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PREPARATION AND EVALUATION A FERMENTED FORMULA FOR ELDERLY

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ABSTRACT

The aim of this study was to prepare and evaluate a fermented formula for elderly by using bioactive ingredients, which are locally available, inexpensive and can be easily prepared. After fermentation, the final product was chemically evaluated, the protein content was 24.42%, the crude fat was 5.50%, the moisture was 6.19%, and pH was 6, while the mineral contents were as following: Ca 220 mg%, Fe 2.93 mg%, Mg 80.3 mg% and Se was 7.8 μg%. The fermented formula was a good source of essential amino acids. Microbiological quality reflects the safety of the final product, after fermentation no harmful organisms were detected. The biological assessment was accomplished by feeding twenty female and male Sprague Dawley rats for four weeks. The effect of the fermented formula on the feed efficiency ratio was significantly decreased than that of the control group. The level of serum lipids was insignificantly decreased compare to the control group. Fasting serum glucose and AST were significantly decreased, while zinc and cupper increased significantly when compared with the control group. Feeding rats on the fermented formula enhanced serum immunoglobulin, IgM. We conclude that the fermented formula showed beneficial effects on liver function and immune system.

Keywords: Fermented formula, Plasma lipid profile, Immunity, Rats.

INTRODUCTION

Fermentation is the oldest known form of food biotechnology. Many fermented foods had longer storage times and improved nutritional values compared to their unfermented equivalents (Compbel, 1987). According to Steinkraus, (1995), the traditional fermented food contains high nutritive values and develops a diversity of flavors, aromas, and textures in food substrates. Many foods including vegetables, fruits, cereals, meat and fish have been converted into desirable food products by fermentation and are still being consumed throughout the world today (Farnworth, 2004). The new trend is to produces fermented product from cereals in combination with legumes to improve the overall protein quality of the fermented product. It could also decrease certain anti-nutritional factors like phytates and proteases inhibitors and flatulence factors (Mital and Garga 1990). Addition of yeast (*Saccharomyces cervisia*) and Lactobacillus in combination with mixture of cereals and legumes are the most important fermentative microorganisms (Gobbetti, 1998). Microorganisms contain certain enzymes which are incapable of being synthesized by humans. One of these important microorganisms (Lactobacilli) and other probiotic organisms (Bifidobacterial) in the fermented food appear to be beneficial in the treatment of some gastrointestinal diseases by improving the ability of the gut to prevent invasion by pathogens (Martenau *et al*., 2001).

One of the most remarkable changes with aging is the frequent development of atrophic gastritis and the inability to secrete gastric acid. This may lead to small intestinal bacterial overgrowth and influences the absorption of a variety of micronutrients.

Lactobacillus acidophilus, Bifidobacteria increase indices of immune response (Portier *et al*., 1993). Lactobacilli may stimulate intestinal mucosal immunity in elderly humans (Van de Water *et al.*, 1999). So, the use of fermented food in elderly would yields good results.

The purpose of this work was to prepare and evaluate a fermented formula for elderly to solve some of their health problems through studying the effect of this formula on serum lipids, liver function and immunity of senile rats.

MATERIALS AND METHODS

Materials:

All ingredients of the fermented product (wheat, soybean, whey protein, carrot, yeast, milk, and flavor) were supplied from local market and Ministry of Agriculture.

Preparation of the bioactive ingredients:

- **Soybean:** Soybean seeds were cleaned by tap water, and soaked overnight at room temperature in water. Soaking is necessary to remove inhibitors from the soybean and loosen the halls. It was boiled for 5 minutes in water with added 1% sodium bicarbonate to inactivate trypsin inhibitor and any other anti-nutritional factors present (Hafez and Hamed 1984). The soybean seeds were dried in air oven at 45 ºC then ground to powder and stored in polyethylene bags at 4 ºC to be used in the formulation.
- **Germinated wheat:** Whole wheat seeds were soaked in tap water (1:3 w/v) for 8 h. Germination was carried out by spreading the soaked seeds in wet blotting paper and kept at 25 ºC for 72 h. to germinate with 8 hr of day light per day. The materials were kept wet throughout germination by spraying them with water every 12 h. The germinated wheat was then dried in air oven at 45 ºC, then ground into fine powder and kept at 4 ºC to be used in the formulation.
- **Carrots:** Carrots were washed with tap water and cut into small slices then dried in air oven at 45 ºC then ground into fine powder and kept at 4 ºC until used in the formulation.
- **Yeast, yoghurt and whey protein** were used without any processing.

