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Research Article
Formulation and Evaluation of Functional Cookies for Improving Health of Primary School Children

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Abstract

Background and Objective: School children especially in Egypt need a safe meal which is able to meet their daily nutrient needs and ameliorate cognition. So, the current study aimed to evaluate formula prepared as cookies to be served as a meal for primary school children. **Materials and Methods:** Wheat, roasted chickpea, milk protein concentrate, cinnamon and brewer's yeast were used to prepare cookies which have been exposed to sensory, chemical and biological evaluation. Peroxide number, amino acids, vitamins (D, B_{12}, folic acid and E) and minerals (calcium, zinc, iron and selenium) were determined. Twenty four rats of weaning age were used, 12 rats to estimate the true protein digestibility and 12 rats to estimate the protein efficiency ratio and the effect of feeding on cookies (28 days) on hemoglobin, glucose, total protein, liver and kidney functions and antioxidant status. **Results:** Palatability and acceptability of cookies were insured via the sensory evaluation results. The value of peroxide number indicated that there is no possibility of rancidity during the storage. The cookies showed high contents of protein (14.88%), fat (16.83%) and carbohydrate (55.1%). Also cookies showed acceptable levels of amino acids, minerals and vitamins that meet a large amount of daily requirements of children. Results of the animal experiment declared the complete safety of the cookies and high nutritional and biological quality. **Conclusion:** Cookies can serve as a meal for the governmental school children to provide them with their needs from nutrients that reducing hunger and improving health benefits and scholastic achievement.

Key words: Nutrient needs, protein digestibility, proximate composition, biological assessment, peroxide number

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

School feeding programs (SFPs) are designed to mitigate short-term hunger, ameliorate nutrition and cognition of children[1]. Since children spend long time (at least 6 h) every day at school in this time children obtained up to 47% of their calories from meals and snakes intake[2], formulation of healthy and nutritious meals to be served for school children is very important. Intake of such healthy and nutritious meals not only provides children by their requirements from the essential nutrients including amino acids, minerals and vitamins but also avoid children from health risks in the short and long term[3] especially that previous studies declare that insufficient nutrition in the childhood is related to an unhealthy state in the adulthood[4]. Anemia, obesity, heart disease, type-2 diabetes, osteoporosis and other health problems are associated with an unbalanced nutrition[5]. Also nutrition in the childhood is associated with the educational achievement and attendance rates[6]. Although selection of available, cheap, non-traditional, high nutritive and tasty ingredients for preparation of school meals is a major challenge, there are a lot of ingredients with high nutritional value can meet the nutritional needs of school children and contain several bioactive compounds with healthy effects among these ingredients roasted chickpea which considered a good source of macro and micro nutrients and contains high amount of essential amino acids[7]. Hubert and Arabie[8] and Vidmar[9] stated that chickpea would promote many macro and micro elements and good balance of essential amino acids when added to the bakery products. Chickpea has many effects by reducing the health problems of some diseases[7], so it can be used as good ingredient to fortify the beverages and foods[10]. Milk protein concentrate is a tasteless ingredient but may allow the taste of other ingredients to appear and develop. Milk protein concentrate is considered a good source of protein (40-90% protein)[11]. It can use as good ingredient to fortify the beverages and foods[12]. Cinnamon is a spice usually used in food throughout the world. Cinnamon is not only a flavored ingredient in many products but also is a source of antioxidants, flavonoids and phenolic compounds, in addition to its content of vitamins such[13] as A, C, K and B_3 and minerals such as calcium, iron, zinc, potassium, manganese and magnesium[14]. Cinnamon possessed anti-diabetic, anti-inflammatory effects and neuroprotective properties[15]. Brewer's yeast has excellent effect on immunity system and it is considered a good source of vitamin B and some minerals such as chromium, potassium, selenium and zinc[16]. So, the aim of the current study was to prepare a formula that contains the ingredients mentioned above and can serve as a meal for the primary governmental school children in Egypt. Also the aim of the current study was to evaluate the nutritive and biological value of this prepared formula.

