

Introduction

Consumption of foods rich in whole grains and cereal fibers has in epidemiological studies been shown to reduce the risk of chronic diseases such as diabetes, cardiovascular disease and certain cancers, as reviewed by **Murtaugh *et al.*, (2003)** and shown by **Larsson *et al.*, (2005)**. Cereal brans are important ingredients providing dietary fiber. Wheat bran is one of the most common raw materials for increasing the level of insoluble dietary fiber which influences the digestibility and bioavailability of nutrients and phytochemical. The outer layers of grain contain cellulose and lignin which influence both the taste and mouthfeel of the bran (**Ross *et al.*, 2004**). Moreover, analogous carbohydrate was reported to be resistant for digestion and absorption in the human small intestine with complete or partial fermentation in the intestine. Dietary fiber includes polysaccharide, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation and blood cholesterol attenuation (**Anon, 2001**). Furthermore, **Harris (2000)** noticed that dietary fibers may cause dietary starch to escape digestion in the small intestine and enter the large intestine. If these results from the dietary fibers reducing the gastro intestinal transit time those dietary fibers that's reduce this the most would be expected to cause the most starch to escape digestion. Resistant starch is defined as the same of starch food and products of starch degradation not absorbed in the small intestine of healthy individuals because resistance low reduce caloric content and is characterized by physiological effects that make it similar to dietary fiber .

Hung *et al.* (2005) studied the quality of bread and formation of resistant starch in bread with high amylase wheat flour substitutions was evaluated. The results showed that the amount of resistant starch in bread with high amylase wheat flour substitutions with up to 50% high amylase wheat flour did not significantly change the quality of bread.

Gorecka *et al.* (2002) reported that the absorption of bile acids by fiber carrots was depended on kind of plants material as well as, type of heat treatment and type of bile acids. Among all examined

plant carrots fiber showed the lowest adsorption one. Dietary fiber of backed carrots adsorbed the most bile acids and the cholesterol was adsorbed in the highest degree by fibers. Also, **Doweidar and Mohamed (2005)** reported that the chemical composition of dry carrot contained 9.3%protein, 2.9% fat, 6.93% ash, 7.06% crude fiber and 73.81% total carbohydrates, respectively.

Owing to the importance of dietary fiber for human health, the present study was conducted to produce biscuits rich in fiber. Also, a set of experiments were performed to elucidate the effect of biscuits produced using equal volume from resistant starch, carrot powder and wheat bran to give four blends on some blood constituents such as total lipids, triglycerides, cholesterol levels, alanine (ALT) and aspartate (AST) aminotransferase activities of rats.

Materials and Methods

Materials:

Wheat bran (a by-product of wheat milling) and wheat flour (72% extraction) were obtained from south Delta Mills Company, Giza- Egypt. Whereas, corn starch was purchased from starch and Glucose Company, Cairo-Egypt. Meanwhile, carrot (*Daucus carrot*) was brought from local market, Cairo, Egypt.

Kits for determination of serum cholesterol and other parameters were purchased from Alkan-Medical Division Biocon, Germany.

Methods:

Preparation of raw materials:

Resistant starch was prepared according to the method described by **po-ying et al. (1994)**. Corn starch weighted 200 g. Into a beaker one liter and mixed with distilled water 700 ml. of suspension was autoclaved at 125°C for 1 hr. After autoclaving, the sample was cooled to room temperature and stored in a refrigerator over-night at 4°C., a product (resistant starch) was determined according to the method described by **Sambucetti and zuleta (1996)**. A product

(resistant starch) containing approximately 30% resistant starch was obtained.

The green portion of carrots was discarded and the yellow roots were washed with tap water and cut into slices. The slices were soaked in boiled water for 5 min. and dried in an oven at 70°C for 6 hr and then the complete drying process at 40°C until the moisture content reached to 7.0%. Finely, the slices were milled using Quodmat Lenior Laboratory Mill to a fine powder and stored in a refrigerator until use. As described by **Park (1987)**.

Chemical analysis of raw material:

The raw materials (resistant starch, wheat bran and carrot powder) and wheat flour (72% extraction) were analyzed for their ether extract, protein, fiber and ash contents according to the methods of **A.O.A.C. (2000)**. Total carbohydrates content were calculated by differences. Whereas, cellulose and hemicelluloses content of raw materials were determined according to the method described by **Chahal et al. (1979)**. Moreover, total dietary fiber was determined according to the method **Prosky et al. (1988)**.

