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Studies on bioavailability of iron from iron fortified commercial edible mushroom *Hypsizygus ulmarius* and standardization of delivery system for human nutrition

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ABSTRACT

Iron is one of the most important micronutrients for human health. Iron deficient diet and defective iron absorption are among the major reasons for iron malnutrition. Mushrooms are edible fungi, which are a very good source of iron and can be easily grown on agricultural residues at home scale, dehydrated and stored in the powder form, which can supplement the daily diet. Although mushrooms in general and oyster mushrooms in particular are a rich source of iron, yet, have not become a recommended diet by the nutritionists due to lack of data pertaining to its bioavailability from mushrooms. Data has been generated in the present study on the *in vitro* bioavailability of iron from non-fortified and iron fortified *Hypsizygus ulmarius,* which is a commercially grown species. This is the first report pertaining to the bioavailability of iron from iron fortified mushrooms. A delivery system for human nutrition was also standardized in the form of Arka Mushroom Fortified Rasam Powder using iron fortified and non-fortified mushrooms, which can be used to mitigate iron malnutrition. The data generated in this study will help in providing application techniques to use mushrooms as dietary source of iron in everyday diet to mitigate iron deficiency.

Key words: Arka Mushroom Rasam, Bioavailability, *Hypsizygus ulmarius* and Iron fortified

INTRODUCTION

Iron is one of the most important micronutrients for human health. It is required in the process of oxygen transport and storage (as a constituent of hemoglobin and myoglobin), for electron transfer (in cytochromes), desaturation of fatty acids, tyrosine iodination (in thyroid peroxidase), prostaglandin synthesis, formation of erythrocytes, maintenance of heat balance, as well as humoral and cellular resistance (Burke *et al.,* 2014; Nadadur and Mudipalli, 2008). Iron deficiency can also result in decreased cognitive health. Nutrition Monitoring Bureau Survey (NNMBS, 2006) showed that anemia prevalence in adolescent girls in India was 69.7% which caused 1.8% loss of GDP. Indian government has been working towards reducing the load of anaemia in girls and women by 50%. Numerous interventions like biofortification and fortification of diet are being undertaken to mitigate this problem. A very important intervention can be adoption and popularization of alternate foods rich in iron, which have not yet become a part of daily diet. Although mushrooms in general and oyster mushrooms in particular are a rich source of many minerals including iron, yet, have not become a recommended diet by the nutritionists due to lack of data pertaining to bioavailability. Any iron fortification method is incomplete until a proper delivery system is also standardized so that the benefit can reach the target population. The innovation for the process of production of iron fortified mushroom was taken to a logical and meaningful conclusion through the standardization of recipe for iron rich Arka Mushroom Rasam Powder which is available as licensed technology from ICAR-IIHR. The data

generated in this study will fill this gap on bioavailability of iron from mushrooms and provide application techniques to use mushrooms as dietary source of iron in everyday diet.

MATERIALS AND METHODS

Hypsizygus ulmarius (Elm oyster) was cultivated on sterilized paddy straw-based substrate. Paddy straw was cut into small pieces (about 2.5-3.0 cm) using a motorized chaff cutter and soaked in clean water for 4 h. The soaked straw was drained for removal of excess water and spread on meshed tray under sun to reduce the moisture content to 65-70%. One kg wet straw was filled in polypropylene (PP) bags measuring 304 x 406 mm, plugged with non-absorbent cotton and sterilized in autoclave at 121°C, 15lb pressure for 25 min. The moisture content of the wet straw was determined by gravimetric method by drying 100 g of wet straw to a constant weight in oven at 70°C. The sterilized bags were cooled to room temperature and spawned (seeded) using grain spawn of *H. ulmarius* @ 5% (50g spawn per bag) in an aseptic bag inoculation chamber. Spawned bags were incubated at 26±2°C for spawn running (vegetative growth). After 25 days of completion of spawn running, the bags were shifted to cropping room where a temperature of 26±2°C and humidity of 80-85% was maintained throughout the cropping period of 30 days. Four to five holes were made in each bag to induce mushroom initiation. Iron fortified mushroom was developed through an innovative technique using iron salt (Patent application No -201841022601). Mature mushrooms of both the non-fortified *H. ulmarius* (NFHU) and iron fourtified *H. ulmarius* (IFHU) were harvested, hand shredded and dehydrated in a tray dryer covered with PP sheet at 48±2°C for 12-14 h. Dry mushrooms were powdered in wooden pestle mortar. Necessary precautions like using gloves while handling mushrooms, using plastic knife to cut mushrooms, were taken in handling of iron enriched mushrooms to avoid external contamination. NFHU powder and IFHU powder prepared was used for the *in vivo* iron bioavailability studies.