MATERIALS AND METHODS

Materials: The prepared formula ingredients, wheat flour 72% extraction, roasted chickpea (*Cicier arietinum*), brewer's yeast, sugar, skimmed milk, butter, egg and cinnamon powder were purchased from local market. Milk protein concentrate 78% was purchased from the ministry of agriculture, Cairo, Egypt.

Animals: Twenty four male weanling albino rats with average initial weight of 55-65 g (21 days) were used. Animals were obtained from the animal house of National Research Centre, Cairo, Egypt. The animals were kept individually in stainless steel cages at temperature 24±2°C, a relative humidity of 55±10% and a 12 h light cycle/12 h dark cycle.

Methods

Preparation of the meal: The ingredients of the prepared meal were presented in Table 1. The meal was manufactured in the pilot plant of the Food Technology and Nutrition Division, National Research Centre, Cairo, Egypt. Butter and powdered sugar were creamed in mixer with a flat beater for 2 min at 5 rpm to obtain a homogenous cream, then eggs were added and mixed for 2 min, dry raw materials were added slowly to the above cream and mixed for 5 min at 60 rpm, skimmed milk was poured to form meal dough which was sheeted to thickness of about 0.25 cm using rolling machine and cut into a round shapes (cookies) of 5 cm diameter then baked in an electric oven at 170°C for 10 min.

Physical properties of the prepared meal: Weight of cookies was measured. Diameter and thickness were measured with vernier caliper at two different places in each cookie and the average was calculated for each. The spread ratio was calculated according to Zoulias *et al.*[17] using the following equation:

Table 1: Composition of the prepared meal (g/100 g)

Ingredients	Concentration
Wheat flour (72% extraction)	37.0
Chickpea flour	9.0
Sugar powder	16.0
Butter	10.6
Egg	4.9
Milk protein concentrate 87%	5.0
Yeast	2.3
Cinnamon powder	0.5
Skimmed milk	14.7

$$\text{Spread ratio} = \text{Diameter/ Height}$$

Color of cookies was determined using Hunter Lab color (model, CIE lab color scale, scan XE-RestonvA, USA). Color degrees: L*(lightness), a*(redness) and b*(yellowness) according to Hunter[18]. Apparatus Ametek/Mansfield and Green div. largo, Florida was used to measure the texture of cookies according to Herring[19].

Sensory evaluation of the prepared meal: Organoleptic characteristics of cookies were evaluated according to Penfield and Campbell[20] where cookies were subjected to sensory evaluation by 10 panelists. Each panelist was asked to assign scores 1-10 for taste, odor, color, texture, flavor and appearance.

Peroxide value of the prepared meal: peroxide value of the packed cookies was estimated according to AOAC[21] at 0, 90 and 180 days of the storage.

Chemical composition of the prepared meal: Moisture, protein, fat, crude fiber and ash of the cookies were determined according to AOAC[22]. Carbohydrate was calculated by difference. Calorie content was calculated by multiplying the fat, carbohydrate and protein contents by the Atwater's conversion factors. Vitamins D, E, B_{12} and folic acid were determined by HPLC as described by Pyka and Silwiok[23]. Amino acids was determined in the Central Service Unit, National Research Centre, Cairo, Egypt using LC3000 amino acid analyzer (Eppendorf-Biotronik, Germany. Minerals (calcium, selenium, zinc and iron) were determined using atomic absorption spectrophotometers (perkin-Elmer 3300) according to the method of AOAC[24].

Preparation of experimental diets: Using casein and the prepared meal, the experimental diets (Table 2) were prepared according to the AOAC method[25] with the following composition: 10% protein, 8% corn oil, 5% water, 5% AIN-salt mixture, 1% AIN-vitamin mixture, 1% cellulose, 35% sucrose and 35% corn starch. To estimate the metabolic nitrogen low protein diet (4%) was prepared[26].