Preparation of blends and making biscuits:

Equal volume from resistant starch, wheat bran and carrot powder as a sources of dietary fibers were mixing. The mixture was fortified wheat flour (72% extraction) at levels 20, 30, 40 and 50% to give four blends. The control sample was prepared from 100% wheat flour 72% extraction. Four blends dough and control sample were prepared separately to make biscuits. Blend (1) mad from 20% dietary fiber (resistant starch, carrot powder and wheat bran) plus 80% wheat flour 72% extraction. Blend (2) mad from 30% equal weight dietary fiber sources and , 70% wheat flour 72% extraction. Blend (3) mad from 40% dietary fiber plus wheat flour 72% extraction. Blend (4) mad from 50% dietary fiber sources and wheat flour 72% extraction according to **Manohar and Rao (1997)** as follows: Wheat flour 100g, sugar 30g, sodium chloride 1.0g, sodium bicarbonate 0.5g, ammonium bicarbonate 1.09, backing powder 0.39g and 18 ml water were added to different ration of bland.

The biscuits were baked at 150°C for 20 min-in an electric oven and tested by ten panelists according to **A.A.C.C. (2002)**.

Nutritional experiments:

Male albino adult rats (36 rats) weight ranging 95-105g were brought from Helwan Experimental Animal, Station Ministry of Health, Egypt. Animals were housed in individual cages with screen bottoms and fed on basal diet for eight days. The basal diet consisted of corn starch 70%, casein 10% corn oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% according **A.O.A.C(2000)**.

After feeding on basal diet for eight days, rats were divided into tow groups. The first group (6 rats) was fed on the basal diet for another four weeks and considered as negative control. The second group (30 rats) was fasted overnight and injected by alloxan solution (150 mg active alloxan/1Kg rat weight) according to **Buko et al. (1996)** to induce hypercholesterolemia then the whole rats injection were fed on basal diet for 48 hr. where hypercholesterolemia was developed. After that, the rats were divided into five sub groups.

The first one (6 rats) was continued to be fed on basal diet and considered as positive control. The second , third, fourth and fifth sub group (6 rats for each) were fed on the basal diet after native the dietary fiber sources mixture at different levels 20,30,40 and 50%, respectively.

The body weight and food consumption recorded every three days for four weeks. At the end of experimental, the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera. After that, the sera were kept on a deep-freezer at -20°C until their analyses.

Total lipids; cholesterol and triglycerides in serum were determined using the method described by **knight et al.(1972)**, **Allain et al. (1974)** and **young et al. (1975)**, respectively. Whereas, serum trans amino ferases avidities (alanine, ALT and aspirate. AST transaminases) were measured according to the methods reported by **Bergmeyer (1983)**.

Statistical analysis:

Statistical analysis for each of the collected data was done following the procedure outline by **Gomez and Gomez (1984)**. The treatment means were compared using the least significant difference test (LSD) at 5% level of probability as out line by **Waller and Duncan (1969)**.

Results and Discussion

Chemical composition of raw materials:

From the results presented in Table (1) it could be noticed that wheat bran has the highest fat 3.46%, protein 12.94%, total dietary fibers 43.19% and hemicelluloses 24.31% respectively. However, resistant starch had the highest carbohydrates 98.69% and higher total dietary fiber 39.56% than wheat flour and carrot powder. Whereas, Carrot powder has the highest crude fibers 10.39% , cellulose 14.01% and ash 4.77%, respectively.

The wheat flour 72% extraction contained higher total carbohydrate than wheat bran and carrot powder. The obtained data were found to be in agreement with **Doweidar and Mohamed (2005)** who reported that the percentage of protein, lipids and ash of carrots were 5.1, 1.5 and 6.2%, respectively.

