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ORIGINAL ARTICLES

Formulation and Production of Dietary Supplementation to Health Promotion of Elderly

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ABSTRACT

Elderly people in Egypt need food supplement that can substitute the possible dietary deficiency of any nutrient and promote general health status. In the present study one functional food was prepared in the form of biscuits on a pilot scale in the pilot plant of NRC in Egypt. The biscuits are supplemented with four probiotic strains *Bifidobacteria breve*, *lactobacillus rhamnosus GG*, *Lactobacillus rhamnosus Reuteri* and *propionibacterium freudenrichii spp*. *Shermanii*. The four probiotic strains were subjected to encapsulation, and then subjected to freeze drying process. In this study, the freeze drying process increased the ability of the encapsulated strain to survive up to 42 days without changing in viable count. Each biscuits weight 8 g, which contains 0.3g (10^7 CFU), encapsulated probiotic strains. Every 100 g of the functional food provides the elderly with 38.82% and 30.81% of protein requirements and 20.52%, 16.53 % of the caloric requirements for women and men, respectively. In addition it provides 14.64% of calcium RDA for both sexes; 47.29% of zinc for women and 37.8% for men RDA. Also provides vitamins A, E and C (8.6%, 10.75%), (10%, 8%) and (1.67%, 1.43%) for women and men respectively. The sulphur amino acid and tryptophan exist with higher percentage than the RDA. The sensory evaluation provides remarkable palatability and acceptance of the functional food. The biological evaluations of biscuits incorporated with four encapsulation probiotic bacteria and biscuits without encapsulated probiotic bacteria were studied in albino rats. The results showed that the final body weight of rats fed on biscuits with probiotic significantly increased as compared with rats fed on biscuits without probiotic, while there was non-significant change compared with control group. The feed efficiency ratio significantly increase between the control group and the another two groups. In conclusion; the prepared functional food in form of biscuit provide the elderly with most of the nutritional daily requirements and are characterized by being palatable and of high nutritional and health value.

Key words: Elderly, Functional foods, Biscuit, Encapsulation probiotic, Rats.

Introduction

Ageing is associated with increased susceptibility to degenerative and infectious diseases, which may be exacerbated by poor nutritional status. Also there is a problem that the ageing population does not consume the specific nutritional daily requirements. Hence it is essential and timely that strategies of preventive nutrition aimed at maintaining or improving the quality of life of the ageing population be developed (Saunier and Dore 2002).In the same time, it is emerging to formulate functional foods product which contain functional food ingredients with versatile health benefiting properties.

There are a lot of food sources that are highly nutritive and at the same time contain appreciable amount and type of compounds with health promoting values (Marie *et al*., 2006). Among these sources probiotic, *Lactobacilli*, *Bifidobacterium* and *Propionibacterium* are normal components of the healthy intestinal microflora, also can reduce cholesterol in plasma and reduce the risk of cancer in addition to antiaging, antioxidant and other important physiological functions (Goa, 2012).

Probiotic need to resist the manufacturing process and storage in order to be viable in the commercial product at the end of the shelf-life above a threshold of 10^6 CFU/g (Kurmann and Rasic, 1991). Microencapsulation is often mentioned as ways to protect bacteria to survive during processing and storage and to be released at appropriate sites (e.g. small intestine) in the digestive tract.

The most widely used encapsulation materials is alginate because of its cheapness, simplicity and biocompatibility (Krasaekoopt *et al.,* 2004). Drying encapsulated probiotic bacteria, in order to produce cell powder granules can be achieved by freeze drying.

Some food sources such oat (as prebiotic source) that contain 3.7% of β-glucan, functional foods enriched with β-glucan decrease serum LDL-cholesterol and blood glucose (Braaten *et al*., 1994). Other source is whey

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protein, which has a high concentration of branched chain amino acids that is easily digested to nourish muscle tissues (Madureira *et al.,* 2007). In addition it has high level of tryptophan which increase brain serotonin and improve different cognitive functions including memory (Markus *et al.,* 2000). Whey protein is mostly free of cholesterol and also increases glutathione level in blood that helps to promote immunity (Micke *et al*., 2001). Carrots are a good source of beta carotene (pro vitamin A) which can protect phagocytic cells from autoxidative damage and enhance T and B lymphocyte proliferative responses, promote the production of cytokines and increase macrophage cytotoxine T cell and natural killer cells (Neurath *et al*., 1996). Another source is baker's yeast; in fact one of the richest sources of beta glucan in the cell wall of baker's yeast *saccharomyces cerevisae*. The cell wall is considered an ideal raw material for the manufacture of β-glucan (Ezz El-Arab *et al.,* 2009). Also yeast is a source of vitamin B and has a role in the improvement in cognitive functions.