Preparation of the fermented product:

- The ground material of germinated wheat, soybean, carrot, vanilla, sodium chloride and yeast (*Saccharomyces cervisia*) were mixed with hot water 30-35 ºC. Fermentation was carried out for 20 h at 28-30 ºC, then dried in air oven at 45 ºC.
- The milk was pasteurized at 85 ºC for 5 min. and rapidly cooled down to 4 ºC until it was mixed (after removing its film which was formed on the surface to the mixture) with whey protein, yoghurt (source of Lactobacillus and Bifidobacterium) and the prepared ground dried powders. The fermentation was carried out for 20 h at 28-30 ºC. After the fermentation the dough was dried in air oven at 40-50 ºC until the moisture content was

reduced to 6%. The final product was stored in a glass jar and refrigerated until used for analysis. The composition of the new fermented formula is shown in table (1).

Table (1): A Proximate analysis of the new fermented formula

Ingredients	Weight / 100 g sample
Moisture (g)	6.19
Protein (g)	24.42
Fat (g)	5.50
Fiber (g)	2.48
Ash (g)	4.92
Carbohydrate (g)	56.49
Energy (Kcal)	373.14
pH	6
Minerals	
Zinc (mg)	1.69
Copper (mg)	0.54
Iron (mg)	2.93
Magnesium (mg)	80.3
Calcium (mg)	220
Sodium (mg)	490
Potassium (mg)	450
Selenium (μg)	7.8

Analytical procedure:

Moisture, protein, fat, crude fiber and ash contents were determined according to AOAC, (1995). Carbohydrates were calculated by difference. Minerals (Ca, Fe, Mg, Cu, Na, K and Zn) content of the fermented product was determined by atomic absorption spectrophotometer (Varian spectr AA 220). The pH value of the fermented product was determined as described by Ibanoglu *et al*. (1999). Amino acid profiles of the fermented product were determined by amino acid analyzer system technique (Amino Acid Analyzer LC 3000). Cystiene and methionine were determined as cysteic acid and methionine sulfone, respectively after oxidation with performic acid (Schram, *et al.*, 1954). Tryptophan was completely destroyed during acid hydrolysis so alkali hydrolysis was performed by heating the sample (0.5 g) with 60 ml. of 14% barium hydroxide solution according to McFarren, (1951) in a sealed pyrex tube at 110 °C for 20 hrs. Microbiological quality assessment was done following the method of James, (2000).

Biological evaluation of the fermented formula:

Twenty male and female senile Sprague Dawley rats aged 26 months were used throughout these studies. Rats were in good health and showed no signs of diseases with an average body weight 345 ± 5g. Rats were purchased from the animal house of the National Research Centre of Egypt. All rats were initially given a commercial diet for one week. The rats were divided in two groups, on the basis of equal mean body weight. Rats were individually housed in metal cages at room temperature. The first group was

given the control diet, while the second group was given the fermented diet (table 2). Diet and water were allowed ad-libitum for 4 weeks. The weight of each rat as well as its food intake was recorded twice weekly and residual diet were recorded. No diarrhea, loss of appetite or discomfort was observed during the feeding trial. At the 28 day feeding period, the rats were deprived of food for 14 h and then anesthetized by diethyl ether.

Fasting blood samples were collected from all rats, placed into sterile tubes and centrifuged for 20 min at 3000 rpm. The obtained serum samples were analyzed for determination of total protein (Henry, 1964), total cholesterol (Watson, 1960), triglycerides (Megraw *et al.*, 1979), albumin (Doumas, *et al.*, 1972), creatinine (Houot, 1985), aspartate transaminase (AST) and alanine transaminase (ALT) (Reitman and Frankel, 1957). Serum zinc was also determined according to (Homsher and Zak (1985)) and serum copper according to (Zak, 1958). Fasting serum sugar was determined according to Tinder, (1969). The serum levels of IgG and IgM were measured by using immunodiffusion plate according to Fahey and Meckelvey (1965).

Statistical analysis:

The results obtained were expressed as the mean ± SE and the significance of the difference (p value) was assessed by Student's t- test.

RESULTS AND DISCUSSION

The new trend in food processing promotes the use of biotechnological methods over chemical methods as well as addition from natural sources rather than synthetic ones (Ashraf, 2006). From this point of view, this study aimed to investigate the application of germination and fermentation as a mean to improve the nutritional value of the new formula.

The result of our chemical analysis on the dried fermented formula sample is given in table (2). Crude protein, fiber, calcium, zinc, iron and selenium concentration in the sample were 24.42g, 2.84g, 220mg, 1.69mg, 2.93mg and 7.8ug, respectively. The whole wheat grain was a good source of selenium and during germination several enzymes become active and brought about profound changes in the nutritive value of cereal (Subramanian, 1976). Also soybean was an excellent source of minerals including Ca, iron and copper. During fermentation bacterial enzymatic hydrolysis enhance the bioavailability of protein and increase the production of free amino acids and short chain fatty acid (Parvez, *et al.*, 2006). The result of chemical analysis showed that moisture of the fermented product was 6%; in another work this level was suitable for long term storage without deterioration for 2 and 3 years Degirmencioglu *et al*. (2005). The pH of the fermented product was 6 where (Ashraf, 2006) found that the combination treatment of germination and fermentation brought an increase in nitrogen solubility both at acid and alkali pH.

