Chemical and nutritional comparison between school's biscuits and supplemented biscuits with another protein sources

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Abstract: This study aimed to study the chemical and nutritional value of school's biscuit and different fortified samples of biscuits. Whey protein and okara were added to biscuits at the level 10% and compared with school's biscuits. The chemical contents, lysine score and antimicrobial properties of tested samples were determined. **The results** showed that fortified biscuits with whey protein helped to improve the protein content and lysine score. Adding okara didn't effect on the biscuit weight and its volume when compared with school's biscuits. Also, sample with okara helped to improve the antimicrobial properties of biscuits sample followed by protein and the last one was school's sample. So, adding whey protein and okara to the bakery products as biscuits can saved wheat flour and increase the nutritional value and antimicrobial properties of the products.
[Hoda, M. El Gezery. **Chemical and nutritional comparison between school's biscuits and supplemented biscuits with another protein sources.** *J Am Sci* 2017;13(12):84-89]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 10. doi:10.7537/marsjas131217.10.

Keywords: Biscuits; whey protein; okara.

1. Introduction:

Animal or plant protein is very important for growth and repairing the injured cells and tissues of human beings. The animal protein (meat and milk) are the best source of proteins, where it supply the body with essential amino acids. The meat is considered the main source of proteins which can be obtained from the meat of poultry, beef, pork, fish and mutton. (**Ministry of Agriculture, 2009**). Amino acid composition of meat products can play a significant role in meat identification; the ratios of amino acids arginine, histidine and lysine for the investigated species of animals have been obtained. These ratios do not depend on age or weight of the animal (**Irina *et al.*, 2011**). The chemical and nutritional composition of each meat product is greatly varied from one product to another as it contains different kinds of tissues and sometimes a mixture of meat of various organs (**Lawrie *et al.*, 1998**).

Whey is one of the contents of milk, analytically, it contains many essential materials which posses proteins which play an important functions as nutritional and biological properties such as support of health condition and protection from diseases and improving the health conditions (**Ana *et al.*, 2007**). Whey protein contains many components such as carbohydrates, fat, lactose, minerals and immunoglobulins which may vary in their values. ractions vary in their values. Whey protein is valuable for sports nutrition and for athletic youths. Also, whey products are used in preparation of salad dressings, emulsifiers, infant formulas, medical nutritional formulas and baked goods**) Ha *et al.*,2003**). In addition, whey protein when added to diet called healthy diet and is suitable for nutrition of all ages because it contains a protein of high quality. Usually, Whey protein contains some of growth promoters and stimulate the growth of muscles, therefore, whey protein was used by most of body builders and athletics for its effects on muscle growth (**Fry *et al.*, 2003**). Beside the great value of whey protein in improving the nutrient value of food, it is applied therapeutically for treatment of tumours, wound healing, loss of weight and infant health (**Hoffman *et al.*, 2004**).

Some investigators (**McClements *et al.*, 2010**) reported that whey protein have an effect on the nervous system and stimulate the release of serotonin in the circulation which helping in the relief of restless during sleep or in treat of some psychological disorders, others, found that whey protein can promote the level of energy, have anti-stress action, elevate the basal metabolic rate and decrease the fat percent in the muscles, thus helping in building of the lean body mass. In addition, whey protein can improve the memory lost during stress conditions, also whey protein elevate the immune status and the resistance to diseases, increase the mass of muscles, increase the basal metabolic rate and generally improve the health condition) **Layman *et al.*,2002**).

Soybean considered a main source of plant protein beside it contains a huge quantity of dietary fiber and oil in its by-product what is called okara. Some investigators estimated the contents of soybean seeds and its by-product (okara) (**O'Toole *et al.*, 1999**) and has shown significant differences in fibre levels apparently due, to the advanced methods for estimation of fibre and due to, to the adjustment of the

fibre model and its role in human health (**Guillon *et al.*, 2002**). Determination of dietary fiber (DF) can be carried out by several methods. The official AOAC method is applied widely for determination of DF level (**Rinaldi *et al.*, 2008**). Okara are used widely as meat extenders due to its superior nutritional and functional values that are found in soybean proteins. In addition to the mentioned properties of soybean proteins, economic point view, it consider a cheap source of proteins not coasty, also can be consumed as safe and healthy for the body and can be added safely to most of meat products and by products. On the other hand, in spite of the good characters of soybean proteins, some countries restrict its uses as meat extender or as replacement for animal proteins and permitted only for soybean protein to be added with limited amounts to the meat. Thus, urgent need for accurate analytical methods for the detection of the plant protein (soybean protein) which added to the meat or meat-by products (animal proteins). Many methods are used nowadays for examination of meat /meat by products for alteration in proteins from plant origin, this methods include: the microscopic examination, electrophoresisi, ELISA (immunologic) and fractionation on chromatography which are applied for this purpose (**Belloque *et al.*, 2002 and Villanueva, 2011).**