The last food source namely black chocolate, regarding that chocolate have several evidences correlate the flavonoids and stearic acid naturally present in cocoa to improved antioxidant status and benefit for cardiovascular health (Dillinger *et al.,* 2000).

The aim of the present study was to design, prepare and evaluate functional food formula for elderly population which is based on food sources known to be of high nutritional and health value. The aim also includes production of this formula on a pilot scale, trying to reach a palatable tasting to insure palatability and acceptance. The proximate analysis and biological evaluation of product were also studied.

Materials and Methods

Materials:

- Bacterial strains: *Bifidobacterium breve* ATCC 15700, *Lactobacillus rhamnosus GG*, *lactobacillus reuteri*a ATCC 20016 and *propionibacterium Freudenrichii* ATCC 1907 were obtained from Microbiological Resources Centre Cairo MIRCES, Faculty of Agriculture, Ain Shams University, Egypt.
- Purchased the compounds required for encapsulation from naturally source (Alginate).
- The ingredients used for the preparation product were purchased from local market Giza, Egypt. These include wheat flour was obtain from North Cairo and Giza Milling Co. (72% extraction), corn oil, sugar, yeast, carrots, oat were obtain from Agriculture Research Center, Cairo, Egypt.
- Animal: Male and female rats Sprague Dawely strain of body weight 134.89±7g were used in the present study. The animals were kept individually in stainless steel cages. Water and food were given ad-libitum

Methods:

- *Preparation of microorganism:*

Lactobacillus rhamnosus GG and *Lactobacacillus retueria* (ATCC 20016) were inculated into MRS broth (DeMan Rogosa sharp) and incubated at 37 ºC for 24h. *Bifidobacterium breve* (ATCC 15700) was grown in MRS broth supplemented with 0.05% L-cysteine hydrochloride (Ventling and Mistry, 1993) to provide an anaerobic condition using the gas pak system, while Propionebacterium frendeureichii (ATCC 1907) was inoculated into sodium lactate broth and incubated at 30^{O}C for 72 h. The cells were harvested by centrifugation at 5000 rpm for 15 min. at 25 ºC (Krasaekoopt *et al*., 2004).

- *Microencapsulation of bacterial strains:*

The extrusion technique of microencapsulation was used (Zhou *et al*., 1998; Krasaekoopt *et al*., 2003). After washing the culture were suspended in 5 ml of sterile 0.1% peptone solution and mixed with 20 ml of 4% (w/v) sodium alginate solution (JUDEX Laboratory Reagent, the General Chemical Pharmaceutical Co. LTB, sub BURY MIDDLE England), sterilized at (121ºC for 15 min). The cells suspension was injected through 0.11 mm needle into sterile 0.05M $CaCl_2$ and 0.2 M NaCl. The beads were allowed to stand for 30 min for jellification and then raised and subsequently kept in sterile 0.1% peptone solution at 4 ºC.

- Freeze dry microencapsulated beads:

Microencapsulated beads containing of four strains were freeze dried according to McDouough *et al*. (1982).

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- Preparation of plant materials:

Carrots were washed and cut into small pieces then dried in an air circulated oven at 40 ºC till completely dryness. They were converted into powder form. All oat grains were clean and wash by tap water and dried, then powdered in a mill to pass through a 60 mesh sieves (60 meshes per inch) and stored in air tight polyethylene bottles for formulations.

Development of formula:

The composition of biscuit formula was prepared as in table (1) and manufactured in a pilot plant of Food Industry and Nutrition Division NRC. The different ingredients were mixed together to form a dough. The resulted dough was divided into pieces that were baked at 220 ºC for 15-20 min. Baked biscuits were cooled down at room temperature for 60 min and coated with syrup of dark chocolate supplemented with 0.3g (10^7CFU) of microencapsulated probiotic strains. Another part of biscuits was cooled at room temperature for 60 min without supplemented with encapsulated probiotic bacteria, every biscuit weight 8 g.

