



## Sensory and Nutritional Properties of Protein Enriched Biscuits

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

Malnutrition remains a major nutritional problem nowadays. So there is a need to develop protein-rich products. Biscuits are extensively used for protein fortification vehicles due to their long shelf life and high acceptability. In the present study, biscuits were produced by substituting the refined wheat flour (RWF) with the foxtail millet flour (FMF), pearl millet flour (PMF), defatted soy flour (DFS) and whey protein isolate (WPI) at different levels. The formulations developed were FMWIB (Foxtail millet whey isolate biscuit), PMWIB (Pearl millet whey isolate biscuit) and MMWIB (Mixed millet whey isolate biscuit) and control (RWB-Refined wheat flour biscuit) contained 100% RWF. There was a significant difference ( $p < 0.05$ ) found among the formulations for all sensory parameters. Proximate analysis of the experimental (MMWIB) and RWB biscuit revealed that addition of millet flours, DFS and WPI significantly ( $p < 0.01$ ) improved the moisture (2.47%), protein (16.6%), ash (1.39%), crude fiber (1.9%) and fat (24.74%) content of the biscuits compared to RWB which were 2.36%, 5.56%, 0.63%, 0.21% and 23.11% respectively. The highest protein value was observed for MMWIB (16.6%) followed by PMWIB (15.81%), FMWIB (15.18%) and RWB (5.56%). MMWIB could contribute 38% of the recommended daily protein intake of 3 to 10 years old and 18% of RDA for adolescents group. Nutritional composition revealed that MMWIB had high TDF (15.49%) and IVPD (62.65%) values than RWB. Therefore, millet flours, DFS and WPI has a good potential for use in biscuit formulation to enhance its nutritional quality and also sensory properties.

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## 1. INTRODUCTION

Biscuit is a well-known product and represents the largest category of snack items among baked foods all over the world [1]. Refined wheat flour is a product of refining which contains higher proportion of starch, low in dietary fiber, minerals, and protein and are deficient in some amino acids such as lysine and certain others, the resultant cookies would be characterized with low proteins and mineral content [2].

Cookies prepared from composite flours have been extensively used for protein fortification vehicles due to their long shelf life and high acceptability [3]. These characteristics make protein-rich cookies attractive in countries where protein-energy malnutrition is prevalent [4] and also in areas which need child feeding programs, among low income and for disaster relief operations [5].

In order to improve its nutritive value, there is a need for replacing RWF with flour of better nutritive quality grains like millets; pearl millet [6], foxtail millet [7], Legumes such as soybean [8] which are higher in proteins (18 to 24%) than cereal grains and could be used to support certain amino acids such as lysine, tryptophan, or methionine [9] and whey proteins [10].

Millets are nutritionally comparable or even superior to staple cereals such as rice and wheat [11]. Foxtail millet (*Setaria italica*) is a good source of protein (12.3 g/100g) and dietary fiber (14 g/100g). The carbohydrate content is low (60.9 g/100g). Besides, it is rich in crude protein (12.3%), minerals (3 g/100g) and phytochemicals. Its essential amino acid profile suggests that it can be used as a supplementary protein source to most cereals since it is rich in lysine [12].

Pearl millet (*Pennisetum glaucum*) is one of the four most important cereals grown in tropical semi-arid regions of the world. The carbohydrate content of pearl millet is 67.5 g/100g, high fiber content (1.2 g/100g), protein-rich (11.8 g/100g), richer in fat content (5 mg/100g), high amount of iron (8 mg/100g) and Zinc (3.1mg/100g) [13] which may help to increase the hemoglobin levels.

Defatted soy flour is a cheaper, convenient, conventional and richest source of protein for the

fast expanding population worldwide [14]. Soybeans contain 30–45% protein with a good source of all indispensable amino acids [15]. The whey proteins are potentially nutritional and functional food ingredients for use in a wide range of food types and can replace expensive ingredients such as egg white and milk proteins [16].

Protein-rich supplementary foods are important to prevent protein energy malnutrition in children [8]. People who are overweight can also benefit from a diet with increased protein levels because protein lead to an increased satiety compared to carbohydrates [17]. Biscuits can be easily fortified [18] with protein-rich flours in order to supplement protein in the diet in a convenient form. Therefore, the present investigation was carried out an aim to develop protein enriched biscuits by substituting the RWF with protein-rich millet flours, DFS and WPI.

## 2. MATERIALS AND METHODS

All the ingredients used such as foxtail millet flour (FMF), pearl millet flour (PMF), defatted soy flour (DFS) and refined wheat flour (RWF) were procured from Millet Processing center of professor Jayashankar Telangana State Agricultural University. Fat, sugar, baking powder and ammonia were procured from standard local shops. Whey protein isolate (WPI) was procured from online market.

