International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 365-368

Received: 07-09-2019
Accepted: 09-10-2019

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Nutritional quality of spinach (*Spinacia oleracea* L.) incorporated foxtail millet (*Setaria italica*) based masala biscuits

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Abstract
The present study was conducted to study the nutritional quality of Foxtail millet based spinach incorporated masala biscuits. These biscuits were prepared by incorporating spinach at 10, 15 and 20% to improve the micronutrient and antioxidant content. Nutrient analysis of best-accepted biscuit i.e. 20% spinach fortified biscuits showed an increase in the amount of crude fibre (5.35±0.05 g/100 g), zinc (8.18±0.09 mg/100g), copper (0.87±0.03mg/100 g), antioxidant activity (21.37±0.43%) compared to control biscuits. Besides, these biscuits contained low fat (20.7±0.26 g/100 g), carbohydrates (53.84±0.58 g/100 g) and calorific value (455.6±5.8 K. Cal/100g) compared to control biscuits. Based on the acceptability and nutrient analysis it is concluded that spinach incorporated foxtail millet masala biscuits serve as a vehicle for imparting micronutrients and health-promoting phytochemicals into the diet.

Keywords: Spinach, foxtail millet, biscuit, masala, nutrient composition

Introduction

Biscuit is the most popular snack, reasons being acceptability in all age groups, extended shelf life, easy packaging and good taste. Despite all these merits biscuits mainly provide calories due to high fat, sugar and refined wheat flour as base ingredients. Protective nutrients like vitamins, minerals, dietary fibre, phytochemicals and antioxidants are usually lacking. There are many ways to improve the quality of biscuits one of which is replacement or blending of refined wheat flour with another nutrient-rich millet flour, pulse and vegetable or fruit. Foxtail millet contains a pertinent amount of nutritional components especially starch, protein, vitamins and minerals.

Moreover, it is nutritionally superior to rice in crude fibre content and also contains good quantities of calcium and iron. Spinach (*Spinacia oleracea*) is a green leafy vegetable, low in calories is considered as a good source of vitamins (ascorbic acid, riboflavin, niacin and folic acid), minerals (iron and calcium) and dietary fibre. Soybean has been used effectively in bakery products (e.g. biscuit, bread etc.) to improve protein content. Soy protein is the only vegetable source of complete protein, of a quality comparable to meat and eggs, which contains all the essential amino acids required by humans and animals. Different spices are in use for bakery products due to rapidly increasing consumers awareness on functional foods. Hence, incorporation of nutrient-rich ingredients in the baked products such as biscuits will improve their nutraceutical properties and help to cater to the health needs of various cross-sectionss of the population.

Materials and methods

Materials

Foxtail millet flour, refined wheat flour, soy flour, icing sugar, shortening, spinach, green chillies, cumin seeds and curry leaves were procured from the local market of Hyderabad.

Preparation of spinach puree

Fresh spinach leaves were washed, sorted, stalks were removed and leaves were cut into pieces, ground in the mixer grinder (Philips, HL1632) to fine mass in the grinder and was passed through a muslin cloth to get a homogenous puree.

Formulation of spinach incorporated foxtail millet masala biscuits

Formulations used for biscuits are given in Table 1. All the dry ingredients were mixed and sieved twice to incorporate air. Hydrogenated fat was creamed until light and fluffy, the mixed flour was added to the creamed paste along with spices and mixed until the uniform smooth dough was obtained. The dough was rolled out and cut into a round shape with a biscuit cutter, placed in a greased baking tray and baked in a preheated oven at 180 °C for 10 minutes according to the method of AOAC in the Millet Processing Incubation Centre, Rajendranagar, Hyderabad.

