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Evaluation of iron-rich snacks using liver powder and wheat flour blends for Women of reproductive age

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Abstract
Due to increase in need for iron by the body during menstruation and pregnancy, there is need for food modification to prevent iron deficiency and iron deficiency anaemia. This study investigated the formulation of blends using of liver powder and wheat flour. It also evaluated the Proximate, Iron compositions and Sensory properties of the blends with a view to increasing the Iron quality and quantity of the blends. Beef liver was dried in cabinet drier at 40°C for 12hrs before milling to powder form. Both liver powder and wheat flour were mixed into different proportions 100:0, 70:30, 60:40 and 100:0, 70:30, 60:40 for cake and cookie samples respectively. The samples were evaluated using standard methods. Iron content ranges from 6.64 ± 0.004% to 0.96 ± 0.003%. The Protein content ranges from 20.36 ± 0.02% to 14.38 ± 0.02%, Moisture content ranges from 16.46 ± 0.05% to 0.52 ± 0.02%, Fat content ranges from 16.54 ± 0.02% to 4.83 ± 0.03%, Ash content ranges from 2.82 ± 0.02% to 1.78 ± 0.02%, Crude Fibre ranges from 2.85 ± 0.01% to 0.76 ±0.02%, Carbohydrate content ranges from 72.13 ± 0.01% to 47.40 ± 0.02%. Sample B & D was the most preferred by the panelist based on Colour (7.40 ± 1.14), sample B & C was the most preferred based on Flavour (7.28±1.26) and sample B was the most preferred with respect to Taste (7.26±1.16) and General acceptability (7.54±1.30). The developed Cakes and Cookies have a better nutritional value than the controls and could be used to combat iron deficiency.

Keywords: Iron-rich snacks, reproductive age, nutrients, menstruation, nutritional and sensory evaluation

Introduction
Malnutrition among women not only has a major impact on their own health, but also on their children's [1]. It is well known that an undernourished mother inevitably gives birth to an undernourished baby. Undernourished girls also have a greater likelihood of becoming undernourished mothers who in turn have a greater chance of giving birth to low birth weight babies, perpetuating an intergenerational cycle. This cycle can be compounded further in young mothers, especially adolescent girls who begin childbearing before they have grown and developed enough. When mothers take only short intervals between pregnancies and have many children, this can exacerbate nutrition deficits, which are then passed on to their children [2].

Iron deficiency is the most common nutritional disorder affecting about 20–25% of the world's population, predominantly children and women [3]. Women of reproductive age (WRA) are at particular risk because of menstruation, whereas pregnancy and childbirth result in a net iron loss of 580 to 680 mg because of fetal and placental requirements and bleeding during delivery [4]. The primary causes of iron deficiency include 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 [5].

Women's higher risk of Iron deficiency can be attributed to menstrual losses, which can contribute significantly to ongoing iron depletion during the reproductive years. Women also often have lower overall dietary intake and, in turn, iron intake when compared to men. This is particularly important during pregnancy as during the third trimester iron requirements increase substantially to support the growth of the foetus. Young women, in particular, are at risk of low iron intake due to the high proportion engaging in dieting behaviours, such as energy or food group restriction, and disordered eating which may increase an individual's risk of nutrient deficiencies [6].

Pregnant women with hemoglobin level less than 11g/dl and non-pregnant women with a level less than 12g/dl are considered anemic [7]. An individual's iron status falls on a

continuum, ranging from depleted iron stores, through to depleted iron stores, iron deficiency and iron deficiency anaemia. Individuals with iron deficiency are, therefore, at increased risk of developing iron deficiency anaemia [8]. Women's eating habits contribute to the prevalence of anaemia, which can lead to a high maternal mortality rate in females of reproductive age [9].

Micronutrients are dietary components, often referred to as vitamins and minerals, which although is only required by the body in small amounts, are vital to development, disease prevention, and wellbeing. Micronutrients are not produced in the body and must be derived from the diet. This nutrient-dense organ meat provides a wide range of essential nutrients, and it offers numerous health benefits [10]. Beef liver is well known as a source of iron and a 3-ounce serving supplies 31 percent of women's and 70 percent of men's recommended daily allowance [11].