In vivo studies

The experiment was conducted in the Laboratory Animal House at Experimental Livestock Unit, National Institute of Animal Nutrition and Physiology (NIANP), Bangalore, India. The animal experiment protocol was as approved by the Institutional Animal Ethics Committee (IAEC) and carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

Experimental design

Forty-eight numbers of healthy weaned (40 days old) Wistar strain (*Rattus norvegicus*) albino rats were randomly divided into four dietary groups, with four replicates of three rats in each group. All the rats were housed in polypropylene cages and maintained under similar managemental conditions. Temperature and humidity were maintained at 23±2ºC and 50 to 70% respectively in proper ventilated animal house. All rats were fed pelleted feed and offered purified drinking water *ad libitum*.

Preparation of experimental diets

Commercial pelleted rat feed was served as the control diet (A). The control diet pellets were finely powdered and mixed well separately with dried NFHU and FHU mushroom powder in 75:25 proportion to prepare experimental diet B and C respectively. The experimental diet D comprised of control feed supplemented with ferrous sulfate (Analytical grade, SD fine chemicals, Mumbai, India) equivalent to the iron content of fortified mushroom. Diets B, C and D were again pelleted in a manual pelletizer, air dried for 24 hours, later oven dried at 60ºC for 24 h and stored in respective air tight containers until fed to the experimental rats. Necessary precautions were taken at each step of diet preparation, pelletizing and storage to avoid contamination.

Sample collection

All the rats of different dietary groups were offered daily weighed amount of respective experimental diets in the morning at 9:30 h. They were provided with fresh and clean deionized water *ad libitum.* The feeding experiment was continued for 30 days and initial as well as final body weight of each rat was recorded. At the end of experiment, a digestibility trial of 5 days duration was conducted to record dry matter (DM) intake, DM digestibility and apparent Fe absorption in gut.

Analytical technique for iron bioavailability studies

The samples of diets offered, residues and faeces were analyzed for DM as per standard procedures of Association of Official Analytical Chemists

(AOAC 2000). The feeds and faeces were taken in pre-weighed silica crucibles and oven dried at 80°C for 24h, decarbonized and ashed at 550-600°C in a muffle furnace. The total ash was digested with 5N HCL over a hot plate for 15 min, cooled and filtered through Whatman filter paper (No. 41) into volumetric flasks of desired volume. Iron content in mushroom, diets and faeces was estimated using Optima 8000 inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Perkin Elmer, Shelton CT#064840, USA).

Statistical analysis

The data were analyzed using the statistical Package for Social Sciences (SPSS, version 20.0 Chicago, USA) by one-way ANOVA and comparison of means was tested using Duncan's multiple range tests (Duncan 1995).

Standardization of delivery system for human nutrition

Standardization for a socially acceptable value-added product with iron fortified mushroom was undertaken. A local daily food accompaniment called 'Rasam or Soup' which is used in every Indian home of all strata was standardized using powder made by dehydration of both iron fortified and non-fortified mushrooms. The mushroom fortified rasam powder was made with traditional spices like coriander, jeera, pepper, fenugreek and tomato powder, chili powder, tur (*Cajanus cajan*) and urad dal (*Vigna mungo*), crushed garlic, salt and fresh coriander. This traditional mix was fortified with mushroom powder made from dehydrated non fortified and iron fortified Elm oyster mushroom. Ready to serve rasam or soup was made by boiling 10g of the rasam or soup powder in 350ml of water for 15 minutes and subjected to acceptance test by 501 people of rural and urban backgrounds. The rasam powder was also analyzed for its nutritive values. Taking a cue from the available literature on low availability of iron in human from legumes (Lynch et al 1984), the Arka rasam powder was also prepared with and without two legume ingredients (*C. cajan* and *V. mungo*). The five kinds of products *viz*. Rasam without legumes + iron fortified mushroom, Rasam with legumes + iron fortified mushroom, Rasam without legumes+ non fortified mushroom, Rasam with legumes+ non fortified mushroom and Rasam without mushroom (traditional recipe) were analyzed for iron content as per the standard AOAC method.