Table 1: Formulations of Spinach incorporated Foxtail millet based masala biscuits with spices

Samples	Foxtail millet flour %	Refined wheat flour %	Soy flour %	Sugar %	Fat%	Spinach puree %	Salt %	Spices		
								Green Chill (%)	Cumin seeds (%)	Curry leaf (%)
CB	35	25	15	3	20	---	2	---	---	---
$SIFB_{10}$	30	15	12	3	20	10	2	3	3	2
$SIFB_{15}$	28	15	10	3	20	15	2	3	3	1
$SIFB_{20}$	27	15	8	3	20	20	2	1	2	2

CB: 0% Spinach incorporated foxtail millet masala biscuit
$SIFB_{10}$: 10% Spinach incorporated foxtail millet masala biscuit
$SIFB_{15}$: 15% Spinach incorporated foxtail millet masala biscuit
$SIFB_{20}$: 20% Spinach incorporated foxtail millet masala biscuit

Evaluation of Nutritional composition

Evaluation of nutritional properties was done for the best accepted product among all the variations i.e. $SIFB_{20}$, following standard AOAC methods. Moisture by (AOAC, 1990) [6]; Fat by soxhlet extraction method (AOAC, 1981) [2]. Ash was determined using muffle furnace (AOAC, 1984) [3]; Crude fibre was analysed by AOAC 2000 [4], the protein was estimated by combustion method (AOAC 992.23 – 2005) [5]. Difference method was followed for per cent carbohydrate content estimation (AOAC 2006). Energy value was expressed in kcal/100 g and determined by adding lipid, carbohydrate and protein contents with the formula: (9×lipids) + (4×carbohydrates) + (4×Proteins). Atomic absorption spectroscopy was used to measure mineral (Iron, Zinc and Copper) content. Antioxidant activity was estimated by the DPPH method (Blois, 1958). Total carotenoids, Total Phenolic content were assessed by Zakaria *et al*., 1979 [18] and Slinkard and Singleton, 1977 [16] respectively.

Statistical Analysis

All data were presented as means ± the standard deviation of the mean. As for multiple group comparison, the significance of the differences among the treatment groups and their respective control groups were analysed using Window stat 9.1 software. Statistical significance was assessed by one-way analysis of variance (ANOVA).

Results and Discussion

Results of the nutritional composition of the samples revealed that a significant increase in the moisture, ash, crude fibre content due to spinach incorporation (p<0.05) Table 1. The higher moisture content in the $SIFB_{20}$ was due to the presence of fibre in the spinach which has characteristic water holding capacity during dough formation. Galla *et al*., 2017 [13] also reported that an increase in the moisture due to the incorporation of spinach powder in wheat biscuits. Shere *et al*., 2018 [15] reported that incorporation of spinach puree resulted in increased moisture content in the experimental noodles.

Protein content in the CB was 14.5 g whereas $SIFB_{20}$ had 13.4 g of protein. Lower protein content in $SIFB_{20}$ might be due to the replacement of the other ingredients with the spinach puree in equal amounts and generally, spinach is considered to be low in protein. Decrease in the protein content from 88.0 g/kg to 86.0 g/kg was recorded by (Rekha *et al*., 2013) [14] in spinach puree incorporated bread wheat pasta. The mean values of the estimated fat of the samples were shown in Table 1. The results of the fat were found to be non-significant with that of control. CB had the highest (22.6 g) while $SIFB_{20}$ had the least (20.7 g) fat content. Ramu *et al*., 2016 also reported that fat and ash content enhancement with the level of spinach substitution in the instant spinach fortified wheat noodles when compared to the control. Ash content of the CB was 2.26 g and found to be lower than the $SIFB_{20}$ with 3.16 g. Increased incorporation of spinach powder (5-15%) has shown good enhancement of the ash in the biscuits when compared to control biscuits (Galla *et al*., 2017) [13]. These results are in agreement with those described by Shere *et al*., 2018 [15] that addition of spinach puree at 10-50% in the wheat noodles caused a gradual increase in ash content from 2.85 to 3.6% while control noodles had 1.57% of ash content.

It is evident from the data that there is a significant increase in the crude fibre content in the $SIFM_{20}$ with 5.35 g/100g than CB with 3.39 g/100 g. Displacement of the carbohydrate rich ingredients with spinach puree showed an inversely proportional relationship with the increase in the level of carbohydrate content. However, this change was found to be non-significant.