### 2.1 Product Formulation

Biscuits were prepared by substituting the RWF with the FMF, PMF, DFS and WPI at different levels and the formulations developed were: RWB- Refined wheat flour biscuit (RWF-100%), FMWIB-Foxtail millet whey isolate biscuit (RWF-20%, FMF-50%, DFS-20% and WPI-10%), PMWIB- Pearl millet whey isolate biscuit (RWF-20%, PMF-50%, DFS-20% and WPI-10%) and MMWIB- Mixed millet whey isolate biscuit (RWF-20%, FMF-25%, PMF-25% DFS-20% and WPI-10%).

### 2.2 Preparation of Biscuits

Initially, all ingredients were weighed according to set formulations of RWB, FMWIB, PMWIB and MMWIB. Flour and baking powder was sifted

together (twice). Fat was creamed till light and spreadable then powdered sugar was added and creamed again till light and fluffy. Ammonia and essence were added to cream and mixed. All the ingredients like flour mix, whey protein isolate were added and rubbed to get uniform and pliable dough. The dough was spread into a sheet and cut into different shapes and baked at 180°C for 20 minutes. Finally, biscuits were cooled and packed [19].

### 2.3 Sensory Evaluation of Protein Rich Biscuits

Sensory evaluation of biscuits was carried out in Post Graduate and Research Center, PJTASU. A semi trained panel of 21 members evaluated the biscuits for its appearance, color (evenness of color), texture (crispiness, hardness, roughness and denseness), taste (after taste), flavor and overall acceptability using 9 point hedonic scale. Written instructions were given to panelists and scores were given based on hedonic scale of 1 to 9 (1= dislike extremely to 9= like extremely) as described by Meilgard et al. [20] with few modifications.

### 2.4 Statistical Analysis

All the results were statistically analyzed to test the significance of the results using percentages, means, standard deviations, t-test and analysis of variance (ANOVA) technique [21].

### 2.5 Nutritional Analysis of Control and Experimental Biscuits

The biscuit samples were analyzed by using standard procedures of moisture AOAC [22], protein AOAC [23], Fat AOAC [24], crude fiber AOAC [25], ash AOAC [22], carbohydrate AOAC [26] and total dietary fiber (TDF) AOAC [27] and *in-vitro* protein digestibility was estimated as per Schechterle and Pollak [28].

## 3. RESULTS AND DISCUSSION

### 3.1 Sensory Evaluation of Control and Protein Rich Biscuits

Sensory evaluation was conducted for FMWIB, PMWIB and MMWIB along with RWB to see the acceptability. Analysis of variance of RWB and whey isolate biscuits showed that there was significant difference ( $p < 0.05$ ) found for all parameters. Textural quality is a very important and desirable quality attribute for biscuit [29]

which is highest for MMWIB (7.90). Sensory attributes of MMWIB was comparable to RWB even superior in some attributes like appearance, taste, texture and evenness of color. The sensory attributes of biscuits are shown in Table 1.

### 3.2 Nutritional Analysis of Control and Experimental Biscuits

#### 3.2.1 Protein quality of developed formulations

##### 3.2.1.1 Protein

There was a significant difference among all the formulations at 1% level of significance. Highest protein content was observed in MMWIB (16.63%) than PMWIB (15.81%), FMWIB (15.18%) and RWB (5.56%). The highest protein content of MMWIB (16.63%) was because of the 92% protein content of whey protein isolate, 50% protein content of DSF and protein-rich millet flours. Since the IVPD value of MMWIB was 62.65%, it can contribute 38% of the recommended daily protein intake of 3 to 10 years old and around 18% of RDA (recommended daily allowance) for adolescents [30]. As these biscuits were formulated with millets, that are rich in essential amino acids like lysine, threonine, valine, sulfur containing amino acids and the ratio of leucine to isoleucine is about 2 [31]. Based on the protein content of the different formulations MMWIB was selected for further analysis.

In a similar study of Sambavi et al. [32] who reported that in the best formulation (55% foxtail millet, 45% wheat flour sample) protein content was increased to 13.1%. In another study, biscuits were enriched with whey protein concentrate (WPC) at different proportions 0% (T0), 2% (T1), 4% (T2), and 6% (T3). The highest value for protein content (13.22%) was observed in T3 (6% WPC supplemented cookies) while the lowest amount of 9.08% was in T0 (control sample) [10].