Snack is a portion of food, often smaller than a regular meal, generally eaten between meals [12]. Snacks come in a variety of forms including packaged snack foods and other processed foods, as well as items made from fresh ingredients at home [13]. Snacks are popular and consumed by a wide range of population across all age groups. They are consumed primarily for pleasure rather than for nutritive purpose. Sustainability of energy throughout the day is one of the benefits of snacking. Snacks can provide what may be missing from meals [14]. Snack foods have been part of human diet for a long time and have contributed tremendously to economy of every nation [15].

In women of reproductive age, heavy periods and pregnancy are the most common causes of iron deficiency anaemia as the body needs extra iron for replenishing blood loss during menstruation and for the baby during pregnancy [16]. One of the biggest problems with so many baked foods is that, they are made with highly processed white flour. Majority of flour based-products are made from wheat. The nutrient rich germ and the fibre wheat bran are removed during processing of white flour, leaving only the nutrient poor, fibre-free starch. Consumption of unhealthy (energy-dense) and Poor availability of iron in snacks gives rise to iron deficiency & its anaemia and has also increase death rate in developing and under developing countries [17].

Methods

Collection of materials

Liver was purchased from the central slaughter slab in Ile-Ife, Osun State, south western Nigeria. Wheat flour, butter, eggs, flavours, salt were purchased at the main market in Ile-Ife, Osun State, Nigeria. Oven, weighing scale, balance, bowl, turning stick, baking pan, measuring cups, measuring spoons and other equipment were provided by food and nutrition laboratory of the department of Family, Nutrition and Consumer Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

Production of liver powder

The liver was cleaned using clean water, sliced and diced. The diced liver was dried in a cabinet dryer at 40ºC for 12 hours. The dried liver was milled into fine powder, sieved using a sieve of 250µm aperture size and then stored in a low density polyethylene bag at room temperature.

Fig 1: Flow Chart for the Production of Liver Powder

Formulations for the snacks

Formulations for cake

Sample A: Wheat 100%, Beef liver 0%
Sample B: Wheat 70%, Beef Liver 30%
Sample C: Wheat 60%, Beef Liver 40 %

Formulations for cookies

Sample D: Wheat 100%, Beef liver 0%
Sample E: Wheat 70%, Beef Liver 30%
Sample F: Wheat 60%, Beef Liver 40 %

Production of cake

Recipe for the production of cake

Ingredients	Sample A	Sample B	Sample C
Wheat flour (g)	200	140	120
Liver flour	-	60	80
margarine (g)	120	120	120
Sugar (g)	80	80	80
Eggs	4	4	4
Milk (ml)	60	60	60
Baking powder (g)	3	3	3
Salt (g)	2	2	2
Vanilla essence flavour(ml)	2.5ml	2.5ml	2.5ml

Cake Preparation

Cake samples were produced by replacing wheat flour with different levels of composite flour in the basic formulation of cake. Cake was produced according to the method described by [18] with minor modification. The fat and sugar were creamed together until fluffy (doubled its size) using a wooden spoon in a bowl followed by addition of the liquids (beaten eggs, milk & flavour). The sieved flour with salt and baking powder were added into the mixture gradually with a spoon and poured into creamed baking pan. The mixture was baked at temperature 220 °C for 20min.

