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Comparative study on nutritional and microbiological quality analysis of supplied fortified high energy biscuit for school feeding in poverty prone areas in Bangladesh with World Food Programme nutritional requirements

Suvasish Das Shuvo[1]✉, Md. Ashrafuzzaman Zahid[1]

Dept. of Nutrition and Food Technology, Jessore University of Science and Technology, Jessore, Bangladesh

✉**Corresponding Author:**
Suvasish Das Shuvo
Dept. of Nutrition and Food Technology,
Jessore University of Science and Technology, Jessore,
Bangladesh
Email: suvasish.shuvo@yahoo.com

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ABSTRACT

School feeding program (SFP) has been popular in developing countries as an instrument for achieving food and nutrition security in poverty prone areas. These programs are frequently targeted towards schools that face poor attendance and enrollment of students. SFP biscuits are the most important source of energy, protein, and vitamins in the diet of program participants. SFP improves child nutritional status and academic performance. The purpose of this study is to assess the nutritional and microbiological quality of fortified high energy biscuit with WFP limits. Various nutritional parameters of fortified high energy biscuit (HEB) samples examined such as moisture, ash, protein, fat, crude fiber, sugar and energy by AOAC methods. Iron and vitamin A content were also assessed by Flame Atomic Adsorption Spectrophotometer (FAAS) and High Performance Liquid Chromatography (HPLC). In order to evaluate the microbial spoilage and shelf life *mesophyllic aerobic bacteria, coliforms, escherichia coli, salmonella, staphylococcus, bacillus cereus, enterobacter spp.* and *yeasts* and *moulds* were tested for microbial contamination. The result revealed that energy value 456.5 Kcal, protein 10.9%, fat 16%, ash 1.9%, sugar 13.6%, moisture 2.8%, vitamin A 236.29 µg and peroxide value4.0meq/kg fat of fortified high energy biscuits which was in World Food Program (WFP) permissible limits. Though iron and vitamin A did not comply with the WFP permissible limits and microbial loads of most of the fortified high energy biscuits were in the permissible limits. The proximate analysis shows that there was no significant difference of sample biscuit in moisture, ash, protein, fat, crude fiber, sugar, energy. But significant difference observed in iron and vitamin A content. No significant difference of sample biscuits in microbial loads.

Key words: School feeding program, High energy biscuit, Nutritional Properties, Microbial properties

1. INTRODUCTION

School feeding programs are familiar with both developing and developed countries. The objectives of school feeding programs are to supply meals or snacks to decrease malnutrition, short-term hunger in the classroom so that the students can give attention to read and learn better, and to motivate children to school and have them attend regularly (Ahmed, 2004). In Bangladesh, feeding children in school is a recent phenomenon. In July 2002, in order to reduce malnutrition and hunger in the classroom as well as to promote school enrollment and retention rates; the Government of Bangladesh (GOB) and the World Food Programme (WFP) launched the School Feeding Program (SFP) in chronically food insecure areas of Bangladesh (Ahmed, 2004; Meng and Ryan, 2003; Ahmed et al., 2000). Widespread under nutrition in Bangladesh remains a critical barrier to children's learning. The newly introduced school feeding program has the potential to improve children's learning performance. The program distributes nutrient-fortified biscuits to all children in the intervention schools (Ahmed, 2004).

Biscuits are popular food products, consumed by a wide range of populations, due to their varied taste, good eating quality, long shelf life, easy availability and relatively low cost (Vitaliet al., 2009; Gandhiet al., 2001). It is a small thin crispy cake made from unleavened dough (Okpala and Chinyelu, 2011). The principal ingredients are flour, fat, sugar and water; while other ingredients include milk, salt, flouring agent and aerating agent (Wade, 1988). Long shelf-life of biscuits helps to give large scale production in short time and distribution of this biscuit in whole over the country is possible for a long time (Hossain et al., 2013).

Wheat (Triticumaestivum) is a cereal grain grown all over the world for its highly nutritious and useful gain. It is one of the top three most produced crops in the world, along with corn and rice. According to Okaka (2005), only wheat contains substantial amount of gliadin andglutenin (special protein) which when kneaded with water give gluten, the elastic material important in yeast or aerated baked goods (Okaka, 2005). The enrichment of protein may be achieved through inclusion of protein-rich non-wheat flours (Gandhi et al., 2001; Patel and VenkateswaraRao1996; Sharmaand Chauhan, 2002; Singh et al., 1996).