##### 3.2.1.2 *In-vitro* protein digestibility (IVPD)

*In-vitro* protein digestibility is very important in assessing the quality of protein in a given product. It also helps to assess the effect of any processing on the same. The biological utilization of protein is primarily dependent on its digestibility. Significant difference ( $P < 0.01$ ) was found between the biscuits (Table 3). IVPD value

**Table 1. Mean sensory scores of control and whey isolate biscuits**

Parameters	RWB	FMWIB	PMWIB	MMWIB	CD at 5%
Appearance	7.47±0.17 <sup>b</sup>	7.95±0.23 <sup>b</sup>	6.90±0.21 <sup>c</sup>	8.04±0.18 <sup>a</sup>	0.57
Color	7.57±0.19 <sup>a</sup>	7.95±0.17 <sup>a</sup>	7.04±0.21 <sup>c</sup>	7.09±0.83 <sup>a</sup>	1.26
Evenness of color	7.42±0.20 <sup>a</sup>	7.66±0.17 <sup>a</sup>	7.00±0.22 <sup>b</sup>	7.90±0.22 <sup>a</sup>	0.58
Flavor	7.66±0.25 <sup>a</sup>	7.09±0.34 <sup>a</sup>	6.19±0.32 <sup>b</sup>	6.90±0.34 <sup>b</sup>	0.89
Texture	7.76±0.18 <sup>a</sup>	7.66±0.21 <sup>a</sup>	7.00±0.23 <sup>b</sup>	7.90±0.20 <sup>a</sup>	0.59
Crispiness	7.76±0.18 <sup>a</sup>	7.71±0.22 <sup>a</sup>	6.85±0.31 <sup>b</sup>	7.66±0.21 <sup>a</sup>	0.67
Hardness	7.52±0.24 <sup>a</sup>	7.38±0.27 <sup>a</sup>	6.81±0.29 <sup>b</sup>	7.75±0.23 <sup>a</sup>	0.73
Roughness	7.33±0.23 <sup>a</sup>	7.57±0.24 <sup>a</sup>	6.61±0.26 <sup>b</sup>	7.52±0.21 <sup>a</sup>	0.67
Denseness	7.61±0.23 <sup>a</sup>	7.52±0.22 <sup>a</sup>	7.04±0.22 <sup>b</sup>	7.28±0.20 <sup>a</sup>	0.62
Taste	7.61±0.21 <sup>a</sup>	7.14±0.37 <sup>a</sup>	6.47±0.30 <sup>b</sup>	7.66±0.25 <sup>a</sup>	0.82
Aftertaste	7.66±0.25 <sup>a</sup>	7.09±0.27 <sup>a</sup>	6.52±0.24 <sup>b</sup>	7.0±0.35 <sup>a</sup>	0.80
Overall acceptability	7.76±0.19 <sup>a</sup>	7.57±0.23 <sup>a</sup>	6.61±0.29 <sup>b</sup>	7.47±0.22 <sup>a</sup>	0.67

Note: Means having different superscripts in the same row are significantly different at 5% level of significance.

RWB: Refined wheat flour biscuit, PMWIB: Pearl millet whey isolate biscuit

FMWIB: Foxtail millet whey isolate biscuit, MMWIB: Mixed millet whey isolate biscuit

**Table 2. Proximate composition of control and experimental biscuit**

Biscuits	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude fiber (%)	CHO (%)	Energy (Kcal)
RWB	2.36	5.56	23.11	0.63	0.21	68.10	506.46
MMWIB	2.47	16.63	24.74	1.39	1.99	52.76	501.27
t value	1.88	302.22	2.99	31.42	229.60	29.62	4.89
Probability	0.07 <sup>**</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.00 <sup>**</sup>	0.00 <sup>*</sup>

Note: Values are expressed as mean, <sup>\*\*</sup> (significantly different at  $p<0.01$ ); RWB: Refined wheat flour biscuit; MMWIB: Mixed millet whey isolate biscuit

RWB was 53.34% and MMWIB was 62.65%, this increase in the value might be due to the high biological value of whey protein isolate and DFS. Heat processing is reported to improve the digestibility of seed protein by destroying protease inhibitors and opening the protein structure through denaturation [33]. These results were in accordance with Sudha et al. [34]. The IVPD, which was 57.62% for control, increased to 75–86.67% for samples where DSF and WPC were used in combination.

**Table 3. Nutritional quality characteristics of control and experimental biscuit**

Biscuits	Total dietary fiber (%)	IVPD (%)
RWB	5.90	53.34
MMWIB	15.49	62.65
t value	80.62	3.8
Probability	0.00 <sup>**</sup>	0.00 <sup>**</sup>

Note: Values are expressed as mean, <sup>\*\*</sup> (significantly different at  $p<0.01$ ).

RWB: Refined wheat flour biscuit

MMWIB: Mixed millet whey isolate biscuit

### 3.2.2 Moisture

Biscuits are probably a better vehicle of fortification with protein because of their popularity, high nutrient density and long shelf-life because they are very low in moisture [35]. There was significant difference between the biscuits ( $p<0.01$ ). The moisture content of RWB was 2.36% and MMWIB was 2.47%. However, the values of moisture were very low showing good storage stability. Biscuits generally have low moisture content and it is advantageous as to microbiological aspects, resulting in long shelf-life if stored in appropriate conditions (packaging material and environmental conditions) [36]. Biscuits typically have a moisture content of less than 4% and have a long shelf-life of six months or more [37] thus, indicating that MMWIB and RWB had good storage stability.