Proximate nutritive analysis of Arka Rasam

The total soluble carbohydrates of the dry powders of rasam, rasam powder with mushroom and rasam powder containing iron rich mushroom was determined by the phenol-sulfuric acid method as described by Dubois *et al*. (1956). Protein content was calculated by multiplying the total nitrogen value by the factor 6.25. Fat percentage was determined in the samples by the Soxhlet procedure using petroleum ether (60-80 ^{0}C) (AOAC, 1984). Dietary fiber percentage was estimated in dry, defatted samples by the Official AOAC Method No. 982.29 (Prosky *et al*., 1992). All the results were expressed as mg/100g dry weight or fresh weight basis. The extraction procedure of water-soluble vitamins from mushroom was followed as described earlier by Esteve *et al*. (2001). A composite sample of 2 g of freshly cultivated mushrooms was homogenized using a domestic blender with 5 ml of MilliQ water to which 10 mL of 0.1 N HCl was added and incubated in a water bath at 95–100°C for 30 min. The extract was then allowed to cool at room temperature and the pH was adjusted to 4.0–4.5 using 5 M sodium acetate solution. One ml of takadiastase enzyme solution, contain 200 mg/ml was added to this extract and incubated in a water bath at 45–50°C for 8 hrs. After the enzymatic hydrolysis, 1 mL of 50% trichloroacetic acid was added to the extract and incubated at 95–100°C in a water bath for 5 min. After cooling it was centrifuge at 10000 rpm for 10 min, supernatant was collected and made up the volume to 25ml with 0.1N HCl. To the 5 mL of the stock solution 300 µL of potassium ferricyanide (1% in 15% NaOH) was added and left to react for 10 min in the dark. The extract was then neutralized with 200µL of 15% ortho-phosphoric acid, filtered through a nylon membrane syringe disc filter with a pore size of 0.45 µm followed by 0.2 µm and 1µl was injected immediately into the UPLC-MS/MS.

Mineral analysis of Arka Rasam

Mushroom samples were dried in the oven at 70ºC to constant weight and analyzed for mineral nutrients as per the methods described by Piper (1966). Nitrogen content was determined by Kjeldahl method (Kjeltek Auto-Analyzer, Gerhardt, Germany),

phosphorus was estimated calorimetrically by vanado molybdophosphate method and potassium content by flame photometer. Calcium, magnesium, iron and zinc were determined using atomic absorption spectrophotometer (AAS) by Wet digest method with HNO_3 and $HCLO_4$ in 10:4 ratio.

Water soluble vitamins analysis

Reagents

The water-soluble vitamin standards biotin (B7), niacin (B3), pyridoxine (B6), pantothenic acid (B5), folic acid (B9), cyanocobalamin (B12), thiamine (B1) and riboflavin (B2) were from Sigma Chemical Co., USA. The standard vitamin solutions were prepared in 0.01 N HCl. The enzyme takadiastase was purchased from Fluka, Switzerland. The organic solvents used for mobile phase in liquid chromatography were of chromatographic/MS grade and all other reagents were of analytical grade. Milli-Q water (Millipore system) was used to prepare the mobile phases. All mobile phases were filtered through 0.45 µm pore size membranes before use.

Equipment

Freshly harvested mushroom samples were used for the validation of the methodology. An Acquity UPLC-H class coupled with TQD-MS/MS from Waters, USA with ESI source was used for vitamin determinations; the system was equipped with a degasser, quaternary pump, automatic injection system (0–10 µL), with a diode array detector and a temperature control compartment for the analytical column. The detection system allowed for the simultaneous detection at various wavelengths and MRM for individual masses. The overall system was controlled by the Mass lynx software, which also administered the data collection and treatment system.