Some improvements in the nutrition of school students through adding of some microelements (trace elements) for school meals which are deficient from the original constituents for the purposes of increasing the nutritive value and restore the deficiency in essential minerals and finally improving in the health condition, and decreased morbidity for students (**Tran *et al.*, 2009**).

School meal programmers afford complete meals while other schools afford biscuits or snacks of high content of energy, High energy biscuits is the most known meal provide at school. School feeding program used to prevent micronutrients and vitamins deficiency of school age children (**Zhang *et al.*, 2010**)). School meal fortification is the addition of micronutrients during or after processing a food, raising micronutrient levels above the amounts in the original food product Fortification is sometimes also called enrichment, levels of fortification should be set so that vitamin or mineral added will make significant contribution to nutritional requirements, but not lead to a micronutrient intake above the safe upper limit (**Elizabeth *et al.*, 2016**).

2. Materials and methods

Materials:

Okara and whey protein powder were obtained from international centre for research, Cairo, Egypt.

School's biscuits were obtained from schools at El -Menofia Governorate which offered to school's pupils, every packet weight (50gm) consists of (4) pieces, Wheat flour and other ingredients used in sweet biscuits were obtained from the local markets. Corn oil, wheat flour 72% extraction, eggs and sugar were obtained from local market to prepare the fortified biscuits.

Methods:

Preparing of biscuits

Biscuits were prepared using the following:

Ingredients	Amount
Corn Oil	25g
Wheat flour 72% extraction	150g
Eggs	one
Sugar	50g

The fat and sugar are creamed, mixed in egg, then the flour added. The dough is kneaded lightly until smooth, rolled out thinly, pricked and shaped. baking was carried out for 10-15 minutes at 180ºC (**Panel and Sonthgate, 1978)** to obtain the prepared samples which fortified with 10% okara and whey protein. okara and whey protein were added at the expense of wheat flour.

Baking quality of biscuits:

Weight:

The average weight of one biscuit was recorded after cooling it.

Volume:

The biscuit volume was measured by rapeseed displacement method as described by **A.A.C.C. (1987).**

Specific Volume:

Specific volume (g/cm3) was calculated by dividing volume of the biscuit by its weight (**Collins et al., 1982).**

Analytical Methods:

Proximate composition was estimated by **A.O.A.C. (1999).**

Lysine

Lysine was determined using the ninhydrin colorimetric analysis as described by **Rosen (1956).**

Amino acid score (A.S.) as mentioned by **FAO/WHO (1973)** was as follows:

$$\text{Amino acid score (\%)} = \frac{\text{Amino acid (9/16gN) of sample}}{\text{Amino acid (9/16gN) of Pattern}}$$

It has been indicated; in the same reference that lysine concentration in references casein protein is 55 mg per g of protein.

Determination of iron, selenium and zinc:

These minerals were determined by using atomic absorption spectrophotometer (PYE Unican 929)

according to the method of (**Jorhem, 2000**); the sample was ashed in the muffle. Furnace at 525C° for 6 hours until white or light grey ash. The ash was dissolved in 0.1 N HCL and diluted to 50 ml. Then the concentration of sample was determined using standard curve.

Microbiological detection:

Biscuit samples with (10%) of whey protein okara powder were examined weekly for total count of mesophilic bacteria, coliform bacteria, molds and yeasts according to the methods mentioned in the **Difco (1985).**

Sensory evaluation:

Made biscuit samples and school's biscuits were subjected to organoleptic testes according to **Watts et al., (1989)**.