Table (2): Composition of diet provided during the study (g/100g)

Ingredients	Control	Fermented formula
Casein (86% protein)	15	--
Fermented formula	--	51.19
Corn starch	65	35.42
Corn oil	7	5.18
Saturated fat	5	4.0
Cellulose	3	1.73
Salt mixture (1)	4	1.48
Vitamin mixture (2)	1	1
Total	100	100

(1) Briggs and Williams (1963). (2) Morcos, (1967).

The essential amino acids composition of fermented formula together with the recommended FAO/WHO (1991) requirement is presented in Table (3). The contents of essential amino acids such as lysine, valine, isoleucine and aromatic acids in sample were found to be nearest to FAO/WHO recommended pattern. We used whey protein because the essential amino acids consists of branches chains amino acids, lysine, phenylalanine, methionine and tryptophane which are essential to tissue growth and repair and promote the healing of bones, skin and muscle, they are also regulate blood sugar level.

Table (3): Amino acids profile of the new fermented formula

Amino acids	fermented formula	FAO/WHO (1991)	%
	mg/g protein		
Theronine	30.71	34	90.32
Cystine	10.85	25	97.44
Methionine	13.51		
Valine	40.83	35	116.66
Isoleucine	35.91	28	128.25
Leucine	59.38	66	89.97
Tyrosine	30.26	63	107.44
Phenylalanine	37.43		
Lysine	42.18	58	72.72
Tryptophan	11.06	11	100.55

The microbiological quality was done on the final product, which reflects the hygiene during production (as table 4). The basic organisms are LAB and streptococci from yoghurt and Saccharomyces cervisia from the baker's yeast and we added bifidobacteria. These microorganism groups are responsible for the fermentation of the product. Yeast may produce vitamins that enhance the growth of LAB (Degirmencioglu *et al*., 2005).

Table (4): Microbiological evaluation of the fermented formula

	Complete process of fermintation
Total bacterial count (CFU/g)	10×10^5
Yeast/mould count (CFU/g)	0/0
Coliform count (CFU/g)	Free
Escherichia coli	Free
Salmonella and shigella	Free
Bacillus cereus (CFU/g)	Nil (0)
Staphylococcus aureus (CFU/g)	Nil (0)
Listeria monocytogenes	Free

The effects of the fermented product on weight gain, food intake and food efficiency ratio in rats are shown in table (5). Rats that received the fermented product had significantly lower body weight gain than those in the control group. Food intake and food efficiency ratio were the same in the fermented rat group and the control rat group. Bernardeau *et al*. (2002) found that lactobacillus acidophilus added to the drinking water did not change the weight gain or feed intake in mice.

Table (5): Nutritional parameters of senile rats fed the new fermented formula

Parameters	**Control (Mean ± SE)**	**Fermented formula (Mean ± SE)**
Final body weight gain (g)	88.0 ± 6.9	67.5 ± 9.1
Total food intake (g)	501.5 ± 19.2	505.0 ± 17.4
Feed efficiency ratio	0.175 ± 0.01	0.134 ± 0.01 ^{C}

Statistically significant as compared with control, a: P<0.05; b< 0.025; C: P<0.01.

The results of the present study demonstrated that rats given the fermented product had a lower cholesterol concentration than that of control group. Sautier *et al*. (1983) reported that whey protein concentrate similar to soybean has a high serum cholesterol lowering action in animal experiments. Nagaoka *et al*. (1992) also reported that the serum cholesterol lowering action of whey protein concentrate is more powerful than that of soybean in several animal experiments. Nagaoka, (1996) described that β-lactoglobulin inhibited cholesterol solubility in the intestine and this was accompanied by an increase of fecal steroid excretion. Furthermore increased bacterial activity in the large intestine results in enhanced bile acid deconjugation. Deconjungated bile acids are not well absorbed by the mucosa of the gut and are excreted. Consequently, cholesterol being a precursor of bile acids is used to a greater extent for de novo bile acid synthesis (Chikai *et al.* 1987; Driessen and de Boer 1989; and De Rodas *et al.*, 1996). These results agree with Foo *et al.*, (2003) who observed that the plasma cholesterol concentration for rats fed with fermented fruit were significantly lower than that of the control. The level of serum triglycerides for the fed fermented product rat group was lower than that of the control rat group but the difference was non-significant.