MS-MS Methodology

The methodology for estimation of vitamins by UPLC-MS/MS system was validated for linearity, quantification and detection limits, and repeatability; recoveries of standards were 85-95%. The most sensitive, Multiple Reaction Monitoring (MRM) mode was employed for detection. The optimal operational parameters of the MS/MS system, to select the most abundant mass-to-charge ratio (m/z), was determined for each vitamin by direct sample infusion, using positive ionization mode (ES^+): the precursors ion and the product ions, the MS/MS capillary voltage, extractor voltage and RF lens values were set at 3.2 kV, 4 V and 0.1 V respectively, the gas flow for de-solvation and cone was set at 550 and 50 L/h. The cone voltage and the collision energy for individual vitamins were optimized (Table 1).

Table 1. MRM of water-soluble vitamins standards

Water soluble vitamins	Formula/Mass	Parent m/z $[M+H]^+$	Cone Voltage	Daughters	Collision energy (CE)	Ion Mode
Thiamine (B1)	264	265.03	20	122.06(Q)	16	ES+
		265.03	20	144.02	14	ES+
Riboflavin (B2)	376	376.97	40	243.05	24	ES+
Niacin (B3)	123	123.9	34	80.523(Q)	20	ES+
		123.9	34	77.47	18	ES+
Pantothenic acid (B5)	219.03	220.01	28	202.21	12	ES+
		220.01	28	124.16	20	ES+
Pyridoxine (B6)	169	169.97	24	152.09(Q)	12	ES+
		169.97	24	134.04	20	ES+
Biotin (B7)	244	245.03	26	227.14	14	ES+
Folic acid (B9)	441	442.1	24	295.16	16	ES+
Cyanocobalamin (B12)	677	678.29	38	147.18	68	ES+

* "Q" taken as quantified ion.

LC and MS/MS conditions

The mobile phase consisted of the aqueous phase 0.1% formic acid in water (A) and organic phase acetonitrile (B). The initial gradient of 60% A and 40% B was held for 0.5 min; at 6.0 min the gradient was changed to 5% A and 95% B, held for 0.5 min, and a linear gradient then followed, and later 30% A and 70% B in 8.0 min, held for 0.5 min. The system was then returned to initial conditions at 14 min and held for 1 min for equilibrating before the next injection. The flow rate was 0.3 ml/min. The analytical column 2.1x50 mm UPLC BEH-C18 column (Waters) with 1.7 µm particle size, protected by a Vanguard BEH-C18 with 1.7 µm. guard column (Waters). The column temperature was maintained at 25 °C and sample injection volume was 1.0 µl. The vitamins eluted were monitored using a PDA detector and the UPLC column effluent was pumped directly without any split into the TQD-MS/MS (Waters, USA) system, optimized for the vitamins analysis.

Extraction of Water-Soluble Vitamin

The extraction procedure of water-soluble vitamins from mushroom was followed as described earlier by Esteve *et al*. (2001). Two gram of composite sample of freshly harvested mushrooms was homogenized in a domestic blender with 5 ml of MilliQ water, added 10 mL of 0.1 N HCl and incubated in a water bath at 95–100 °C for 30 min. The extract was cooled to room temperature and pH was adjusted to 4.0–4.5 with 5 M sodium acetate solution. 1 ml of takadiastase enzyme solution (200 µg/ml) was added to this extract and incubated in a water bath at 45–50 °C for 8 hrs. After the enzymatic hydrolysis, 1 mL of 50% trichloroacetic acid was added to the extract and incubated at 95–100 °C in a water bath for 5 min. After cooling, the extract was centrifuged at 10000 rpm for 10 min; supernatant was made up to 25 ml with 0.1 N HCl. To 5 mL of this stock solution 300 µL of potassium ferricyanide (1% in 15% NaOH) was added and reacted in the dark for 10 min. The extract was then neutralized with 200 µL of 15% *ortho*-phosphoric acid, filtered through a nylon membrane syringe disc filter (0.45 µm pore size), followed by 0.2 µm filter, 1 µl was injected immediately into the UPLC-MS/MS.