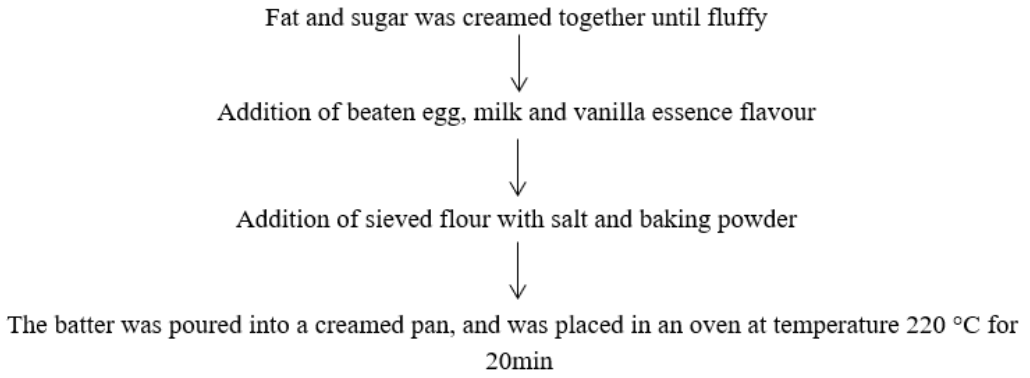

Fig 2: Flow Chart for the Production of Cake

Production of cookies

Recipe for the production of cookies

Ingredients	Sample D	Sample E	Sample F
Wheat flour (g)	200	140	120
Liver flour (g)	-	60	80
Sugar (g)	75	75	75
Margarine (g)	125	125	125
Vanilla flavour (ml)	3	3	3
Milk (powder, peak) (g)	105	105	105
Baking powder (g) 5	3	3	3
Egg (whole) 1	1	1	1

Cookie production

The method described by [19] with minor modification was used in the production of cookies. Sugar (75 g) was added to 125g of margarine and mixed at medium speed until fluffy. Whole egg and milk powder were added during mixing and then mixing continued for about 30min. Sifted flour, baking powder and flavor were slowly added to the mixture and kneaded to form dough. It was then be rolled on a flat rolling board sprinkled with flour and cut out into required shapes and baked in an oven at 160 ºC for 15 min.

Fig 3: Flow Chart for the Production of Cookie

Sensory Evaluation

The six samples (3 cake samples and 3 samples of cookie) were presented to 100 female member panellists of reproductive age within the university community. Each panellist was asked to rate the samples for colour, texture, taste, flavour and overall acceptability using 9 point hedonic scale. The panellists were supplied with water to rinse their mouth between samples. Samples were rated alongside the control samples.

Proximate analysis

Proximate analysis was carried out on the 3 cake samples and on the 3 cookie samples.

Determination of Ash Content

The ash content of the products and flour samples was determined according to the standard methods of [20]. The crucible was thoroughly washed and afterwards dried in an oven at 100 ^{0}C for 1hour. The hot dried crucible was cooled in the desiccator. The weight W1 was noted. Two gram of the sample was weighed into the crucible and the total weight (W2) was noted. The sample was charred on a Bunsen flame inside a fume cupboard. The sample was transferred into a preheated muffle furnace 550^{0}C for 2 hours until a white or light grey ash was obtained. It was cooled in a desiccator and weight (W3) was noted.

The ash content was calculated mathematically as follows:

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} = \frac{W3 - W1}{W2 - W1} \times 100$$

Fat determination

The fat content of the sample was determined using the standard method of [20]. A soxhlet extractor with a reflux condenser and a 500ml round bottom flask will be fixed. Two gram (2g) of the sample was weighed into a labeled thimble. Petroleum ether (300ml) was filled into the round bottom flask. The extraction thimble was sealed with cotton wool. The soxhlet apparatus after assembling will be allowed to reflux for about 6 hours. The thimble was removed with care. The petroleum ether that was collected in the top was drained into a container for reuse. When the flask is free of ether, it was removed and dried at 105 ^{0}C for 1 hour in an oven. It was cooled in a desiccator and then weighed.

Calculation: %fat content

$$= \frac{\text{weight of fat}}{\text{weight of sample}} \; x \; 100 = \frac{\text{weight of fat}}{\text{weight of sample}} \; x \; 100$$

Crude Protein Determination

The protein content of the samples was determined according to the standard method of [20] using kjeldahl method.

Digestion of Sample: The sample (2g) was weighed into kjeldahl flask. Anhydrous sodium sulphate (5g) or 4 tablets of kjeldahl catalyst was also added. Addition of kjeldahl tablet followed up. Addition of twenty-five milliliter (25ml) of concentrated tetraoxosulphate (vi) acid (H_2SO_4) was done

with few boiling chips. The flask with the content was heated in the fume chamber until solution becomes clear. The solution was cooled to room temperature after which it was transferred into a 250ml volumetric flask and made up to level with distilled water.