Statistical Analysis:

Triplicate samples were analyzed for each property. Data were assessed by analysis of variance (ANOVA) as outlined by (**Sendecor and Cochran, 1987).**

3. Results and Discussion:

Sensory properties of school biscuits and fortified biscuits:

A five member taste panel scored color, odor, flavor, texture and overall acceptability of biscuits baked with 100% wheat flour and fortified biscuits as shown in table (1). Data from table (1) show that school's biscuits were better in organoleptic qualities but fortified biscuits with whey protein was higher in sensory properties followed by biscuits with okara when compared with school's biscuits. Statistical analysis of the results indicated a significant increase (P<0.05) of all tested sensory properties except color for fortified biscuits with whey protein as compared to control biscuits. The decreasing in appearance scores with high protein replacement levels was mainly due to the Egyptian panelists who usually prefer white than the darker grades (**Abd El-Basir *et al.*, 1989).** The same results reported by **Sharma (2002**) reported that bakery products with high protein as supplementation did not effect on the odor and flavor properties.

Table (1): Sensory properties of school biscuits and fortified biscuits.

Organoleptic Properties	School biscuits	Biscuits with 10% whey protein	Biscuits with 10 % okara
Color	8±1.16	8±0.08	8±0.11
Odor	8±1.03	9±1.34	9±0.92
Flavor	8±0.12	9±1.32	9±1.76
Texture	9±1.01	9±0.78	9±0.21
Overall acceptability	8±1.04	9±1.42	9±1.32

values with different superscripts differ significantly, $P \leq 0.05$.

2-Chemical composition of school biscuits and fortified biscuits /100g dry weight basis. School biscuits and fortified biscuits with protein sources were analyzed for their chemical composition (protein, fat, ash, carbohydrates, iron, zinc, fiber and selenium). The obtained results are shown in table (2). showed that protein increased in all fortified biscuits samples reached to 9.48 and 7.31 while protein was lower in school biscuits and reached to 5.88.

For fat there is no significant changes between school biscuits and the other samples while ash content increased in all fortified biscuits with whey protein and okara. There were significant changes between tested samples and school's biscuits in ash and fiber contents. In case of carbohydrates, iron and zinc, there is no significant differences between fortified biscuits and scholar's biscuits although okara increased iron level when compared with other samples. Finally, selenium content increased in biscuits fortified with okara while, there is no significant changes between biscuits fortified with whey protein and school's one. This finding matched with which reported by (**FAO, 2002),** Food Fortification is the addition of nutrients during or after processing a food, raising nutrient levels above the amounts in the original food product and Fortification is sometimes. Levels of fortification should be set so that the vitamin, mineral or protein added will make significant contribution to nutritional requirements. Also according to **Mari *et al.* (2008)** who found that fortification meal with nutrients in a school meal caused changes in children's growth, morbidity, and cognitive function compared with no fortification.

3-Effect of supplementing wheat flour with 10% of whey protein and okara on the protein quality of biscuits.

Data mentioned in table (3) clearly show that supplementation of biscuits with 10% of whey protein and okara increased both the amount of total crude protein and the quality of the protein evaluated by its lysine content. The protein content reached 5.88, 9.48 and 7.31 % in school biscuits and in supplemented with 10% of whey protein and okara, respectively. In table (3) Lysine content also increased from 11.9 mg/g protein in school's biscuits to 16.3 mg/ g protein when biscuits were supplemented with 10% of whey

protein but it was 9.5 mg/g protein in biscuits with okara. The lysine score shown in the same table has increased in the supplemented biscuit with whey protein followed by school's biscuits and the last one was biscuits with okara due to the improvement in lysine contents, which was presented at high concentrations in whey protein (Table 3). It was calculated using the following equation.

$$\text{A.A.S. (\%) for lysine} = \frac{\text{mg of lysine in 1 g test protein}}{55} \times 100$$

Also, **Faheid and Hegazi, (1991)** have improved the protein quality of biscuits by adding fish protein flour and legume flour which are rich in lysine content.

Table 2-Chemical composition of school biscuits and fortified biscuits /100g on dry weight basis.