### 3.2.3 Ash

There was a significant difference between both values at 1% level of significance. The ash content of RWB and MMWIB was 0.63%, 1.39% respectively. As millets are rich in mineral

content, the ash content of MMWIB was found to be higher. Similar findings were observed by Kulthe et al. [38] who reported that substituting refined wheat flour with DSF at 0, 10, 15, 20 and 25% levels increased the protein, crude fiber and ash content in biscuits. The crude fiber and ash contents increased from 0.14 to 0.84% and from 0.85 to 1.95% respectively.

### 3.2.4 Fat

There was a significant difference between the biscuits ( $p < 0.01$ ). MMWIB had high fat (24.74%) content than RWB (23.11%). About 75% of the fatty acids in pearl millet are unsaturated and linoleic acid is particularly high (46.3%) [39]. Pearl millet is richer in fat content (5 mg/100g) [13] as compared to most grains and 75% of the fatty acids are unsaturated. Omega 3, linolenic acid (C18:3 n-3) (LNA) comprises 4% of the total fatty acids in this oil [40], giving it a higher content of n-3 fatty acids than other cereal grains.

### 3.2.5 Carbohydrates

Results from statistical analysis found that there was significant difference between them ( $p < 0.01$ ). High carbohydrate content was found in RWB (68.10 g) than MMWIB (52.76 g). The lowered carbohydrate content in MMWIB was might be due to their lower contents in millets (foxtail and pearl millet) and DSF than in refined wheat flour. Similar results were found by Kulthe et al. [38]. The fat and carbohydrate content of cookies was found to be significantly decreased from 23.77 to 20.96% and from 66.82 to 61.40% respectively with increasing levels of defatted soy flour in cookies.

### 3.2.6 Crude fiber

There was a significant difference between the biscuits at 1% level of significance. Crude fiber content of RWB was 0.21% and MMWIB was 1.9% (Table 2). This increase in crude fiber content is might be due to the replacement of refined wheat flour with FMF and PMF which are rich in crude fiber content (6.7% and 2.3%). The results of the present investigation in accordance with the results of Anju and Sarita [7]. The nutrient composition of biscuits indicated that on replacing refined wheat flour with 45% millet flour in FMB (foxtail millet biscuit) and BMB (barnyard millet biscuit), the crude fiber content increased by 10 times in FMB and BMB compared to

CRWFB (control refined wheat flour biscuit).

### 3.2.7 Energy

RWB was found to have high energy content (506.46%) than MMWIB (501.27%). When statistically tested there was a significant difference between the biscuits at 1% level of significance. The lower carbohydrate content of soy composite biscuits was a result of the low carbohydrate content of DSF because soybean stores energy as oil [41]. Results of the present study was on par with the results of Anju and Sarita, [7] who reported that energy (%) content was lower for 45% millet incorporated FMB (346), BMB (339) than CRWFB (350).

### 3.2.8 Dietary fiber

There was a significant difference between the biscuits at 1% level of significance. The TDF content found to be high in MMWIB (15.49%) than in RWB (5.90%). These results show that dietary fiber content (total, soluble and insoluble) was significantly higher for the millet flours compared to refined wheat flour.

During baking of biscuits, 1, 6 anhydro D-glucose units might have been liberated from the starch and other polysaccharides to form enzyme resistant complexes that are different from resistant starch [42]. Several authors have reported that during baking, a fraction of starch rendered itself inaccessible to amylases [43] and total dietary fiber content increases due to retrogradation [44].

Mineral absorption enhancing properties were observed for some soluble dietary fibers such as pectins and fructo oligosaccharides while no such effect was observed for insoluble ones [45]. PCOS (poly Cystic Ovary Syndrome) is the most common endocrine disorder in women, affecting an estimated 5–10% of women of reproductive age [46]. Based on the evidence to date, a diet low in saturated fat and high in fiber from predominantly low-glycemic-index-carbohydrate foods is generally suitable for women with PCOS [47]. As these developed biscuits are high in protein and dietary fiber helpful for women with PCOS.

## 4. CONCLUSION

Therefore, Protein rich biscuits can be prepared by replacing refined wheat flour with foxtail millet

flour at 25%, pearl millet flour at 25%, defatted soy flour at 20% and whey isolate at 10% level, which will dramatically increase the protein content and sensory quality characteristics of the biscuits. Compared to RWB, MMWIB was found to be nutritionally superior in terms of protein, ash, crude fiber, dietary fiber and IVPD, which can be recommended for people with protein deficiency, life style disorders and degenerative diseases.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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