Design of the animal experiments: The animal experiments were designed to evaluate the protein quality of the prepared cookies as well as its health effect. In the first experiment, true protein digestibility (TPD) which indicates protein bioavailability was estimated according to the method of Miller and Bender[27] using 12 rats which were fed casein diet for an acclimation period of 2 days then 6 rats were fed baked cookies diet and the other 6 rats were fed low protein diet for 9 days including 4 first days of the preliminary period and 5 final days of balance period. Water provided *ad libitum* but food were limited to 15 g per day. During the 5 days of the balance period, faeces were collected daily for each rat. At the end of the 5-day balance period, nitrogen intake by rats fed baked cookies diet were calculated and the collected faeces were dried overnight in a vacuum oven at 100°C, weighed, grind and analyzed for nitrogen. The nitrogen intake of rats fed baked cookies diet and the fecal nitrogen of both rats fed baked cookies diet and rats fed low protein diet were used to calculate the true protein digestibility of prepared cookies through the following equation:

Table 2: Composition of the experimental diets (g/100 g)

Diet ingredients	Control	Prepared meal	Low protein
Prepared formula	-	67.2**	-
Casein*	10.5	-	4.21
corn oil	8	-	8
water	5	-	5
AIN-salt mix.	5	3.8	5
AIN-vitamin mix.	1	1	1
cellulose	1	0.5	1
sucrose	34.75	13.75	38
corn starch	34.75	13.75	37.79

*10.5 g: casein has been estimated to contain 10 g protein , **: Containing 10% protein

$$\text{TPD} = \frac{\text{Ni} - \text{NF1} - \text{NF2}}{\text{Ni}} \times 100$$

where, Ni is nitrogen intake by rats fed baked cookies diet, NF1 is nitrogen excreted in faeces of rats fed baked cookies diet, NF2 is nitrogen excreted in faeces of rats fed low protein diet

In the second experiment, another 12 rats were used for estimating the effect of feeding on the cookies on blood parameters. Rats were fed casein diet for an acclimation period of 5 days then divided into two groups (6 rats per each). Group one was fed casein diet (normal group), while group two was fed diet containing the baked cookies (prepared meal group) for 28 days. Water and food were provided *ad libitum.* At the end of the feeding period blood samples were collected for the determination of hemoglobin[28] and glucose[29]. The plasma levels of creatinine[30] and urea[31] were determined as indicator of kidney function, while the activity of aspartate transaminase (AST) and alanine transaminase (ALT)[32] were determined as indicator of liver function. Also the plasma levels of total protein[33] as indicator to the nutritional status, malondialdehyde (MDA) as indicator to lipid peroxidation[34] and total antioxidant capacity[35] as indicator to antioxidant status were determined. Also total food intake and body weight gain of rats fed diet containing the baked cookies were calculated to calculate the protein efficiency ratio (PER) of prepared cookies according to the AOAC method[25]. The PER calculation was performed through the following equation:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Body weight gain (g)}}{\text{Rotein consumed (g)}}$$

Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Statistical analysis: The data of animal experiment are expressed as the Mean±SE and they are analyzed statistically using the student's t-test. The treatment means were compared at a 5% significance level.

RESULTS

Physical properties of the prepared meal: The physical properties (weight, diameter, thickness, spread ratio, color and texture) of cookies are shown in Table 3. Results showed that diameter, thickness and spread ratio were 50, 5 mm and 10, respectively. It was noticed that the lightness (L*) of cookies was low while there was an increase in a* (redness) and b* (yellowness) values.

Sensory evaluation of the prepared meal: Results of the sensory evaluation of cookies (Table 4) illustrated that the scores of flavor, odor and color of the cookies were higher than those of taste, texture and appearance. Generally, cookies were highly accepted.

Peroxide value of the prepared meal: With respect to the change of the peroxide value during the storage period as shown in Table 5, peroxide value increased with the increase in storage period from 0.20 (me O/kg oil) at zero time to 1.10 (me O/kg oil) after 180 day.

Chemical composition of the prepared meal: Proximate composition of the cookies is shown in Table 6. It is clear that the cookies showed high contents of protein (14.88%), fat (16.83%) and carbohydrate (55.1%) which make it supplying high calories (431.44 Kcal/100 g).