Distillation: The distillation unit was cleaned and the apparatus set up. A 100ml conical flask (receiving flask) containing 5l of 2% boric acid was placed under the condenser, with the addition of drops of methyl red indicator. The digest (5ml) was pipetted into the apparatus through a small funnel, washed down with distilled water, followed by the addition of 5ml of 60% NaOH (sodium hydroxide) solution. The digestion flask was heated until 100ml of distillate (ammonium sulphate) will be collected in the receiving flask. The solution from the receiving flask was titrated with 0.049M H_2SO_4 to a pink colour. The same procedure was carried out on the flask.

Calculation:

$$\% \ of \ Nitrogen \ Sample \ (\%N) = \frac{Vs - Vb}{W}$$

$$\% \ of \ Nitrogen \ Sample \ (\%N) = \frac{Vs - Vb}{W}$$

x N acid x 0.01401 x 100
Where Vs = volume (ml) of Acid required to titrate the sample
Vb= volume (ml) of acid required to titrate the blank
N acid = Normality of acid (0.1 N)
W= Weight of sample in gram
% Crude protein = % N x 6.25 (conversation factor)

Crude fibre determination
The crude fibre content of the sample was determined using the standard method of [20]. Petroleum ether was used to defat 2g of the sample. This was put in boiled 200ml of 1.25% $H_2SO_4$and boiled for 30minutes. The solution was filtered through linen or muslin cloth on a fluted funnel. It was washed with boiling water until it is free of acid. The residue was returned into 200 ml boiling NaOH and allowed to boil for 30minutes. It was washed with 1% HCl and then with boiling water, to free it of acid. The final residue was drained and then transferred to silica ash crucible (porcelain crucible) and dried in the oven at 100^0C to a constant weight. The sample was cooled and incinerated or ashed in a muffle furnace at 600^0C for 5hours, cooled in a desiccator and weighed.

Calculation
% Crude fibre =

$$= \frac{\text{loss of weight after ignition}}{\text{weight of original sample}} \ x \ 100$$

$$= \frac{\text{loss of weight after ignition}}{\text{weight of original sample}} \ x \ 100$$

Moisture content determination
The moisture content of the products and flour samples was determined according to the standard method of [20]. The crucibles was washed thoroughly and afterwards dried in the oven at 100^0C for 1 hour. The hot dried crucible was cooled in the desiccator. The weight (W1) was taken when cooled. Two grams of the sample was weighed into the crucible and the total weight (W2) will be taken before and during drying at 100 ^{0}C until a constant weight (W3) was obtained.

$$\% \text{ moisture content} = \frac{W2 - W3}{W2 - W1} = \frac{W2 - W3}{W2 - W1} \text{ x } 100$$

Where W1= Initial weight of empty crucible.
W2= Weight of crucible + weight of sample before drying.
W3= Weight of dish + weight of sample after drying.

Carbohydrate content determination
The carbohydrate content of each of the sample was determined by difference according to [21] as follows:
% Carbohydrate= 100 - (% moisture + % ash + % protein + % fat + %crude fibre).

Iron content Determination
The iron content was determined based on the method described by [22]. Ten milliliter (10ml) of concentrated HNO3 was added to 1g of the sample and left overnight. The sample was carefully heated until the production of red nitrogen dioxide fumes ceased. The sample was cooled and 4ml of 70% HClO4 was added and evaporated to a smaller volume (7ml) by careful heating. The resulting solution was quantitatively transferred into 50ml volumetric flask and diluted to the mark with distilled water. The solution was sprayed into an atomic absorption spectrophotometer (Perkin Elmer, model 5100 PCAAS, USA) at 248.3nm to determine the concentration of iron. The iron standards to be used were 0ppm, 1ppm, 2ppm, 3ppm and 4ppm.

Statistical Analysis

The results were expressed as mean$\pm$standard deviation and the test for statistical significance was carried out with the use of one-way analysis of variance (ANOVA). The statistical package used to determine the significant differences was Statistical Package for Social Science (SPSS, Version 20). Significant means was separated using Turkeys Least Significance Difference (LSD) test. Differences was considered significant at $p<0.05$.

Results
Sensory Evaluation
Sensory evaluation was carried out on six different snacks produced using liver powder and wheat flour blends at different proportions.

