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RESEARCH ARTICLE

PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF FORTIFIED HIGH ENERGY BISCUITS SERVED IN SCHOOL OF POVERTY PRONE AREAS IN BANGLADESH

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ABSTRACT

Two hundred nine fortified high energy biscuit (HEB) samples from seven different locations were collected and examined for moisture, ash, protein, fat, crude fiber, sugar, carbohydrate and energy by AOAC methods. Iron was estimated by Flame Atomic adsorption spectrophotometer (FAAS) and vitamin A by High Performance Liquid Chromatography (HPLC). While *mesophyllic aerobic bacteria, coliforms, escherichia coli, salmonella, staphylococcus, bacillus cereus, enterobacter spp.* and *yeasts* & *moulds* were tested for microbial contamination. In the physicochemical examinations such as food energy value, protein, fat, iron and vitamin A content of some fortified high energy biscuits did not comply with the World Food Programme (WFP) permissible limits but moisture, sugar and microbial loads of most of the fortified high energy biscuits were in the permissible limits. The physicochemical analysis showed that there was no significant difference observed among the industry wise category of biscuit samples in the analysis of moisture, protein, sugar and ash. While significant differences ($p<0.05$) were observed in fat, carbohydrate, vitamin A, iron and energy content. It was concluded that a substitution of biscuit ingredients by biscuit industries can improve the quality of fortified high energy biscuit and thus the acceptability of these biscuit will increase to the WFP.

Key words: World Food Programme (WFP), AOAC, FAAS and HPLC.

INTRODUCTION

Biscuits are nutritive snacks produced from unpalatable dough those are transformed into appetizing product through the application of heat in an oven[1]. They are ready-to-eat, convenient and inexpensive food product those containing digestive and dietary fibre with principles of vital importance[2]. The principal ingredients are flour, fat, sugar and water while other auxiliary ingredients such as milk, salt, flouring agent and aerating agent are used for making biscuit[3]. Biscuit is a rich source of fat and carbohydrate and it is high energy giving food. They are also a good source of protein and minerals[1]. The nutritional value of biscuits varies with the type of cereal used. Biscuit generally contains fat (18.5%), carbohydrate (78.23%), ash (1.0%), protein (7.1%) and salt (0.85%) [4].

Among ready-to-eat snacks, biscuits possess several attractive features including wider consumption base, relatively long shelf-life, more convenience and good eating quality[5&6]. 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. Good eating quality makes biscuits attractive and therefore, protein fortification and other nutritional improvements are essential for good quality of biscuit. Development of fortified biscuits or other composite flour bakery products is the latest trend in bakery industry. 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[7].

Biscuits are the most popularly consumed bakery items in the world. Some of the reasons for such wide popularity are their ready to eat nature, affordable cost, good nutritional quality, availability in different tastes and longer shelf life[8].

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human

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consumption due to changes in sensory characteristics. Spoiled foods may be safe to eat if they may not cause of illness because there are no pathogens or toxins present but changes in texture, smell, taste, or appearance cause them to be rejected[9,10,11&12]. A food borne infection involves the ingestion of the microbial pathogens followed by growth in the host, including tissue invasion and release of toxins. 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*[13&14].

The present study aimed to assess the nutritional and microbiological quality of fortified high energy biscuit and to identify their safety for human consumption in terms of bacterial pathogens.

MATERIALS AND METHODS

Sample Collection: A total of 209 biscuits samples were randomly obtained from seven different industries in Bangladesh. These samples are commercial named as "Fortified High Energy Biscuit" and collected from different biscuit industries such as (1) Mona Food Industries, Jessore, (2) New Olympia Biscuit Factory Pvt. Ltd., Savar, (3) Hooghly Biscuit Company, Khulna, (4) Central Marketing Company. Ltd., Jessore, (5) Resco Biscuit & Bread Factory (Pvt) Ltd., Jessore, (6) Masafi Bread & Biscuit Industries Ltd., Tangail and (7) Pran Foods Ltd., Narsingdi. Samples were collected from November 2011 through August 2012. A 1000 gm 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: Biscuit samples have been analyzed for moisture, ash, protein fat and crude fibre content according to AOAC methods[15]. All determinations were carried out in triplicates. The carbohydrate was determined by difference[16]. Food energy was calculated using the Atwater factor 4 × protein, 4 × carbohydrate, 9 × fat[17]. Vitamin A was determined by Shimadzu high performance liquid Chromatography[18]. The iron (Fe) content in biscuits was determined by the flame atomic absorption spectrophotometric method using Shimadzu spectrophotometer[19].

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[20]. After removal of ethanol, the solution is hydrolyzed and determined the total sugar content by volumetric lane-eynon method[21] and calculated the sugar content by using this formula, Sugar (as Sucrose), % = (Total sugar % - reducing sugars %) × 0.95[22].

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[23].