Results of minerals content (Table 7) showed that the cookies contained high amounts of iron (8.5 mg/100 g) and zinc (3.23 mg/100 g). Thus 100 g of cookies can achieved about 94.40% iron and 49.69% zinc from the amounts provided by the world healthiest food (2016) for children aged from 4-13 years.

Results of vitamins contents (Table 8) showed that the cookies contained high amounts of vitamin E (7.0 mg /100 g) and vitamin D (11.9 µg/100 g). Thus 100 g of cookies can achieved about 79.8% vitamin E and 79.3% vitamin D from the amounts provided by the world healthiest food (2016) for children aged from 4-13 years.

Results of amino acids profile (Table 9) showed that the cookies contained high amounts of tryptophan (9 mg g^{-1} protein) and aromatic amino acids (22.7 mg g^{-1} protein). Thus each gram protein of cookies can achieved about 18.8 and 36.03% of the daily requirements from sulfur amino acids and aromatic amino acids, respectively. Leucine is the first limiting amino acids score and lysine is the second limiting amino acid.

The nutritive and biological value of the prepared meal. Data in Table 10 illustrated that protein efficiency ratio, true digestibility, amino acid score and protein digestibility corrected amino acid score for the cookies were 1.9, 85, 127 and 108, respectively.

Depending on T ratio values (using student's t-test), it can be ascertained that there are no significant changes of the blood parameters between normal rats fed on casein diet and rats fed on the cookies diet. Data presented in Table 11 declared that there are no changes in hemoglobin, blood glucose levels and total protein.

Table 3: Physical properties of the prepared meal

	Weight (g)	Diameter (mm)	Thickness (mm)	Spread ratio	L* white	a* red	b* yellow	Texture (N)
Physical properties	9.63	50	5	10	19.42	42.38	33.45	12.65

Table 4: Sensory evaluation of the prepared meal (Mean±SD)

	Taste	Odor	Color	Texture	Flavor	Appearance
Organoleptic characteristics	7.84±1.142	8.25±0.808	8.03±0.943	7.37±1.269	8.12±1.184	7.81±1.281

*Mean of 10 replicates

Table 5: Effect of storage period on peroxide value

Storage period (days)	Peroxide value (me O_2/kg oil)
Zero time	0.20
90	0.50
180	1.10

Table 6: Total calories and proximate composition of the prepared meal

Parameters	Calories
Energy (K cal/100 g)	431.44
Moisture (g/100 g)	10.69
Crude protein (g/100 g)	14.88
Crude fat (g/100 g)	16.83
Ash (g/100 g)	1.80
*Carbohydrate (g/100 g)	55.1
Crude fiber (g/100 g)	0.7

*Calculated by differences (non-nitrogenous extract)

Table 7: Minerals content of the prepared meal compared to the world healthiest food (2016)

Minerals	World healthiest food (2016)*	Prepared formula	Percentage**
Calcium (mg/100 g)	1050	200	19.05
Zinc (mg/100 g)	6.5	3.23	49.69
Iron (mg/100 g)	9.0	8.5	94.40
Selenium (μg/100 g)	35	4.0	11.43

*World healthiest food (2016) for children aged from 4-13 years and **: Percentage that achieved by 100 g formula compared to the world healthiest food (2016)

Table 8: Vitamins content of the prepared meal compared to the world healthiest food (2016)

Vitamins	World healthiest food (2016)*	Prepared meal	Percentage**
D (μg/100 g)	15	11.9	79.3
E (mg/100 g)	9	7.0	79.8
B12 (μg/100 g)	1.5	0.6	40
Folic acid (μg/100 g)	250	108.4	43.36

*: World healthiest food (2016) for children aged from 4-13 years, **: Percentage that achieved by 100g meal compared to the world healthiest food (2016)

Table 9: Amino acid profile of the prepared meal compared with provisional amino acid and calculated chemical score

Amino acids	Amino acids (mg/g protein)	*FAO/WHO amino acid requirement mg/g protein	Chemical score (%)
Threonine	6.40	34	18.82
Valine	6.70	35	19.14
Total sulfur amino acids	4.70	25	18.80
Isoleucine	9.90	28	35.36
Leucine	8.40	66	12.73
Total aromatic amino acids	22.70	63	36.03
Lysine	7.60	58	13.10
Tryptophan	9	11	81.82

*: FAO/WHO expert consultation[59]

As shown from the present results (Table 12) feeding rats on the cookies didn't change the liver functions (ALT and AST) and kidney functions (urea and creatinine). Since there are no significant changes of these parameters between normal rats fed on casein diet and rats fed on the cookies diet.