Spoilage can be defined as any change in the state of a food that makes it less palatable at the time of consumption. The causes of spoilage of food products can be classified into 1) physical spoilage (moisture loss, staling), 2) chemical spoilage (rancidity), and 3) microbiological spoilage (yeast, mold, bacterial growth). The major inter-related factors, specifically storage temperature, relative humidity, level of preservatives, pH, packaging material and gaseous environment surrounding the product and, most importantly, by the moisture content and aw are influenced to spoilage food products (Ooraikul, 1991). Microbial growth in food products can result in a food intoxication in which symptoms are produced shortly after the food is consumed because growth of the disease causing microorganisms is not required. Toxins produced in the food can be associated with microbial cell or can be released from the cells. Some of the major bacterial genera which cause food borne infection and intoxication include *Staphylococcus, Bacillus, Escherichia, Shigella, Clostridium* and *Salmonella* (Madigan and Martinko, 2006; Sousa, 2008). *Staphylococcus aureus*is an important food borne disease throughout the world.

Currently the fecal coliforms, e.g., *Escherichia coli*used as indication of the sanitary conditions. It includes a wide range of strains types, ranging from the highly pathogenic strains causing the worldwide outbreaks of severe disease. Above two million deaths occurring each year because of several *E. coli* pathotypes with the diarrheal illness (W.H.O., 2002). Escherichia coli are responsible for serious illness in susceptible humans (Bacon and Sofos, 2003). Symptoms include diarrhea, haemorrhagic colitis and haemolyticureamic syndrome (Doyle et al., 2001; Doyle, 1991). Many high risk pathogens transmitted through various food items that cause diseases in human (Hassanain, 2008). *Salmonella* spp. is one of the most commonly reported causes of the food borne disease in many countries around the world (Varma et al., 2005). It is the most important cause of human food borne infections in Latin America, and poultry meat is one of the main vehicles. Many yeasts can produce visible growth on the surface of bakery products, particularly those products with high water activity. The appearance of some of these yeasts has led to the descriptive term 'chalk mould' ('Kreidschimmel' in German). Unfortunately many authors using this term seem to mean slightly different things (Bloodand Curtis, 1995; Miller, 1979; Seiler, 1982).Several studies provided evidence that flours can contain a wide variety of yeasts and thus could serve as a vehicle for yeast entry into the bakery (Spicher, 1984; Mislivec et al., 1979). The growing interest in these types of bakery products is due to their better nutritional properties and possibility of their use in feeding programs and in catastrophic situations such as starvation or earthquakes (Awasthiand Yadav, 2000). The aims and objectives of this research work were (1) to evaluate the nutritional properties of the biscuits. (2) to evaluate the microbiological properties of the best biscuit.

2. MATERIALS AND METHODS

Sample Collection

A total of 150 packets biscuit samples were randomly obtained from food industry. These samples were commercial named as "Fortified High Energy Biscuit" and collected from biscuit industries as Central Marketing Company Ltd., Jessore, Bangladesh. Samples were collected from January 2015 through January 2016. A 1000 g portion of each sample was immediately transported to the laboratory in chilled packed at ambient temperature and subsequently analyzed in terms of nutritional and microbial parameters. All chemical were analytical grade of Merck (Germany) and media was obtained from Oxoide (Basingstoke, UK).

Nutritional Analysis

According to AOAC methods biscuit samples have been analyzed for moisture, ash, protein fat and crude fiber content (AOAC, 2002). All determinations were carried out in triplicates. The carbohydrate was determined by difference (Bryant, 1988). Food energy was calculated using the Atwater factor 4 × protein, 4 × carbohydrate, 9 × fat (Marero et al., 1988). Vitamin A was determined by Shimadzu high performance liquid Chromatography (Horwitz and Latimer, 2007). The iron (Fe) content in biscuits was determined by the flame atomic absorption spectrophotometric method using Shimadzu spectrophotometer (Kosse, 2001).

Sugar estimation

Before analyzing any class of carbohydrate whether it is monosaccharide or insoluble cellulosic material, the sample must be prepared in such a way that all interfering substances must be removed before analysis. Low molecular weight carbohydrates can then be extracted using a hot mixture of 96% ethanol and water in a ratio 1:1. Glucose is soluble in 96% ethanol-water mixture while polysaccharide material is not (Cui, 2005). After removal of ethanol, the solution is hydrolyzed and determined the total sugar content by volumetric lane-eynon method (Puwastien et al., 2011) and calculated the sugar content by using this formula, Sugar (as Sucrose), % = (Total sugar % - reducing sugars %) × 0.95(AOAC, 2000).