Statistical analysis

The statistical analysis was done by t-test using the Excel (2019) software. The significant differences ($p<0.01$) were evaluated for the mineral and vitamin contents of each parameter and for each sample individually. The statistical analysis of iron content data of Arka Mushroom Rasam using iron fortified mushroom was analyzed using the software Agres.

RESULTS AND DISCUSSION

The data presented in Table 1 shows that the dry matter intake of the experimental animals reduced significantly with both NFHU (diet B) and IFHU mushroom diet (diet C) as compared to diet without mushroom (diets A & D) by 22.09, 21.40 and 10.46, 9.67% respectively. The reduction was more pronounced in NFHU diet (diet B) as compared to IFHU diet (diet C). Mushrooms are known to be low calorie, high fiber diets with high amounts of non-digestible polysaccharides as β glucans, which act as prebiotic. Such diets can generally reduce the dry matter intake and body weight gain as is revealed in the present study. Hence mushrooms have been recommended as a diet to fight obesity as well (Friedman, 2016). However, no significant reduction in the daily weight gain of the experimental animals was observed in the present investigation. The data in table 1 also reveals that the gut absorption of iron was significantly higher both for NFHU (Diet B) and IFHU (Diet C) as compared with diets A and D without mushrooms. The gut absorption of iron from NHFU diet was 36.30% higher as compared to non-mushroom diet A and 39.52% higher as compared to non-mushroom diet D. The iron absorption from FHU was 66.76% higher as compared to non-mushroom diet A and by 70.70% higher as compared to diet D. This observation is of immense importance as despite its abundance in nature, iron still remains one of the major factors of mineral deficiency leading to numerous health issues. An estimated 20% of maternal deaths are directly related to anemia and another 50% of maternal deaths are associated with it (Anand *et al.,* 2014). Regula *et al*. (2016) studied the effect of feeding iron deficient Wistar rats with cereal products enriched with 10 and 20% *Pleurotus ostreatus* powder. The authors reported a complete restitution of iron deficient rats in a short time. They also reported that iron bioavailability from cereal products enriched with mushroom powder was higher in comparison to standard reference Fe (II) gluconate. Such results further corroborate the

results obtained in the present investigation especially in reference to the higher bioavailability of iron from iron fortified mushrooms (IFHU) as compared to non- fortified (NFHU) mushrooms which can become an important strategy to mitigate iron deficiency.

Table 2. Performance of rats fed diets with and without iron enriched mushroom

Group	Dry matter intake (g/r/d)	Fecal dry matter outgo (g/r/d)	Dry matter digestibility (%)	Average daily gain (g/r/d)	Iron intake (mg/r/d)	Fecal iron outgo (mg/r/d)	Gut absorption of Iron (%)
A control	17.20 ±0.13 [a]	3.54±0.10	79.66±0.54 [a]	2.09 ±0.22	8.90 ±0.06 [a]	7.74 ±0.06 [a]	13.00± 0.92 [c]
B (NFHU) non fortified mushroom	13.40±0.98 [b] [b]	3.70±0.34	72.38±1.82 [b]	1.66± 0.25	5.76 ±0.42 [c]	4.74 ±0.35 [b]	17.72 ±0.59
C (FHU) iron fortified mushroom	15.40±1.07 [ab]	4.23±0.41	72.62 ±1.48 [b]	1.53 ±0.07	6.80± 0.47 [b]	5.30 ±0.31 [b]	21.68 ±1.30 [a]
D equivalent of FeSo4	17.05±0.27 [a]	3.68±0.15	78.42 ±1.00 [a]	2.10± 0.04	9.38± 0.15 [a]	8.18 ±0.11 [a]	12.70 ±0.67 [c]
SEM+_	0.51	0.14	1.03	0.10	0.41	0.40	1.04
F value	5.709	1.12	8.510	2.813	27.646	50.033	21.661
P value	0.012	0.379	0.003	0.084	0.000	0.000	0.000