Table 10: Protein efficiency ratio (PER), true digestibility, chemical score and protein digestibility corrected amino acid score of the prepared meal

Parameters	Nutritive values
PER	1.9
True protein digestibility	85.0
chemical score	127.0
PDCAAS	108.0

Table 11: Hemoglobin, glucose and total protein of normal rats and rats fed on prepared meal (Mean±SE)

Groups	Hemoglobin (g dL^{-1})	Glucose (mg dL^{-1})	Total protein (g dL^{-1})
Normal	12.42±0.46	79.27±0.73	6.86±0.20
Prepared meal	12.22±0.47	78.06±2.01	7.02±0.20

Table 12: Liver and kidney functions of normal rats and rats fed on prepared meal (Mean±SE)

Groups	ALT (IU L^{-1})	AST (IU L^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Normal	35.96±0.51	47.84±0.80	30.42±0.53	0.62±0.01
Prepared meal	35.66±1.05	48.16±0.94	31.80±0.51	0.62±0.02

ALT: Alanine transaminase and AST: Aspartate transaminase

Table 13: Plasma malondialdehyde and total antioxidant capacity of normal rats and rats fed on prepared meal (Mean±SE)

Groups	Malondialdehyde (nmol mL^{-1})	Total antioxidant capacity (mM L^{-1})
Normal	5.08±0.19	1.63±0.04
Prepared meal	5.12±0.36	1.67±0.05

The results in Table 13 represented plasma MDA, as indicator to lipid peroxidation and total antioxidant capacity of rats fed on casein diet and rats fed on the cookies diet. It was noticed that the cookies neither elevated MDA nor reduced total antioxidant capacity.

DISCUSSION

The studied cookies were prepared to serve as a meal for primary school children in Egypt. So, it was formulated from available and high nutritive ingredients to help improving health and scholastic achievement of primary school children. The meal was prepared in the form of baked cookies to be attractive to the children. Palatability and acceptability of these cookies were insured via the sensory evaluation results. Even if the cookies tend to be extremely hard, it was generally acceptable. This hardness may be due to fortification with chickpea flour which reduced gluten in the dough thus the formation of gluten matrices was retarded[36]. The value of peroxide number indicated that there is no possibility of rancidity during the storage. Cookies can be safely consumed at least for three months as the peroxide value was less than permissible limit for peroxide value (10 me O/kg). The results indicated that the prepared cookies is a good source of protein which could be safe for school children compared to the healthy diet of adult (0.75 g protein/kg b.wt./day)[37].