Preparation of biscuit samples for microbiological analysis

About 20 gm of biscuit sample were taken and suspended in 180 ml Ringers solution and homogenized by using a homogenizer. Aliquots of each sample were plated for microbiological count. Microbiological analysis was carried out according to APHA (Speck, 2001).

Isolation

Microbiological analysis of foods are based on the detection of microorganisms by visual and biochemical either before enriched by quantitative or enumerative methods or after enriched by qualitative methods also known as presence or absences tests. *Mesophyllic aerobic bacteria, coli forms, staphylococcus, bacillus cereus, enterobacter spp.* and *yeasts* & *moulds* were assessed by quantitative method. On the other hand *salmonella* and *Escherichia coli* were assessed by qualitative method.

Enumeration

Enumeration is made by direct counting of colonies developed on plate and expressed as colony forming unit/gm. For enumeration of total *coliform* results are expressed as MPN/gm.

Statistical analysis

Two sample*t*-tests was used for comparison of proximate and microbial load analyzed value from prescribed value of World Food Programme (WFP) to assess the significance of treatment means at the 5% and 1% significance level.

3. RESULTS AND DISCUSSION

Nutritional analysis

Table 1 show the proximate composition of fortified high energy biscuits and Table 2 explain mean comparison of analyzed sample fortified high energy biscuit with WFP permissible limits. Moisture content was 2.824% which was statistically significant (p>0.001) and under the permissible limits according to WFP requirement. Moisture is a crucial parameter of biscuit product because it significantly affect on growth of microorganisms, shelf life and quality (International Commission on Microbiological Specifications for Foods, 1998). This could be due to increase in protein content as a result of soya flour and protein has more affinity to moisture than carbohydrate and have an advantage to keep quality (shelf life) as most spoilage organisms may not be able to survive (Aguand Okoli, 2014). This finding agreed with work carried out on "effect of sesame seed flour on millet biscuit characteristics (Alobo, 2001). The protein content found in biscuit product was 10.9834g which was statistically significant at 1% level. Protein content enriched with supplementation of soy flour. Biscuit enriched with soy flour contained significantly higher amounts of total and digestible proteins (Vitali et al., 2009). Obtained results are nearly consistent with data obtained by Abdel-Aal (2008), where protein content of wheat based biscuit and its dough was found to be11-11.5 g/100 g dry matters, respectively. The fat content was 16.045g which also statistically significant at 1% level. This increase may be due to the addition of vegetable fat and beniseed flour which was a good source of oil (Akinoso et al., 2006).

Table 1 t-test value of nutritional analysis of fortified high energy biscuit

Parameters	Observation	Mean	Standard Deviation	p-value
Moisture (%)	150	2.824	0.569	0.0000
Food energy value (Kcal/100g)	150	456.54	59.636	0.2206
Protein (%)	150	10.983	1.275	0.0000
Fat (%)	150	16.045	1.707	0.0000
Sugar (%)	150	13.687	1.618	0.0000
Fibre (%)	150	0.447	0.162	0.0000
Total Ash (%)	150	1.916	0.351	0.0000
Vitamin A (µg/100g)	150	236.29	56.54	0.0464
Iron (mg/100g)	150	12.978	2.716	0.0000
Peroxide Value (meq/kg fat)	150	4.098	2.340	0.0000

Significant level *** $p<0.01$, ** $p<0.05$

Sugar, crude fiber contents were 13.687 g and 0.447g under the permissible limits according to World Food Programme (WFP) requirement. Sugar and fiber content may increase due to added wheat and soya flour which was good sources of fiber content. This is an advantage as it helps in bowel movement and easy digestibility. The total ash content was 1.916g which statistically significant at 1% level. The high levels of ash are generally associated with the addition of bran in the wheat (W.F.P., 2010). Ash content is vital part of biscuit product because it provides essential minerals to diet but higher content of ash helps to yeast fermentation (Dewettinck et al., 2008). Vitamin A content was found 236.29µg which was statistically significant at 5% level and lower than WFP requirement. Iron was detected in biscuit about 12.978mg was higher than permissible limits. The energy content of sample biscuit was 456.54 kcal which was higher than permissible limits (W.F.P., 2010). Per-oxide value was 4.098 which were under permissible limit. Figure1 also shows the mean comparison of analyzed sample fortified high energy biscuit with WFP permissible limits.