The delivery system developed in the form of Arka Mushroom fortified Rasam or Soup powder was found to be highly acceptable during the acceptance trials. As evident from Table 2, 91.21% of the respondents liked and accepted the product Arka Mushroom fortified Rasam or Soup while 68.39% liked it very much. None of the respondents disliked the product. This was an important observation as developing iron fortified value-added products acceptable to masses is very challenging due to the color and flavor changes imparted by iron and the protection of the fortified iron from iron inhibitors generally present in cereal-based foods (Hurrell 1997). Both these issues were resolved in the present delivery system through choosing of the right kind of traditional recipe. The traditional recipe of rasam or soup is originally reddish brown and the addition of iron fortified mushroom does not bring about any color changes and is well accepted. The recipe of rasam includes many traditional Indian spices with very strong aroma and flavor which masks any changes brought about in flavor due to the addition of iron fortified mushroom to the recipe.

Table 3. Acceptance score of Arka Mushroom Rasam or Soup by 501 panelists

	9-point Hedonic scale	Acceptance among panelists (Nos)	Acceptance score (%)
9	Like extremely	52	10.379
8	Like very much	291	58.08
7	Like moderately	114	22.75
6	Like slightly	32	6.38
5	Neither like nor dislike	7	1.39
4	Dislike slightly	5	0.998
3	Dislike moderately	NIL	-
2	Dislike very much	NIL	-
1	Dislike extremely	NIL	-
	Total	501	100

The traditional recipe has legumes like tur dal *(Cajanus cajan*) and urad dal (*Vigna mungo*), which contain phytates known to inhibit iron absorption. The recipe was suitably modified without the usage of the legumes to enhance iron bioavailability. The proximate and vitamin B composition of the standardized raw Arka Mushroom Rasam powder with and without mushrooms has been tabulated in table 3a & 3b. As per the data in table 3a, the protein content of the mushroom fortified rasam powder significantly increased by 3.4%, carbohydrate by 2.14% and fiber by 1.43%. The fat content of the sample with mushroom significantly decreased by 3.53%. There was appreciable and significant increase in vitamin B content in the rasam powder with mushroom vis-à-vis without mushroom. (Table 3b).

Table 3a. Comparative proximate composition of raw Rasam or Soup powder

Composition	Raw Arka Mushroom Rasam powder with Mushroom mg/100g raw powder	Raw Rasam powder without mushroom mg/100g raw powder	't' Stat value
Protein	20.85 g	17.45 g	9.89**
Carbohydrate	27.64 g	25.5 g	27.74**
Fat	94.93 g	13.02 g	86.90**
Fiber	10.63 g	92.08 g	5.77**

** Significant at 1%

Table 3b. Comparative vitamin B composition of raw Arka Mushroom rasam or Soup powder

Composition	Raw Arka Mushroom Rasam powder with Mushroom mg/100g raw powder	Raw Rasam powder without mushroom mg/100g raw powder	't' Stat value
Biotin	2.576	0.857	11.15**
Cyanocobalamin	0.003	0	59.00**
Folic acid	0.059	0.003	8476**
Niacin	161.00	39.82	33.61**
Pantothenic acid	270.433	15.09	468.56**
Pyridoxin	8.104	1.829	77.35**
Riboflavin	9.057	1.62	83.90**
Thiamine	2.576	0.396	113.43**

** Significant at 1%

Table 4a & 4b depicts the data obtained of the proximate and vitamin B content of the cooked and ready to use rasam. There was a significant increase in the protein content of cooked and ready to use rasam with mushroom by 7.34%, carbohydrate by 16.19% and fiber by 3.15%. The fat content in the product with mushroom significantly decreased by 7.3%. There was significant increase in the biotin, niacin, pantothenic acid, riboflavin and thiamin content in the cooked product with mushroom as compared to without mushroom.