Table 1: Composition of the biscuit formula (g/100g).

Ingredients	g
Wheat flour 72%	40
Oat flour	10
Whey protein	15
Carrot powder	5
Corn oil	10
Sucrose	10
Yeast	3
Chocolate	6
Baking powder	0.9
Vanillin	0.1
Total	100

- *Sensory evaluation:*

Just after baking a biscuits were cooled to room temperature and subjected to sensory evaluation (Penfield and Campbell, 1990) by a group of expert person (10 members) from the Food Science and Nutrition Division NRC. Each panelists were asked to give a score from 1-20 to each following product character such as appearance crust color, symmetry shape, crumb texture, odors , break and shred , taste and mouth feels. A mean value was calculated for each character, the score value was given to the product.

- *Chemical analysis of biscuits:*

Powder mixture sample was re-dried and sieved through 100-mesh sieve. The sample was analyzed for moisture, protein, fat, crude fiber and ash content using standard AOAC (1990). Vitamins E, C and A, were determined according to AOAC (1990). Amino acids were determined by auto analyzer system technique (Amino acid Analyzer LC3000). Minerals such as calcium, magnesium, potassium, iron, and zinc were measured according to AOAC (1990).

- *Preparation of diets:*

Experimental diets were prepared as in table (2), the control diet content according to AOAC (2000). Salt mixture and vitamins mixture were prepared according to Reeves *et al*. (1993). Oil soluble vitamins were given orally in a dose of 0.1ml/rat per week.

- *Design of the experiment*:

Nutritional assessment was done on experimental animals. Twenty four male and female rats (Sprague Dawly strain) were divided into 3 groups each of 8 rats and housed individually in stainless steel cages, water and food was given ad-libitum, room temperature was adjusted to 25 ºC and light cycle of 12 hours. One of these groups was fed on control diet, the second group was fed on biscuits coated with encapsulated probiotic strains and, third group was fed on biscuits without encapsulation. The experiment continued for 6 weeks, during the experiment food intake was daily recorded, body weight was recorded twice a week. At the end of the study, total food intake, body weight gain and feed efficiency ratio (Body weight gain / total food intake) were calculated.

Table 2: Composition of the experimental diets (g/100g) as recommended by AOAC (1991).

	Control	Biscuit with encapsulated probiotic	Biscuit without encapsulated probiotic
Casein*	11.5	-	-
Corn oil	8	2.3	2.3
Salt mixture	3.5	2.5	2.5
Vitamin mixture	1	1	1
Starch	75	42.02	42.02
Cellulose	1	0.66	0.66
Biscuit	-	49.59	51.52
Encapsulated probiotic strains	-	1.93	-

*11.5g casein has been estimated to contain 10 g protein.

- *Statistical analysis:*

The results of animal experiments are expressed as the mean ± SE and they are analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance.

Results:

Chemical composition of the biscuit are shown in table (3) clarified that biscuit contains 19.41% protein, fat content was 11.14%, carbohydrate was 54.67%, and crude fiber was present as 0.65%, while calcium, zinc, iron, and magnesium were 175.7%, 5.67%, 3.28%, 209.54%, respectively. Vitamins A, C, E and folic acid contents were 86.0%, 1.00%, 0.8%, 41%, respectively.

Amino acids profile of the biscuits is presented in table (4) results show that sulphur amino acids (methionine and cysteine) and tryptophan were higher than FAO/WHO recommended.

The results of sensory evaluation are shown in table (5) total score recorded for biscuits were 91%.

Table 3: Total calories and chemical composition of the biscuit.

	Wt/100g	% RDA	
		Women	Men
Total calories (Kcal)	396.58	20.53	16.53
Moisture (g%)	12.30		
Crude protein (g%)	19.41	38.82	30.81
Crude fat (g%)	11.14	18.57	13.93
Crude fiber (g%)	0.65		
Ash (g%)	1.83		
*Carbohydrate (g%)	54.67	20.63	15.31
Zinc (mg%)	5.67	47.29	37.80
Iron (mg%)	3.28	32.80	32.80
Calcium (mg%)	175.70	14.64	14.64
Magnesium (mg%)	209.54	65.48	49.89
Vitamin A (RE)	86.00	10.75	8.60
Vitamin E (TE)	0.80	10.00	8.00
Vitamin C (mg)	1.00	1.67	1.67
Folate (mg)	41	10.25	10.25

* Calculated by difference.