The effect of the fermented product on the kidney and liver function of rats showed no difference than that of control rats i.e. there was no side effect from the fermented product and it had no toxic effect on rats. The level of fasting sugar was significantly decreased than in the control which might attributed to the presence of the yeast in the fermented product contains the glucose tolerance factor that helps in the regulation of blood sugar. Also whey protein concentration consists of branched chain amino acids which regulate blood sugar level (Walzem *et al*., 2002).

Serum zinc and copper in rats fed with the fermented product were increased than that in the control group, and it is well known that the fermented products have anti-oxidative properties that protect against oxidative damage.

Lactobacilli may stimulate intestinal mucosal immunity in elderly human (Karine and Douglas 2000). Our results suggest that the fermented product enhances the serum levels of IgG and IgM in rats. Increased immunoglobulin secretion appears to be associated with a higher number of antibody secreting cells in the gut associated lymphoid tissue.

Table (6): Biochemical parameters of senile rats fed fermented formula for a month

Parameters	Control (Mean ± SE)	Fermented formula (Mean ± SE)
Glucose (mg/dL)	78.30 ± 1.12	73.60 ± 1.32 [b]
Total protein (g/dL)	7.54 ± 0.74	7.19 ± 0.56
Albumin (g/dL)	3.79 ± 0.31	3.52 ± 0.29
Globulin (g/dL)	3.75 ± 0.49	3.67 ± 0.50
A/G ratio	1.02 ± 0.10	0.98 ± 0.16
AST (IU/L)	88.8 ± 2.58	76.70 ± 2.59 [d]
ALT (IU/L)	28.4 ± 2.76	33.4 ± 2.42
Creatinine (mg/dL)	0.61 ± 0.04	0.54 ± 0.06
Triglycerides (mg/dL)	74.2 ± 1.38	72.30 ± 1.11
Cholesterol (mg/dL)	83.20 ± 1.14	81.0 ± 0.99
Zinc (μg/dL)	79.62 ± 0.91	86.66 ± 1.64 [d]
Copper (μg/dL)	107.7 ± 1.83	137.54 ± 1.91[d]
IgM (mg/dl)	168.0 ± 11.0	226.67 ± 10.9 [d]
IgG (mg/dl)	2461.69 ± 62.8	2330.59 ± 54.7

Statistically significant as compared with control, a: P<0.05; b< 0.025; C: P<0.01; d: P<0.005

Conclusion

We conclude that it is possible to manufacture a fermented formula for elderly with bioactive ingredients using optimized concentration of lactic acid and bifidobacteria, since both have a serum lipid improvement effect and enhance the level of immunoglobulin IgG and IgM in animals. These results indicate the potential of development of fermented formula with multiple therapeutic effects to be used for old people.

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تحضير وتقييم منتج متخمر لكبار السن

إبراهيم محمد حامد ، أمل سعيد عبد العظيم ، منى محمد حسين و ثريا طاهر الدمهوجى
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الهدف من هذه الدراسة هو تحضير منتج غذائى متخمر يصلح لكبار السن باستخدام مكونات ذات فاعلية حيوية من مصادر محلية سهلة التحضير ورخيصة الثمن. وتم تقييم هذا المنتج كيميائياً وبيولوجياً . والتى أوضح التقييم الكيميائى أن البروتين الكلى بلغ 24.42%، الدهن الخام 5.5% أما الرطوبة فكانت 6.19% أما الـ pH فكان قيمته 6 . وبتقدير العناصر المعدنية أظهرت أن الكالسيوم والحديد والماغنسيوم كانت قيمتها 220، 2.93 ، 80.33 مجم فى مائة جرام عينة. أما السيلنيوم فكانت نسبته 7.8 ميكروجرام. واظهر التحليل أن المنتج يعتبر مصدر جيد للأحماض الأمينية الأساسية. تبين أيضاً من التحليل الميكروبيولوجى أنه آمن من الناحية الصحية وخالى من الميكروبات الضارة.

وبتغذية فئران التجارب على المنتج لمدة 4 أسابيع وبقياس كفاءة الاستفادة من هذا الغذاء (FER) أظهرت النتائج تغييراً معنوياً بالمقارنة بتجربة الكنترول . وأيضاً وجد إختلافاً معنوياً فى مستوى دهون مصل الدم وكذلك مستوى جلوكوز الصائم وأيضاً إنزيم الكبد الأسبارتات ترانس أمينيز (AST) عنه فى تجربة الكنترول. وإشار أيضاً إلى زيادة معنوية فى كل من الزنك والنحاس. وأيضاً وجد أن هذا المنتج يعمل على زيادة المناعة وهى (IgM , IgG)

خلصت الدراسة إلى أن المنتج له تأثير إيجابى على كفاءة وظائف الكبد وكذلك على جهاز المناعة.