Table 4. Comparative proximate composition of cooked Arka Mushroom rasam or Soup

Composition obtained after boiling 10g of the powder in 350ml of water	Cooked & ready to use Arka Mushroom Rasam with Mushroom mg/100ml	Cooked & ready to use Rasam without Mushroom mg/100ml	't' test value
Protein	3.19 g	2.46 g	64.90**
Carbohydrate	8.32 g	6.70 g	105.76**
Fat	0.1 g	1.42 g	29.135**
Fiber	4.31 g	3.99 g	40.93**

** Significant at 1%

Table 4b. Comparative vitamin B composition of cooked Arka Mushroom rasam or Soup

Vitamin B	Cooked & ready to use Arka Mushroom Rasam with Mushroom µg/100ml	Cooked & ready to use Rasam without Mushroom µg/100ml	't' test value
Biotin	2.555	0.316	7.88**
Cyanocobalamin	0	0	65535 NS
Folic acid	0	0.0035	65535 NS
Niacin	134.184	34.914	151.18**
Pantothenic acid	49.905	14.50	114.89**
Pyridoxin	4.628	0.677	27.80**
Riboflavin	3.404	0.354	272.92**
Thiamine	2.594	0.268	59.94**

** Significant at 1% NS – Non significant

Table 5 shows the macro and micro element composition of the Arka rasam powder with and without mushroom. As seen from table 5a, there was a significant increase in Phosphorus, potassium and calcium levels in the rasam powder with mushroom as compared to without mushroom. The levels of other minerals remained same in both treatments. As per table 5b, the level of micronutrients Manganese significantly increased in the product with mushroom as compared to without mushroom. The level of Zinc decreased significantly by the addition of mushroom. The levels of iron and copper remained statistically unchanged in both treatments.

Table 5a. Comparative macro and micro element composition of raw Arka Mushroom rasam or Soup powder

Composition	Raw Arka Mushroom Rasam powder with Mushroom mg/100g raw powder	Raw Rasam powder without mushroom mg/100g raw powder	't' test value
Macro-elements			
Phosphorus	516.66	341	10.106**
Potassium	2170	1943.33	6.552**
Calcium	348.33	451	19.479**
Magnesium	120	131.33	4.25 NS
Micro-elements			
Iron	9.23	13.52	4.066NS
Manganese	5.43	2.01	80.781**
Copper	1.11	1.05	1.889 NS
Zinc	1.20	3.20	22.863**

** Significant at 1% NS – Non significant

Lynch *et al*. (1984) reported a low bioavailability of 0.84-1.91% of iron from legumes in human beings. The data in table 6 shows the iron content of the Arka Rasam Powder made without the use of legumes.

Table. 6. Iron analysis of rasam with iron fortified mushroom

Sl. No.	Treatment	Iron content(mg/100g dry powder)
1	Rasam without dal + with iron fortified mushroom	30.8[a]
2	Rasam with dal + iron fortified mushroom	29.2[a]
3	Rasam without dal+ non fortified mushroom	11.36[b]
4	Rasam with dal+ non fortified mushroom	12.06[b]
5	Rasam without mushroom (traditional recipe)	11.83[b]
	Coefficient of variation	8.589
	CD (0.01)	4.234
	CD (0.05)	2.977

As evident from Table 6, the iron content of the product Arka Rasam Powder made with and without legumes but with iron fortified mushroom was statistically at par and significantly higher as compared to the one made with non-fortified mushroom or the traditional rasam without mushroom. The iron content of the rasam with and without non fortified mushroom and without dal were statistically at par. This result is of significance as the bioavailability of iron is higher as depicted in the animal model studies earlier. Bioavailable iron in 10g Arka rosam powder made with iron fortified mushrooms @ 21.68% bioavailability is 0.667m and it takes cae of 28.02% requirement of bioavilable iron (2.3 mg/day) iron measurating women.

Conclusion

The high bioavailability of iron from both non fortified and iron fortified *Hypsizygus ulmarius* is of immense significance as the predominantly vegetarian population of India suffer with iron deficiency due to very poor bioavailability of 5-8% of non-heme iron from plant sources. The bioavailability of heme iron from meat sources varies from 18-25%. The present data of the bioavailability of 21.68% from iron fortified mushroom can play a significant role in mitigating iron malnutrition in the country. The delivery system standardized in the form of Arka Mushroom Rasam powder was not only significantly higher in

iron content with high bioavailability but also showed enhanced nutritional values in terms of protein, carbohydrate, fiber and B vitamins like biotin, niacin, pantothenic acid, riboflavin and thiamin. Thus, this product can play an important role in mitigating iron malnutrition as it fits into the taste, flavor and aroma of an already well accepted form of daily food accompaniment already in vogue.

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