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, coliforms, 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: The Tukey HSD test value was used to determine significant differences among means of moisture, protein, sugar and ash parameters and due to heterogeneity of variance of rest of the parameters, we use Games-Howell test value to separate means at $p < 0.05$ using SPSS package version 15.0 (SPSS Inc., Chicago, IL, USA). *t*-test were used for comparison of physiochemical and microbial load analyzed value from prescribed value of World Food Programme (WFP) to assess the significance of treatment means at the 5% significance level[24].

RESULTS AND DISCUSSION

Physicochemical composition: The physiochemical composition of biscuit samples collected from different locations in Bangladesh is

shown in Table- 1&2. Moisture is an important parameter when considering biscuit quality because it is significantly affect on shelf life and growth of the microbes[25]. Our study showed that the mean values of moisture content in biscuit samples were 2.95±0.78 which were under the permissible limits according to the (P>0.99)[23]. Ash is composed of inorganic matter generally present in biscuit. It includes iron, copper, potassium, sodium, and zinc. Besides, providing needed minerals to the diet, ash increases yeast fermentation by providing minerals to yeast. In this study the biscuit samples have ash with mean values of 1.59±0.39. The high levels of ash are generally associated with the addition of bran in the wheat[26]. While crude fiber was found 0.40±0.02 which is also acceptable limit (P>0.99). The protein content is very important to check the quality of biscuit. It is key factor to determine the stability for different biscuit product. The protein content found in the range of 10.29±1.35 and it was revealed that as compared to the standards which was significantly differed (P=0.00). The fat content found in biscuit samples was 14.78±2.06 which was statically significance (P=0.03). The carbohydrate content was 70.35±2.83%. Changes in the physicochemical properties of biscuit during storage has been widely documented[27]. Some metals like iron and zinc are played a vital role in the biological activity of man but some toxic metals like cadmium arsenic and mercury are hazardous for human health. Only iron was detected in biscuit with a mean value of 11.70±7.87 ppm. Difference between standard and estimated value were insignificant in terms of metals (P = 0.13). Total sugar was found to be 13.55±2.22%. So, it can be concluded that biscuits have good nutritional quality (P=0.23). The energy content of all the biscuit samples was 455.61±11.17 kcal/100g. There was significant difference in the energy content of the biscuits (P=0.00). The increase in energy content of the biscuits resulted from the high fat and carbohydrate contents of the blends used for their production. In the case of mean measured concentrations of vitamin A were 426.41±760.51 µg/100g that sufficiently differ compared to standard (P=0.00).

Proximate analysis of biscuits of multiple comparisons is presented in table -1. In the case of moisture, protein, fat, sugar, carbohydrate, vitamin A, iron and energy contents were significantly differ among biscuit industry where as fibre and ash contents were insignificant.

Microbial Contamination: Cereal or cereal products, especially flour constitute are large part of the daily Bangladeshi diet in the form of biscuits. Studies on the physiochemical composition and microbial contamination of the Bangladeshi biscuit are very valuable from the view point of risk assessment. The microbes present in flour generally consist of yeast & moulds, *thermophilic bacteria, lactic acid bacteria* and *pathogenic bacteria* like *E. coli* and, *Salmonella spp.* Although cereals, milled product have very few cases that they have been implicated in food born diseases[28]. Flour generally produced from wheat that has been significantly exposed to the microorganism and promoted the retrieval of data on frequency of pathogens bacteria and microorganism. This would render the food unfit for the consumer.

Total viable count or total plate count is used as an indicator of bacterial populations on a sample. It is widely used to gain the opinion about the hygienic quality and microbiological load of foodstuffs. Our findings show that the *Mesophyllic aerobic bacteria* (cfu/g) found in mean of all samples of 310.57 (cfu/g) at p-value >0.99, *Staphylococcus* (cfu/g) is 0.01 at p-value 0.34, *Bacillus cereus* (cfu/g) is 0.57 at p-value >0.99, *Enterobacter spp.* (cfu/g) is 0.23 at p-value 0.16, *Yeasts and moulds* (cfu/g) is 111.67 at p-value 0.55 and respective pathogens bacteria are absent which is shown in table-2. From compare according to WFP permissible limit, we show that *Mesophyllic aerobic bacteria, Bacillus cereus, coliforms* and *escherichia coli* are highly insignificant while *Staphylococcus*, *Enterobacter spp.* and *Yeasts* and *moulds* are only insignificant.

CONCLUSION

In conclusion, this study has found that biscuit samples of high nutrition value may be formulated from different combination of wheat flour, defatted soy flour, vegetable oil and baking soda . The composition and nutritive value of biscuit samples represent balanced quantity of carbohydrate, crude fiber, moisture, ash, sugar and iron. Protein and fat content of the experimental biscuit samples were slightly decreased while vitamin A and energy content of biscuit sample were unbalanced quantity.