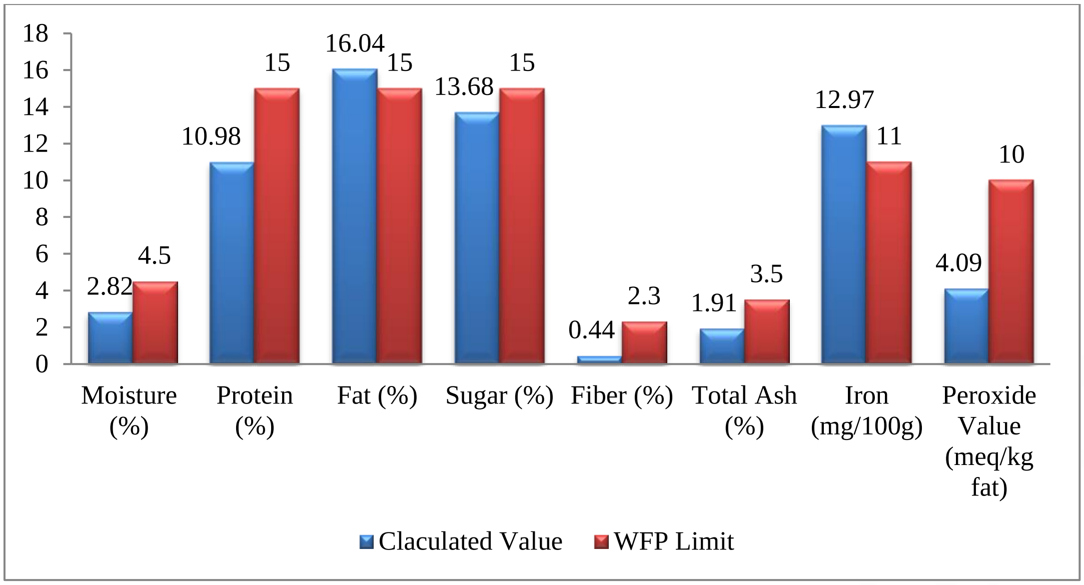

Figure 1 Mean comparison of analyzed sample fortified high energy biscuit with WFP permissible limits

Table 2 Mean comparison of analyzed sample fortified high energy biscuit with WFP permissible limits

Parameters	Mean	WFP limits
Moisture (%)	2.824	Max 4.5%
Food energy value (Kcal/100g)	456.547	Min 450 Kcal/100g
Protein (%)	10.983	10-15g/100g
Fat (%)	16.045	Min 15g/100g
Sugar (%)	13.687	10-15g/100g
Fiber (%)	0.447	Max 2.3/100g
Total Ash (%)	1.916	Max 3.5/100g
Vitamin A (µg/100g)	236.294	250mcg/100g
Iron (mg/100g)	12.976	11mg/100g
Mesophyllic aerobic bacteria (cfu/g)	837.6	Max 10,000 cfu per g
Coliforms (MPN/g)	0.162	Max 10 cfu per g
Staphylococcus (cfu/g)	3.4	>10 cfu per g
Escherichia coli (MPN/g)	0.108	Absent in 10g
Salmonella spp. (cfu/g)	0	Absent in 25g
Bacillus Cerus(cfu/g)	0	Max 10 cfu per g
Yeasts and moulds(cfu/g)	4.18	Max 100 cfu per g
Peroxide Value	4.098	Max 10 meq/kg fat

Microbial analysis

The hygienic standard of food can be assessed by the analysis of the indicator microorganisms (Hayesand Forsythe, 2013). Besides this, there are major microorganisms groups that are used alone or together in order to verify the microbiological characteristics and the hygienic condition of the food. Biscuits are produce from cereal or cereal products, can affected by microbial contamination due to improper handling and use of unsanitary conditions. Total viable count or total plate count method was broadly used to get the opinion about the hygienic quality and microbiological load of foodstuffs. Table 3 shows that the mean value of mesophylic aerobic bacteria (cfu/g) was 837.6 (cfu/g) significant at 1% level, *Coliforms* was 0.162 (MPN/g), *Staphylococcus* (cfu/g) was 3.4 at p-value 0.001, *Escherichia coli* (MPN/g) was 0.108, *Salmonella spp.* (cfu/g) was 0.0, *Bacillus cereus* (cfu/g) was 0.0 at p-value >0.001, *Yeasts and moulds* (cfu/g) was 4.18 at p-value 0.001 and respective pathogens bacteria are absent which is shown in Table-2. According to

WFP permissible limit, the study shows that *Mesophyllic aerobic bacteria, Bacillus cereus, coliforms* and *escherichia coli* are highly insignificant while *Staphylococcus, Enterobacter spp.* and *Yeasts* and *moulds* are only insignificant. Figure 2 also shows the mean comparison of analyzed sample fortified high energy biscuit with WFP permissible limits.