Table (1): Chemical composition of raw materials as sources fiber and wheat flour (72% extraction) on dry weight basis g/100g

Analyses	Wheat flour	Wheat bran	Carrot powder	Resistente starch
Ash	0.51	4.04	4.77	0.30
Fat	1.20	3.46	1.53	0.09
Protein	10.68	12.94	6.72	0.72
Crude fibers	1.13	6.99	10.39	0.20
Total carbohydrates	87.08	72.57	76.59	98.69
Total dietary fibers	3.71	43.19	31.21	39.56
Cellulose	0.95	12.11	14.01	-
Hemicelluloses	1.82	24.31	9.86	-

Sensory characteristics of biscuits:

Equal volume from wheat bran, carrot powder and resistant starch were mixture and fortified with wheat flour at level 20, 30, 40 and 50%, respectively from the mixture sources to produce biscuits. The biscuits were evaluated for their sensory characteristics and the results are reported in Table (2). The results presented in Table (2) show the effect of addition 20, 30, 40 and 50% of the dietary fiber sources to wheat flour on biscuits properties. It could be clearly observed that there no significant difference between wheat flour biscuits (control) and samples supplemented with 20, 30, and 40% of dietary fiber mixture sources for taste, color, texture and odor. Whereas, the addition of 50% dietary fiber mixture to wheat flour 72% extraction, the biscuits product had significantly decreased than control sample and gave lower score in all sensory evaluation parameters than control sample and other addition. The results of statistical analysis indicated that no significant difference was found between control sample and supplemented samples for all evaluated characteristics of biscuits contained 20, 30 and 40% dietary fiber mixture.

Table (2) : Effect of fortified wheat flour using different mixture dietary fiber sources at level 20, 30, 40 and 50% on sensory evaluation of biscuit.

Samples	Shape	Taste	Color	Texture	Odor	Over all acceptability
	20	20	20	20	20	100
Control	19.5	19.4	19.1	19.6	19.8	97.4
D. F. W. F.						
20 80 Blend (1)	18.9	19.3	18.2	19.3	19.7	95.4
30 70 Blend (2)	18.5	18.7	17.7	18.9	19.1	92.9
40 60 Blend (3)	18.0	18.2	16.9	18.2	18.8	90.1
50 50 Blend (4)	17.1	17.5	16.1	17.5	18.7	86.9
L.S.D. at 5%	1.564	1.287	1.713	1.413	1.126	

Control made from wheat flour 72% extraction

Biological evaluation:

Concerning, the changes in body weight, total food intake and food efficiency ratio for rats fed on diets containing different dietary fiber sources mixture as wheat bran, carrot powder and resistant starch. The results are recorded in Table (3).

At the end of experimental period the data showed that loss in final weight of the hypercholesterolemic rats (positive control) the loss reached 18.32 g. As well as the rats hypercholesterolemic fed on 20, 30, 40 and 50% of different dietary fiber sources mixture lost from its initial weight 31.65, 28.67, 23.42, and 18.21 g., respectively . The results are in agreement with **Holm et al (1995)** mentioned that the addition of bile salts to the diet of experimental rat caused inhibition absorption of foods and led to decrement of body weight gain and feed efficiency ratio. Also, at the end of experimental period, the decrease in total food intake may be due to decrease of rate appetite or to the swelling of fiber which caused a feeling of satiety. These results are agreed with **Higgins (2004)** who reported that resistant starch ingestion increase satiety so it is possible that this effect could aid the success of weight loss and maintenance diets. In addition that, it is well known that a high fiber content leads to a low caloric intake.

Total lipid, triglycerides and total cholesterol were determined in blood serum experimental rats to evaluate the effect of hypercholesterolemia diet containing 20, 30, 40 and 50% dietary fiber mixture from wheat bran, carrot powder and resistant starch. The obtained data are given in Table (4). Diets reach in dietary fiber, it could be noticed that there was significant decreased total cholesterol, triglycerides and total lipid in rats fed on dietary fiber mixture. Similar results were obtained by **Cheng and Lai (2000)** who reported that serum cholesterol and triglyceride concentrations were clearly lower in rats fed diet containing amount of corn resistant starch. Also, **Flores et al. (2004)** mentioned that response in hamsters on serum lipidemic when fed on diets containing 2% cholesterol and different dietary fiber sources. Whilst, **Gorecka et al (2002)** reported that the cholesterol was absorbed in the highest degree by fiber and the absorption of bile acid by fiber.

Generally, feeding the rats diet containing 20, 30, 40 and 50% dietary fiber sources mixture as wheat bran, carrot powder and resistant starch led to lower the level of serum cholesterol, triglycerides and total lipids.

Table (3) : Mean of body weight, food intake and food efficiencies ratio of cholestremic rats fed different dietary fiber mixture sources diets.