Biscuit is cooked at a high temperature which is enough to destroy pathogens, post-contamination and cross-contamination that is being promoted by unhygienic food handling, and incorrect storage practices. Microbial contamination occur in flour is widely depending on a number of factors such as initial counts in the grain from crop conditions, milling practices, post-milling handling, moisture content of flour and storage conditions. So this study concludes that the presence of these pathogens in biscuit requires attention of the Ministry of Health and Environmental Health organization. They should be arranged food safety education campaign and quality management

programme for the mill owner and government agencies. Present results recommend that similar surveys for other food products and stored conditions should be carried out to make a clear picture for quality assurance and quality of food and also in the development of advanced quality system by advanced techniques in agriculture of Bangladesh.

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Table 1: The results of the proximate analysis and physical characteristics of biscuit samples*

Parameters	Biscuit samples						
	A	B	C	D	E	F	G
Moisture (%)	3.03±0.79^{a}	2.89±0.62^{a}	3.22±0.75^{a}	3.17±0.63^{ab}	3.26±0.62^{ab}	2.82±0.71^{a}	2.54±1.17^{ac}
Protein (%)	10.97±1.16^{a}	10.36±1.26^{ab}	10.02±1.21^{ab}	9.98±1.62^{b}	9.91±1.29^{b}	10.01±1.30^{b}	10.41±1.52^{a}
Fat (%)	16.97±2.12^{a}	14.99±1.45^{bhi}	12.06±3.06^{chi}	14.00±2.51^{dhi}	13.47±1.08^{ei}	14.74±1.52^{fhi}	14.67±0.74^{ghi}
Sugar (%)	12.56±1.71^{a}	14.88±2.10^{bf}	14.46±2.75^{ad}	12.19±1.60^{ace}	13.36±2.29^{ac}	13.20±1.84^{ac}	13.65±2.32^{af}
Crude fiber(%)	0.27±0.02^{a}	0.47±0.01^{a}	0.24±0.04^{a}	0.40±<0.01^{a}	0.48±0.01^{a}	0.53±0.02^{a}	0.39±<0.01^{a}
Ash (%)	1.74±0.60^{a}	1.69±0.56^{a}	1.80±0.27^{a}	1.46±0.22^{a}	1.55±0.55^{a}	1.48±0.47^{a}	1.72±0.57^{a}
Carbohydrate (%)	67.28±2.32^{a}	70.101.98^{bh}	72.89±3.69^{ch}	71.39±3.51^{dh}	71.80±2.17^{e}	70.91±2.38^{fh}	70.66±2.05^{gh}
Vitamin A (µg/100g)	248.17 ±42.26^{ab}	118.22 ±116.59^{a}	211.20 ±29.04^{ac}	264.90 ±48.67^{a}	175.13 ±142.58^{a}	170.95 ±124.34^{a}	231.55 ±62.99^{a}
Iron (mg/100g)	17.02±12.12^{a}	9.11±6.29^{b}	11.84±1.75^{a}	11.62±4.78^{a}	11.48±7.52^{a}	11.14±7.77^{a}	10.89±1.68^{c}
Food energy value (Kcal/100g)	465.75 ±11.87^{a}	456.73 ±8.03^{bhj}	440.21 ±16.76^{cij}	451.48 ±13.06^{dhij}	448.41 ±6.39^{ei}	456.34 ±7.89^{fhij}	456.32 ±6.92^{ghij}

*Data are mean ± standard deviation. Means within a row with different superscripts letters are significantly different at $P < 0.05$.

A= Mona Food Industries, Jessore

B=New Olympia Biscuit Factory Pvt. Ltd., Savar

C=Hooghly Biscuit Company, Khulna

D=Central Marketing Co. Ltd., Jessore

E=Resco Biscuit & Bread Factory (Pvt) Ltd., Jessore

F=Masafi Bread & Biscuit Industries Ltd., Tangail

G=Pran Foods Ltd., Narsingdi

Table- 2: *t*-test value for parameters in the physiochemical and microbial analysis of fortified high energy biscuit products

Parameters	Mean	Standard Deviation	*t*-value	p-value
Moisture (%)	2.95	0.78	-33.77	>0.99
Ash (%)	1.59	0.39	-	-
Food energy value	455.61	11.17	8.54	0.00
Protein (%)	10.29	1.35	-3.94	0.00
Fat (%)	14.78	2.06	-1.86	0.03
Sugar (%)	13.55	2.22	1.36	0.23
Fibre (%)	0.40	0.02	-2129.81	>0.99
Carbohydrate (%)	70.35	2.83	-	-
Vitamin A (µg/100g)	426.00	760.51	5.13	0.00
Iron (mg/100g)	11.70	7.87	1.54	0.13
Mesophyllic aerobic	310.57	1302.49	-69.79	>0.99
Staphylococcus (cfu/g)	0.01	0.11	0.97	0.34
Bacillus cereus (cfu/g)	0.57	2.33	-38.04	>0.99
Enterobacter spp. (cfu/g)	0.23	1.50	1.42	0.16
Yeasts and moulds (cfu/g)	111.67	1001.03	0.12	0.55