Table 4: Amino acids profile of the formula.

Amino acids	mg/g protein	FAO/WHO (1991)
Theronine	39.38	34
Methionine + Cystine	36.88	25
Valine	55.18	35
Isoleucine	48.04	28
Leucine	38.21	66
Tyrosine + Phenylalanine	89.64	63
Lysine	53.39	58
Tryptophane	22.68	11

Table 5: Sensory evaluation of the biscuits.

Type	General appearance							
	Aroma 20	Taste 20	Mouth feel 15	Color 10	Break and shred 20	Symmetry shape 5	Crumb texture 10	Total score 100
Biscuit	18	19	13	9	18	5	9	91

The four probiotics bacteria were subjected to encapsulation and freeze drying process. The viable of unfreeze and freeze dry encapsulated probiotic bacteria are shown in tables (6), (7). Viability of unfreeze dried of four probiotic bacteria strain has been decreased after 2 weeks then 6 weeks than initial count, where this decrease was considered as a significant difference except for *Lactobacillus rhamnosus GG* which have non-significant after 2 weeks but significant after 4 weeks.

Table 6: The viability of unfreez dry encapsulated probiotic bacteria storage at 7°C for two & Six weeks.

Strains	Initial	Two weeks	Six weeks
Lactobacillus rhamnosus GG	8.4±0.12^{a}	8.4±0.12^{a}	8.1±0.06^{a}
Lactobacillus retueria ATCC 20016	7.6±0.23^{a}	7.5±0.06^{a}	7.2±0.23^{a}
Bifidobacterium breve ATCC 15700	8.1±0.06^{b}	8.0±0.06^{a}	7.5±0.06^{c}
Probionebacterium freudenreichii ATCC 1907	8.2±0.29^{ab}	8.0±0.06^{a}	7.7±0.17^{c}

In each row same letters means non-significant difference; different letter means significance difference at 0.05 probabilities.

Table 7: The viability of freez dry encapsulated probiotic bacteria storage at room temperature for six weeks.

Strains	Initial	Six weeks
Lactobacillus rhamnosus GG	8.6±0.12^{a}	8.2±0.12^{a}
Lactobacillus retueria ATCC 20016	8.4±0.12^{a}	8.1±0.12^{a}
Bifidobacterium breve ATCC 15700	8.7±0.17^{a}	8.2±0.17^{a}
Probionebacterium freudenreichii ATCC 1907	9.6±0.23^{a}	9.1±0.23^{a}

In each row same letters means non-significant difference; different letter means significance difference at 0.05 probabilities.

Further in vivo studies are necessary, this study focused on the body weight gain, food intake, and feed efficiency ratio of rats receiving the experimental diets (Table 8). Body weight gain was increased ($p < 0.05$) rats fed on diet containing the encapsulation probiotic bacteria during 6 weeks period as compared to the biscuits without probiotic bacteria, but average feed intake was non-significant. No difference was found between the control and rats fed biscuits with the probiotic strains in body weight gain and food intake. The feed efficiency ratio reduced significantly in both biscuit groups compared with control group. Feed efficiency ratio showed non-significant change when biscuit coated with ecapsulated probiotic compared with biscuit without.

Table 8: Body weight and food intake of rats fed on experimental diets*.

	control	Biscuit with encapsulated probiotic	Biscuit without encapsulated probiotic
Initial body weight (g)	134.89 ± 6.97^{a}	134.99 ± 6.53^{a}	134.86 ± 6.46^{a}
Final body weight (g)	200.43 ± 6.48^{a}	198.35 ± 5.46^{a}	178.30 ± 5.26^{b}
Body weight gain (g)	65.54 ± 4.20^{a}	63.36 ± 3.96^{a}	43.44 ± 2.61^{b}
Food intake (g)	432.99 ± 12.78^{a}	413.11 ± 6.76^{a}	417.57 ± 8.82^{a}
Feed efficiency**	0.20 ± 0.01^{a}	0.15 ± 0.01^{b}	0.10 ± 0.01^{b}

*: Data are presented as means and standard errors of the means.
**: Feed efficiency = Body weight gain / food intake.
In each row same letters means non-significant difference; different letter means significance difference at 0.05 probabilities.