Significant increase in the zinc and copper content was observed whereas iron content was decreased due to spinach incorporation (p<0.05). As per the results, CB was found to have high iron (8.30 mg/100g) than $SIFM_{20}$ (6.76 mg/100g). As indicated in Table 1 zinc content was 7.50 mg/100g and 8.18 mg/100g in CB and $SIFM_{20}$ respectively. Addition of spinach puree at 20% level resulted in enhancement of copper content in the $SIFM_{20}$ however this was found to be nonsignificant.

$SIFM_{20}$ had the highest total carotenoids content (8.28 µg/g) followed by CB (12.01 µg/g). The increase in the total carotenoids content of $SIFM_{20}$ was attributed to the high inherent carotenoids proportions in the spinach. Similar results were reported by Khan *et al*., 2015 that the addition of dehydrated spinach powder at 5-10% significantly increased total carotenoids from 62.32 to 118.32 µg/g in *chapathi* premixes.

Polyphenolic compounds exert anti-mutagenic and anti-carcinogenic properties in humans when consumed up to 1 g daily (Tanaka *et al*., 1998) [17]. The results of total phenolic content (TPC) of the analysed biscuits were shown that there was no significant variation in the TPC in the samples. The highest TPC was found in CB with 78.65 µg RE/g followed by $SIFM_{20}$ with 73.10 µg RE/g. Fizah, 2014 stated that the addition of spinach from 10-40 g in the blends prepared to develop extrudates showed increased values of Total phenolic content compared to the control. Blend with 20 g spinach had 452 mg CE /100, while corn grits which were the control had 116.52 mg CE /100. Antioxidants are effective in preventing oxidative stress, thus they have a protective role for the human body (Mandis *et al*, 2008). The antioxidant activity of $SIFM_{20}$ (21.37%) was higher than CB (17.60%) this is due to the addition of spinach puree and spice mix in the Spinach fortified biscuits.

Table 2: Nutritional composition of control and spinach incorporated foxtail millet based biscuits

	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Crude fibre (g/100g)	Carbohydrate (g/100g)	Energy (Kcal/100g)
CB	2.66±0.17[a]	14.95±0.19[a]	22.6±1.17[a]	2.26±0.08[b]	3.39±0.05[b]	54.14±0.58[a]	479.70±5.8[a]
$SIFB_{20}$	3.46±0.31[b]	13.49±0.24[b]	20.7±0.26[a]	3.16±0.12[a]	5.35±0.05[a]	53.84±0.58[a]	455.60±5.8[b]
CD	0.65	0.24	5.78	0.24	0.22	2.26	22.66

	Iron mg/100g	Zinc mg/100g	Copper mg/100g	Carotenoids µg/g	Total phenolic content µg RE/g	Antioxidant activity %
CB	8.30±0.17[a]	7.50±0.15[a]	0.83±0.31[a]	8.28±0.15[b]	78.65±2.46[a]	17.60±0.71[b]
SIB_{20}	6.76±0.12[b]	8.18±0.09[a]	0.87±0.03[a]	12.01±0.65[a]	73.10±4.72[a]	21.37±0.43[a]
CD	0.28	0.82	0.21	3.49	30.88	1.90