Sample	Protein	Fat	Ash	Fiber	Carbohydrates	Iron	Zinc	Selenium
Scholar's biscuits	5.88 ±0.16	16.23 ± 0.21	0.81 ±0.02	1.01 ±0.04	75.06 ± 1.08	5.73 ±.02	1.75 ± 0.02	0.98 ± 0.01
Biscuits with10% protein	9.48 ±0.06	16.21 ± 0.17	1.42 ±0.11	1.32 ±0.01	71.57 ±1.25	5.72 ± 0.31	1.71 ± 0.07	0.98 ± 0.15
Biscuits with 10% okara	7.31 ±.022	16.93 ± 0.15	1.69 ±0.03	1.45 ±0.12	72.62 ±1.35	5.81 ± 0.36	1.75 ± 0.1	1.06 ± 0.11

values with different superscripts differ significantly, $P \leq 0.05$.

Table 3-Effect of supplementing wheat flour with 10% of whey protein and okara on the protein quality of biscuits.

Type of biscuits	Crude protein, %	Lysine (mg/g protein)	Lysine score, %
Wheat flour	10.19± 0.87	20.4± 1.21	37.01± 2.78
Okara powder	13.41± 0.93	21.12± 1.32	38.4± 2.93
Whey protein powder	51.34± 5.43	93.6± 7.98	170.2± 8.34
School's biscuits	5.88± 0.03	11.9± 0.54	21.6± 1.22
Biscuits with 10% whey protein	9.48± 0.22	16.3± 1.11	29.64± 1.57
Biscuits with 10% okara	7.31± 0.13	9.5± 0.67	17.3± 0.99

whey protein values with different superscripts differ significantly, $P \leq 0.05$.

The effect of supplementing wheat flour with 10% whey protein and okara on the baking quality of biscuits.

Data in table (4) show that biscuit weight increased with increasing whey protein concentration. It increased from 3 (wheat flour 72% extraction) to 3.12g at the levels 10%. This enrichment due to the very high water retention of very high protein content of whey protein. Biscuit volume and specific volume were decreased by increasing by whey protein. Biscuit volume decreased from 8cm. at school sample to 7.94 cm. with 10% of whey protein. These decreases may due to the dilution of gluten (**Pemeranz et al., 2005),** and high protein content of dairy protein beside the slow formation of gluten network, which the parameter dough development time cleared it. This may be due to the high ability of protein components to swell and absorb more water as cited by **Villanueva (2011).**

The results presented in the same table, biscuits weight, volume and specific volume didn't change with adding okara Same conclusion was found by **Sharaf *et al.* (2002)** who found that the dilution of the functional gluten proteins and high protein can partly explain the poor baking quality.

5- Microbial properties:

Microbiological profile of school biscuits and supplemented biscuits with 10% whey protein and okara after storage at room temperature up to 30 days. The inhibition percentage of microbiological profile in biscuits with whey protein and okara upon storage at room studied in table (5). It could be observed that, the microbiological load of biscuits was clearly affected by the addition of whey protein and okara. The highest percentage inhibition of mesophilic bacteria, coliform bacteria, molds and yeasts was found in case of okara followed by whey protein and the last one was school's one. Yeasts and molds had the highest sensitivity microorganisms to nutritional protein source at tested level. The inhibition percentage levels of the mesophilic bacteria, coliform bacteria and yeasts were ranged between (60 - 90%) for biscuits with whey protein and school's one while, they were ranged between (80 - 96%) for sample with okara. The results of okara products on the inhibition percentages agreement with Katayama and **Wilson**

(2008) who found that Okara (a soya pulp) as a source of LAB (Lactic acid bacteria) to ferment the soya milk and to evaluate its probiotic properties.

Table (4): The effect of supplementing wheat flour with 10% whey protein and okara on the baking quality of biscuits.

Blends		biscuit weight (g)	biscuit volume cm3	specific volume (g/cm3)
Wheat Flour	additives			
100	**0**	3± 0.08	8± 1.23	2.66± 0.62
90	**10 % whey protein**	3.12± 0.07	7.94±1.06	2.54± 0.54
90	**10% okara**	3.0± 0.04	8± 1.44	2.64± 0.76

values with different superscripts differ significantly, $P \leq 0.05$.

Table (5): Microbiological profile of school's biscuits and supplemented biscuits with 10% whey protein and okara after storage at room temperature up to 30 days.

Biscuit samples	Growth inhibition percentage (%)			
	Mesophilic bacteria	Coliform bacteria	Molds	Yeasts
School's biscuits	45	53	36	42
Biscuits with whey protein	78	85	78	86
Biscuits with okara	80	92	85	91

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12/24/2017