Discussion:

In view of increase in the proportion of Egyptian over 60 years old the research community is challenged to contribute improving the quality of life for this age group .The objective of study was to prepare and evaluate a functional food as supplement diet to improve the health status of the ageing population via specific nutritional recommendations that will necessitate the design and provision of functional food ingredients that positively affect the elderly health.

In the present study the four probiotic strains were subjected to micro-encapsulation and freeze drying process. All unfreeze and freeze dry encapsulation probiotic strain exhibited the ability for survival storage period. However the freeze dry process was more effective on the ability of encapsulation strain to survive up to 42 days without changing in the viable count as compared to both unfreeze dry encapsulated cells and the initial cell count. The results obtained indicate that the encapsulated probiotic bacteria strains can be an excellent solution to protect them from environmental condition. The addition of prebiotic to the formula for the advantage of prebiotic on the health that it is contain beta glucan, it can decrease serum LDL-cholesterol and blood glucose (Neurath *et al*., 1996). The chemical composition of our biscuits clarified that every 100g of biscuits can provide the elderly with 20.53 % from the daily calories requirements for women and 16.53 % for men. The protein content was 19.41%, it provides 38.82% and 32.31% for the daily requirements for women and men, respectively. The biscuits were provide by 11.14% of fat, this amount of fat although it is relatively high yet it is safe, since the type of fat is the plant origin that is not high in its saturated fatty acid. In addition, it was necessary to use this amount to give an acceptable taste to the product. Calcium provide 14.64% of the RDA for the both sexes, zinc provides 47.29% for women and 37.80% for men, while iron provides 32.8% for both sexes. Vitamins A, C and E provides (10.75%, 8.6%), (1.67%, 1.67%), (10%, 8%) for women and men respectively.

The results of amino acids profile of the biscuits showed that sulpher amino acids (methionine + cysteine) and tryptophan were higher than RDA (FAO/WHO, 1989). The improvement of amino acids profile was due to the presence of whey protein the best source of essential amino acids and has a high level of tryptophan. It was reported that diet rich in tryptophan increase the serotonin synthesis of brain and improve different cognitive function including memory (Markus *et al*., 2000).

The sensory evaluation of this biscuits showed that it is highly accepted (91%). When produced on a field scale, it has a good chance to success.

Further, this study (in vivo) are necessary and focused on health, body weight gain, food intake and feed efficiency ratio of rats fed on any of the experimental diets. The result showed that the final body weight of rats was increased ($p < 0.05$) by feeding the biscuits coated by probiotic strains during 6 weeks as compared with the rats fed biscuits without probiotics but average feed intake was non-significance.

On the other hand, the results showed that there were no-significant difference in the average of both body weight gain and food intake between the rats fed on control diet and rats fed on biscuits with probiotic strains. There was no- significance difference between the two biscuits diets in feed efficiency ratio but the difference was significant when compare with rats fed on control diet. These findings could not be supported by other studies because up to date there is no similar study being carried out. Throughout the experiment period, there were no diarrhea, sickness nor was death recorded in any of the animals under study. At the end of the experiment period, all animals receiving bacteria were alive and healthy. These observations indicated that the test strains (*Bifidobacterium breve*, *Lacobacillus rhamnosus*, *Lactobacillus Reuteria* and *Propionebacterium freudenreichii*) at dose of 0.3/g/day (10^7 CFU) increases the weight gain of rats, this strains level was determined to be the threshold amount necessary to assess a significant growth effect. Choi *et al*. (2004) found that improvement in growth depended on the dose level of probiotic bacteria. Different result could be due to not only different strain and dose level of strain but also to animals' physical condition and environment (Elleva *et al*., 2010).

In conclusion; this study resulted in formulation and production in a small scale functional food product that can be offered to elderly people .This product proved to be palatable and of high nutritional and health value that help to promote the health of elderly and satisfy a considerable degree of nutritional daily requirements.

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