2018). An author of a study proved that the hydrated distillate of Carica Papaya foliage has phytonutrients which are very effective for blood production and protection due to their potent prospective against nephron damaging agents. Thus the foliage of Carica Papaya may serve as a good natural medicinal source against anemia due to its beneficial effect on major anemia indices (Gheith & El-Mahmoudy, 2019).

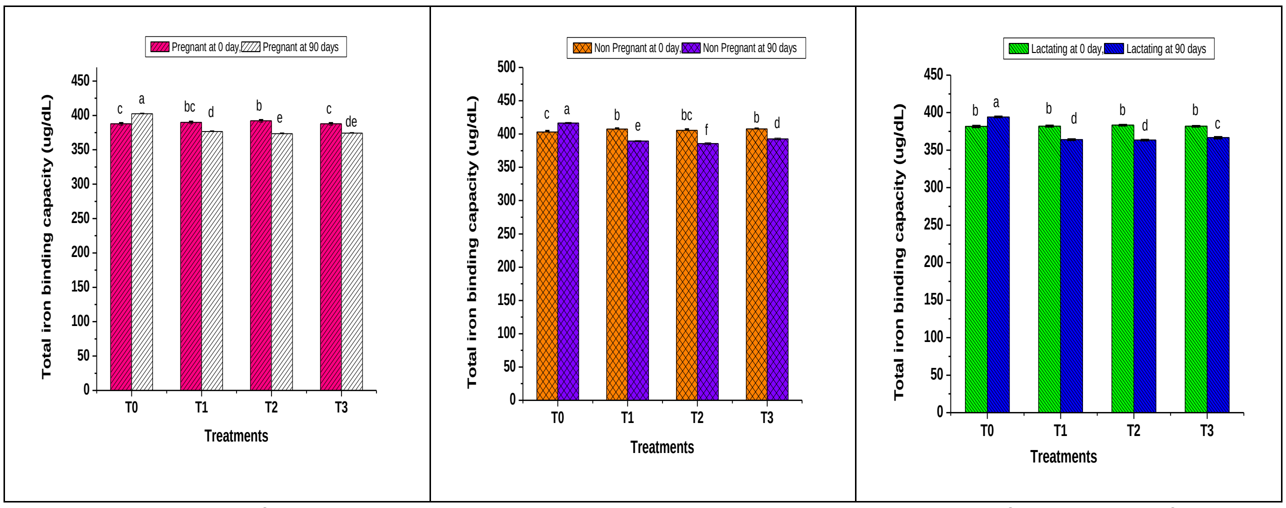

Fig. 2 TIBC (ug/dL) of pregnant, non-pregnant and lactating volunteers at recruitment and after 3 months of study interval

Conclusion

Cheap and common food sources rich in iron especially blackstrap molasses, dried apricots, dried dates and rape seeds were used in the present study. The food sources were compared with common synthetic iron supplements (ferrous sulphate) in patients of iron deficiency anemia (IDA). Four treatments of bioactive iron and synthetic iron were prepared and evaluated for storage stability, nutritional quality, and sensory acceptability that were fed to IDA patients of reproductive age group to fulfill 50% recommended dietary allowance of iron. The proximate composition of different treatments after 60 days of storage depicted the highest ash (%) in T_1 (molasses). Micro-minerals profile of treatments after prescribed storage indicated highest iron in T_3 (ferrous sulphate) followed by control and T_2 (a mixture of molasses with other natural food items). Organoleptic acceptability of treatment T_2 was high that followed a non-significant undulating pattern throughout storage. Hence, treatment of blackstrap molasses along with other food items exposed the highest improvement in IDA indicators particularly Hemoglobin regeneration and reduction in Serum TIBC of women. It can be concluded from the results that blackstrap molasses as waste material (rich in bioactive food iron) of sugar industry can be easily available to vulnerable populations that can be consumed as such and could also be used for value addition by the food industry to develop food products useful in reducing IDA among reproductive age group females.

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