Table 3 t-test value of microbial analysis of fortified high energy biscuit

Parameters	Observ ation	Mean	Standard Deviation	p-value
Mesophyllic aerobic bacteria (cfu/g)	150	837.6	1649.117	0.0000
Coliforms (MPN/g)	150	0.162	0.145	0.0000
Staphylococcus (cfu/g)	150	3.4	4.785	0.0000
Escherichia coli (MPN/g)	150	0.108	0.145	0.0000
Salmonella spp. (cfu/g)	150	0	0	0
Bacillus Cerus(cfu/g)	150	0	0	0
Yeasts and moulds(cfu/g)	150	4.18	7.009	0.0000

Significant level *** $p<0.01$, ** $p<0.05$

Mesophyllic aerobic bacteria, Bacillus cereus, coliforms and *Escherichia coli, salmonella* are mainly grow in unhygienic and improper sanitary condition of raw materials store, production area and working personnel. If the personnel do not maintained sanitary conditions then microbial contamination may increase in biscuit products.

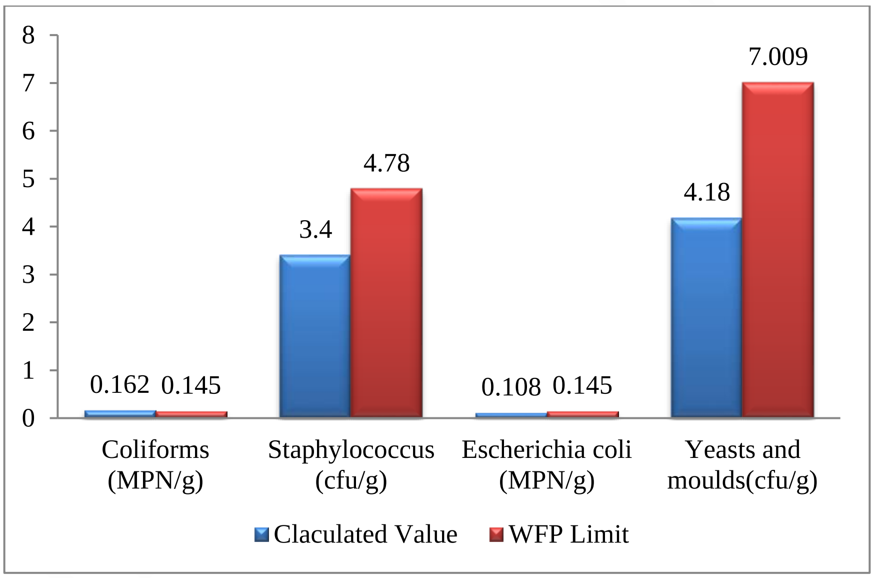

Figure 2 Microbial comparison of analyzed sample fortified high energy biscuit with WFP permissible limits

Studies on the physiochemical composition and microbial contamination of the Bangladeshi biscuit are very valuable from the view point of risk assessment (Hossain et al., 2013). Comparing in order to WFP requirement our study result reveals that *Escherichia coli, Coliforms, Salmonella spp. and Bacillus cereus* are highly insignificant. While *Mesophyllic aerobic bacteria, Staphylococcus, Yeasts and moulds* are only insignificant.

4. CONCLUSION

An acceptable biscuit was formulated from wheat flour, soya flour, vegetable oil and baking soda. Analysis showed that the formulated biscuit was rich in protein, fat, carbohydrate, ash, and energy but vitamin A content was slightly lower than WFP permissible limit. Biscuit was baked at high temperature that's why most of the pathogens are destroyed. Post contamination and unhygienic condition could promote microbial spoilage of biscuit, here analyzed biscuit had low microbial load and therefore had a long shelf life. So it concludes that hygienic and sanitation condition was maintained properly during production and storage area. Since microbial contamination could occur from raw materials milling, handling, transportation, storage and moisture condition of the product so industries should maintain their product safety from initial stage by arranging food safety education campaign and quality management program if they want to produce good quality food for consumer.

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