Diets	Initial weight (g)	Final weight (g)	Body gain weight (g)	Total food intake (g)	Food efficiency ratio
Control negative	104.0 ± 1.47	146.12 ± 3.66	42.12 ± 4.39	472.40 ± 9.88	8.92 ± 0.64
Control positive	99.25 ± 3.25	117.57 ± 2.85	18.32 ± 1.15	384.30 ± 7.07	4.76 ± 0.15
Blend (1)	101.75 ± 2.56	133.40 ± 5.20	31.65 ± 5.02	395.25 ± 9.75	8.00 ± 0.29
Blend (2)	99.25 ± 3.25	127.93 ± 3.28	28.67 ± 1.97	370.35 ± 8.65	7.74 ± 0.45
Blend (3)	99.25 ± 3.25	122.67 ± 4.05	23.42 ± 2.21	340.0 ± 6.51	6.88 ± 0.21
Blend (4)	100.34 ± 2.91	118.55 ± 4.13	18.21 ± 2.45	310.11 ± 7.51	5.87 ± 0.35

Food efficiency ratio was calculated the equation:

$$\text{Gain in body weight} / \text{Food intake} \times 100$$

Effect of different diet composition on serum transamino-ferases:

Table (5) showed the mean values of SAST and SALT as a factor affecting liver function. The basal diet (negative control) showed SAST value 33.18 μ / L. The hypercholesterolemia rats (positive control), resulted a increase in SAST amounts by about 1.53 fold as that of negative control. Whereas, feeding 20, 30 and 40% different dietary fiber sources mixture, resulted was significant decreased in SAST by about 0.81, 0.41 and 0.21 fold as negative control. Mean while, 50% dietary fiber fed on hypercholesterolemia

rats was equal resulted for negative control fed on basal diet. Also, the serum ALT activity on hypercholesterolemic rats fed on different dietary fiber sources mixture, the results showed that significant increased in positive control than negative control. Where as, the rats fed on different blends fiber showed significant decreased than positive control till equal volume of negative control in blend 50% different fiber sources mixture.

Form the afore mentioned results, it can be suggested that the addition of different dietary fiber sources mixture at 20, 30, 40 and 50% levels to wheat flour 72% extraction to produce biscuits had significant acceptability and better sensory evaluation. Also, it can be recommended that the different dietary fiber sources mixture occurred reduction the serum lipid pattern and improvement liver lipids in hypercholesterolemic rats by using biscuits contained 40 and 50% dietary fiber mixtures .

Table (4) : Means of serum cholesterol, triglyceride and total lipids (mg/dl) in rats fed on different sources of dietary fiber mixture.

Diets	Serum cholesterol		Serum triglycerides		Serum total lipids	
	Initial	Final	Initial	Final	Initial	Final
Negative control	119.24 ± 15.85	122.07 ± 10.42	146.04 ± 16.01	137.57 ± 21.18	462.14 ± 43.23	459.37 ± 29.85
Positive control	374.37 ± 19.29	368.16 ± 17.87	235.3 ± 27.93	249.83 ± 36.34	890.72 ± 73.19	877.96 ± 38.70
Blend (1)	345.11 ± 27.28	247.41 ± 23.41	230.69 ± 11.93	215.46 ± 19.79	871.42 ± 59.98	672.07 ± 77.88
Blend (2)	334.53 ± 42.12	225.50 ± 18.99	232.95 ± 35.07	160.30 ± 25.15	840.57 ± 74.70	582.86 ± 33.16
Blend (3)	347.54 ± 32.29	162.74 ± 34.68	235.75 ± 43.35	148.19 ± 28.07	851.74 ± 86.80	466.25 ± 45.73
Blend (4)	331.32 ± 29.79	129.62 ± 28.58	249.18 ± 17.93	125.7 ± 16.63	862.31 ± 87.31	440.19 ± 40.79

Table (5): Serum AST (μ / L) and ALT (μ / L) activities in hypercholesterolemic rats fed on different dietary fiber sources mixture.

Diets	SAST (SGOT) Activity	SALT (SGPT) Activity	SAST / SALT ratio
Control negative	33.15 \pm 3.91	25.43 \pm 3.43	130.35
Control positive	83.92 \pm 6.0	42.63 \pm 7.1	196.86
Blend (1)	60.02 \pm 2.28	35.91 \pm 4.91	167.14
Blend (2)	49.54 \pm 3.46	30.31 \pm 2.89	163.44
Blend (3)	40.26 \pm 4.47	25.39 \pm 2.28	158.57
Blend (4)	34.50 \pm 3.41	24.60 \pm 2.36	140.24

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