Table 1: Sensory evaluation of the cake and cookie samples from liver powder and wheat flour

Sample	Color	Taste	Texture	Flavour	Acceptability
A	8.16±0.84^a	8.18±0.87^a	8.26±0.77^a	8.06±0.99^a	8.28±0.90^a
B	7.40±1.14^b	7.26±1.16^c	7.50±1.16^c	7.28±1.26^c	7.54±1.30^b
C	7.22±1.00^c	7.12±0.98^d	7.54±1.11^b	7.28±1.14^c	7.40± 1.11^d
D	7.40±1.23^b	7.30±1.25^b	7.48±1.18^d	7.48± 1.16^b	7.50±1.25^c
E	6.22±1.54^e	6.66±1.26^e	6.88±1.14^f	6.64± 1.37^e	6.62±1.24^f

F	6.62±1.46^{d}	6.76±1.30^{e}	7.08±1.31^{e}	7.02±1.30^{d}	6.88±1.27^{e}

*Different superscript in the same column indicates significant difference (p<0.05)

Key:
Sample A: Cake produced from 100% wheat flour
Sample B: Cake produced from 70% wheat flour and 30% liver powder
Sample C: Cake produced from 60% wheat flour and 40% liver powder
Sample D: Cookie produced from 100% wheat flour
Sample E: Cookie produced from 70% wheat flour and 30% liver powder
Sample F: Cookie produced from 60% wheat flour and 40% liver powder.

The sensory score for the color of the cake samples ranged from (8.16-6.22) with the 100% wheat cake having the highest scores of 8.16, while sample E (6.22) had the least score. Among the composites, samples B and D (7.40) had the highest score.

The sensory scores for the taste of the cake samples ranged from (8.18-6.66) with the 100% wheat cake having the highest score of 8.18, while sample E (6.66) had the least score. Among the composites, sample B (7.26) had the highest score.

Sensory score for the texture of the cake sample ranged from (8.26-6.88) with 100% wheat cake having the highest score of 8.26 while sample E (6.88) had the least scores. Among the composites, sample C (7.54) had the highest score.

The sensory scores for flavor of the cake samples ranged from (8.06-6.64) with 100% wheat cake having the highest score of 8.06, while sample E (6.64) had the least score. Among the composites, sample B & C (7.28) had the highest score.

The sensory score for acceptability of the cake samples ranged from (8.28-6.62) with 100% wheat cake having the highest score of 8.28, while sample E (6.62) had the least scores. Among the composites, sample B (7.54) had the highest score

Proximate Composition

Table 2: Proximate composition of cake and cookie samples from liver powder and wheat flour blends

Samples	Protein%	Moisture%	Fat%	Ash%	Crude fiber%	Carbohydrate%
A	14.38±0.02^{f}	8.57±0.06^{c}	16.54±0.02^{a}	2.82±0.02$_{a}$	0.76±0.02$_{f}$	56.93±0.10^{d}
B	18.56±0.03^{c}	16.46±0.05^{a}	14.48±0.02^{b}	2.32±0.03^{c}	0.80±0.02$_{e}$	47.40±0.02$_{f}$
C	20.36±0.02^{a}	14.28±0.03^{b}	14.28±0.02^{c}	2.48±0.02^{b}	0.84±0.02$_{d}$	47.77±0.05^{e}
D	16.86±0.02^{e}	0.54±0.02^{e}	6.60±0.02^{d}	1.78±0.02^{f}	2.30±0.02^{c}	71.92±0.04^{b}
E	18.34±0.02^{d}	0.52±0.02^{f}	4.83±0.03^{f}	1.83±0.03^{d}	2.35±0.01^{b}	72.13±0.01^{b}
F	18.84±0.03^{b}	1.86±0.02^{d}	6.25±0.04^{e}	1.80±0.02^{e}	2.85±0.01$_{a}$	68.31±0.02^{c}

*Different superscript in the same column indicates significant difference (p<0.05)

Key:
Sample A: Cake produced from 100% wheat flour
Sample B: Cake produced from 70% wheat flour and 30% liver powder
Sample C: Cake produced from 60% wheat flour and 40% liver powder
Sample D: Cookie produced from 100% wheat flour
Sample E: Cookie produced from 70% wheat flour and 30% liver powder
Sample F: Cookie produced from 60% wheat flour and 40% liver powder.