Bhagavan[38] stated that protein improve the rapid growth and muscles development of children. About 100 g of cookies can provide the child with about 30-40% of his daily protein allowance. The fat content of cookies was relatively high, but fat is necessary not only to improve the taste and texture but also fats are considered as energy source. Glatz *et al.*[39] stated that the fatty acid composition of blood lipids and adipose tissue can be affected by the fatty acid composition of dietary fat. One hundred grams of cookies supplied energy about 431.44 kcal. This covered 30% of the daily energy requirement for children[40]. Cookies can provide the primary school children by their needs from minerals and vitamins. Since 100 g of the cookies covered 19.05, 49.64, 94.4, 11.43 from calcium, zinc, iron and selenium, respectively from daily requirement (mg) compared with the world healthiest food (2016) for children aged 4-13 year. Kapil and Bhavna[41] stated that cognitive functions and scholastic achievement are associated with the micronutrients. Pollitt[42] reported that the iron-deficiency anemia is associated with the poor education performance in school children. Mineral deficiency is associated with a low intake of minerals during the rapid growth or due to the poor absorption of minerals from the diet[43]. Many nutrition survey data in Egypt illustrated that the school children do not get their needs of minerals and vitamins to improve health and educational performance[44]. So, the intake of minerals from the edible foods must be increased also bioavailability of minerals should be taken into account. The prepared cookies contain vitamins B_{12}, E and folic acid due to the presence of chickpea which contain these vitamins[45]. Kennedy[46] stated that vitamins B_{12} and folic acid are essential to the brain health and functions. Huh and Gordon[47] reported that several diseases such as autoimmune conditions, cardiovascular diseases and cancer are associated with the vitamin D deficiency. The presence of vitamin D in the prepared cookies is attributed to the presence of cereal (wheat flour), eggs and milk protein concentrate[48]. It was noted that the cookies provide 23.56% of the requirements of total essential amino acids. The highest were aromatic amino acids; the lowest were sulfur amino acids. The cookies content of lysine was higher than the requirement according to FAO/WHO. The improvement of amino acids in cookies was due to the presence of milk protein concentrate which is an excellent source of essential amino acids. Markus *et al.*[49] reported that the diet which contains rich amount of tryptophan, increase the serotonin synthesis of brain and improve different cognitive functions including memory. Chang and Satterlee[50] stated that by the determination of essential amino acids composition and the digestibility of the protein, the nutritional quality of any food protein can be evaluated. The present study revealed that the prepared cookies is a good source of protein as estimated via the determination of the amino acids composition, true protein digestibility and protein efficiency ratio. The true protein digestibility for the prepared meal indicates to the true amount of protein that hydrolyzed and absorbed in the body. The most widely used measure of protein quality is the Protein Digestibility Corrected Amino Acid Score (PDCAAS). This is used in place of Protein Efficiency Ratio (PER) evaluations for foods intended for children over 1 year of age and for non-pregnant adults. The PER, AAS and PDCAAS for the prepared meal were 1.9, 127 and 108 respectively. These values are higher than those for wheat 1.5, 47 and 42, respectively[51-53]. The PER for the prepared meal was also higher than that for weaning food prepared from 75: 15% tiger nut: soybean and weaning food prepared from 65: 25% tiger nut: soybean since, PER values were 1.40 and 1.36, respectively while TD values were 86.10 and 87.67%, respectively[54]. The safety and health effect of the prepared meal were evaluated in weaning rats. Feeding rats on the prepared cookies didn't cause reduction in blood hemoglobin than the normal value. Also, feeding on the prepared cookies didn't change the levels of blood glucose than normal rats group, which is better than many sweets and fast food that cause obesity and raise the levels of blood glucose in children[55]. But on the contrary, the prepared cookies can adjust the level of blood glucose due to its content of chickpea which has an important role in preventing type-2 diabetes[45]. Results declared the complete safety of the prepared cookies. This was demonstrated by the absence of changes in liver and kidney function values of rats fed the prepared meal from normal rats group. Especially that any injury in the liver or kidneys accompanied by an increase in the functions of each[56]. It cannot be underestimated the role of the antioxidant compounds present in the ingredients of the prepared cookies which prevented the oxidative stress. Antioxidant compounds presents in the prepared cookies are selenium and vitamins E and C in chickpea[45]; flavonoids and phenolic compounds in cinnamon[13] and wheat flour[57]. Mittal *et al.*[58] emphasized the role of chickpea dietary fiber in improvement the antioxidant status of the body.

CONCLUSION

It was suggested that the prepared meal in form of baked cookies can safely serve as a meal for the governmental school children to provide them with part of their needs from macro and micro nutrients that reducing hunger and improving health benefits and scholastic achievement.

SIGNIFICANCE STATEMENT

This study confirmed that the cookies which fortified with roasted chickpea, milk protein concentrate, cinnamon and brewer's yeast had high nutritive value and can serve as a meal for the governmental school children. This meal can participate not only in meeting children's nutrient needs but also in improving their health and scholastic achievement.

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