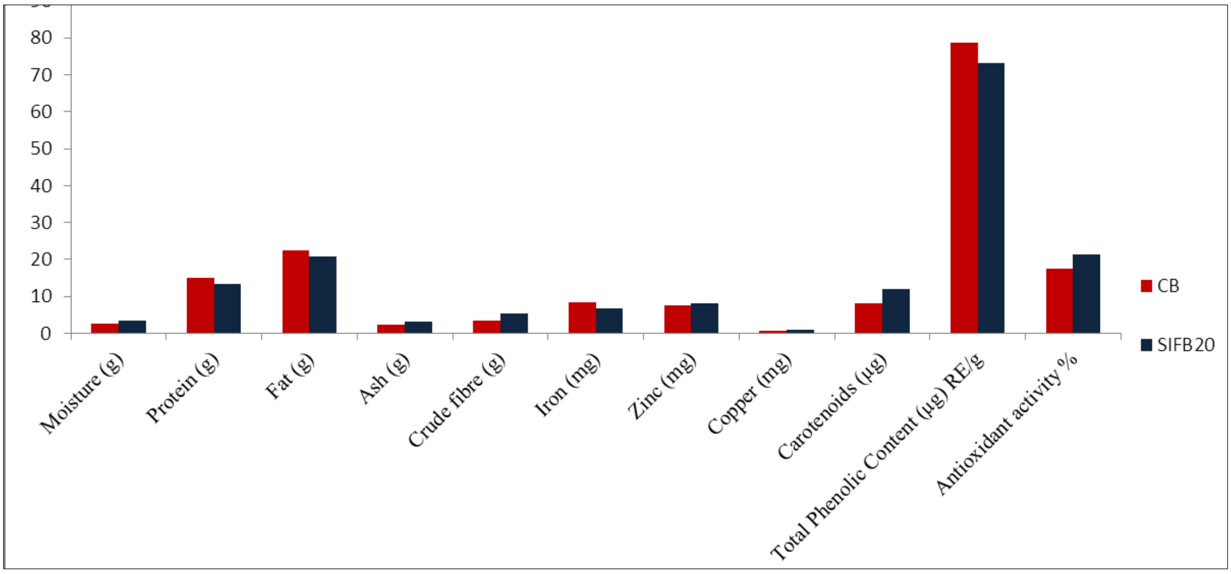

Fig 1: Nutritional composition of biscuits

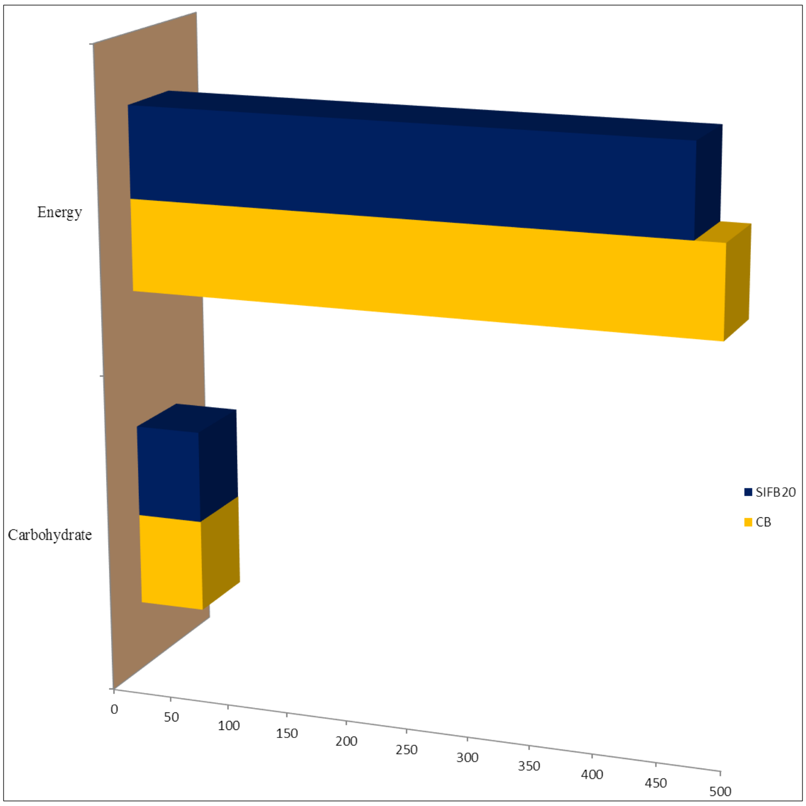

Fig 2: Carbohydrate and energy value of biscuits

Conclusion

It has been demonstrated that spinach incorporation at 20% level in foxtail millet based masala biscuits could be employed as a source of micronutrients and antioxidants. Nutritive value of biscuits improved in terms of crude fibre, zinc, copper, total carotenoids and antioxidant activity due to spinach incorporation. It can be concluded that spinach incorporated masala biscuits have a good potential for promotion as functional food having the potential to prevent micronutrient deficiencies and oxidative stress.

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