Table 2 shows that among the samples, sample C had the highest protein content (20.36%). This was followed by sample F (18.84%). The sample having the least protein content was sample A (14.38%). Among the samples, sample B (16.46%) had the highest moisture content, followed by sample C (14.28%) and sample E (0.52%) having the least moisture content.

Among the samples, sample A (16.54%) had the highest fat content, followed by sample B (14.48%) and sample E (4.83) having the least fat content.

Among the samples, sample A (2.82%) had the highest ash content followed by sample C (2.48%) and sample D (1.78%) had the lowest ash content.

Among the samples, sample F (2.85%) had the highest crude fiber content followed by sample E (2.35%) and sample A (0.76%) had the least crude fiber content.

Carbohydrate content

Among the samples, sample E (72.13%) had the highest carbohydrate content, followed by sample D (71.92%) and sample B (47.40%) had the least carbohydrate content.

Iron Composition

Table 3: Iron composition of cake and cookie samples from liver and wheat flour blends

Samples	Iron
A	0.96±0.003^{f}
B	1.47±0.002$_{e}$

C	$3.48 \pm 0.002_{b}$
D	$1.56 \pm 0.002_{d}$
E	$2.16 \pm 0.002_{c}$
F	$6.64 \pm 0.004_{a}$

*Different superscript in the same column indicates significant difference (p<0.05)

Key:
Sample A: Cake produced from 100% wheat flour
Sample B: Cake produced from 70% wheat flour and 30% liver powder
Sample C: Cake produced from 60% wheat flour and 40% liver powder
Sample D: Cookie produced from 100% wheat flour
Sample E: Cookie produced from 70% wheat flour and 30% liver powder
Sample F: Cookie produced from 60% wheat flour and 40% liver powder.

Table 3 shows that Sample F(6.64) had the highest iron content, followed by sample C (3.47) and sample A (0.96) having the least iron content.

Discussion

Sensory evaluation

There was significant difference (p<0.05) among all the samples with respect to all the sensory parameters (colour, taste, texture, flavor and general acceptability).

There was an observable decrease in preference for colour, taste, texture, flavor and general acceptability of the cake and cookie samples with an increase addition of liver powder. This may be due to the dark brown colour of the liver powder which led to the chocolate colour of cake and cookie samples upon addition of liver powder and is in agreement with [23].

Proximate analysis

The quality of food is determined by the quantity and quality of its protein. The protein content of the cake and cookie samples increased with increased level of liver powder substitution. However liver has been reported to be an excellent source of high quality of protein. The moisture content of the cake and cookie samples increased with addition of liver powder which may be attributed to the water absorption properties of proteins.

The fat contents of food samples serve as an indicator of its durability. The fat content of the cake and cookie samples decreased with addition of liver power [24]. Reported that low fat content in a product will help in increasing the shelf life of the sample by decreasing the chances of rancidity and also contribute to low energy value of the food product while high fat content product will have high energy value and promotes lipid oxidation.

Ash content determines the level of mineral element present in the samples. The ash content of the cake samples increases while that of cookie decreases which was attributed to the method of processing and combination of plants and animal sources of foods. The ash content of the food material could be used as an index of mineral constituents of the food [25].

The fibre content of food sample is related to the roughage content of the sample. The high level of crude fibre of the cake and cookie samples may be as a result of increased level of liver flour substitution.

The carbohydrate content of the cake and cookie samples decreased with addition of liver powder. This may also be a desirable attribute for weight watchers and diabetic patients who require less carbohydrate.

Iron content

There was significant difference (p<0.05) among all the samples. The iron content of the samples increases with increased level of liver powder substitution. Liver has been reported to be rich in highly absorbable iron [26].

Conclusion

This study has revealed that the addition of liver powder to wheat flour can be used to increase the iron content of cake and cookie. It also shown that snacks can be produced using liver and wheat flour blends. These snacks can be used to replace plain wheat cake/cookies because of their satisfactory sensory qualities, proximate qualities and high iron content.

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