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ORIGINAL ARTICLES

Functional Foods for Reduction of Cardiovascular Risk in Type 2 Diabetic Patients

[1]Sahar Y. Al-Okbi, [1]Doha A. Mohamed, [2]Ahmed M.S. Hussein, [3]Seham Sabry, [1]Thanaa E. Hamed and [1]Nihad H. Ahmed

[1]Food Sciences and Nutrition Department, National Research Centre, Dokki, Cairo, Egypt
[2]Food Technology Department, National Research Centre, Cairo, Egypt, [3]Internal Medicine Department, Faculty of Medicine, Al-Azhar University, Egypt

ABSTRACT

Metabolic syndrome has become a major health problem which may lead to cardiovascular diseases. In a previous work we have proved the cardiovascular protective effect of two plant food mixtures in rats. So, the present work was established to study the cardiovascular protective effect of consumption of balady bread containing the two plant food mixtures beside dietary advice in type 2 diabetic patients having the main components of metabolic syndrome. Mixture I was thyme herb, wheat germ and dry carrot. Mixture II was celery seeds, rice bran and dry tomato. The dietary intervention study with the two types of balady bread in addition of dietary regimen continued for a month. Different biochemical parameters were assessed before and after bread consumption, these include plasma lipid profile, testosterone, adiponectin, malondialdehye and glucose. Nutritional status was evaluated through anthropometric measurements and dietary intake. Correlation study was carried out between biochemical and anthropometric parameters in the patients before dietary intervention. Proximate composition, rheological properties, sensory attributes and color quality of the balady bread were studied. Results clarified that basal line of patients showed elevation of plasmaT-Ch/HDL-Ch, TG/HDL-Ch and oxidative stresscompared to normal values which reflected risks of CVD. Also, plasma adiponectin and testosterone have significant negative correlation with total cholesterol (T-Ch), triglycerides (TGs), malondialdehyde (MDA), low density lipoprotein-cholesterol (LDL-Ch), body mass index and waist circumference, together with significant positive correlation with high density lipoprotein-cholesterol (HDL-Ch) level. Mean Dietary intake of patient in the beginning of the study revealed that all diabetic patients were normo-caloric. Mean Dietary intake of patient after a month of dietary intervention revealed that all diabetic patients reduced their caloric, saturated fat and cholesterol intake and increased their minerals and vitamins intake as a result of dietary advice. Consumption of the two types of balady bread resulted in significant decrease in fasting and postprandial plasma glucose and MDA level and significant improvement of plasma lipid profile together with significant increase in plasma testosterone and adiponectin. Kidney function tests showed non-significant change. Waist circumference and the ratio of waist circumference to height were reduced significantly. Physicochemical evaluation of bread revealed that addition of plant food mixture I or II to wheat flour elevated all the proximate composition parameters (except for carbohydrate), increased water absorption, arrival time, mixing tolerance index and weakening. Dough development time and dough stability were reduced on adding mixture I or II. Adding mixture I or II produced decrease in sensory scores of balady bread for crust colour, and taste. **Conclusion:** Dietary intervention with functional balady bread beside dietary advice produced increase in plasma adiponectin and testosterone in type 2 diabetic patients along with reduction of visceral obesity, dyslipidemia and oxidative stress. The observed elevation in plasma adiponectin and testosterone together with reduction of T-Ch/HDL-Ch and TG/HDL-Ch could be taken as good marker of the reduction of cardiovascular risk in diabetic patients.

Key words: Metabolic syndrome, type 2 diabetes, plant food mixtures, adiponectin, testosterone, lipid profile, oxidative stress.

Introduction

Overweight especially that accompanied by accumulation of fat in the abdominal region and visceral fat is very serious since it is associated with an increased risk of Type 2 diabetes mellitus and cardiovascular disease (CVD) (Saad and Gooren, 2011). Low plasma testosterone has been related to visceral obesity and the metabolic syndrome and increased cardiovascular risk in man (Mah and Wittert, 2010). Low testosterone levels may promote insulin resistance and predicts a higher incidence of the metabolic syndrome (Pitteloud *et al*., 2005; Saad, 2009). A potential association was very recently reported between plasma levels of testosterone and the

Corresponding Author: Doha A. Mohamed, Food Sciences and Nutrition Department, National Research Centre, Dokki, Cairo, Egypt
E-mail: dohamohamed@yahoo.com

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risk of developing type 2 diabetes mellitus (Vikan *et al*., 2010). Adiponectin, an adipocyte-derived protein, is produced mainly by visceral adipose tissue and plays an important role in the suppression of many metabolic derangements, including type 2 diabetes mellitus, through its involvement in glucose regulation and fatty acid catabolism (Gastaldelli *et al*., 2009). Low plasma adiponectin level has been shown to be predictive of the future development of diabetes mellitus (Rasul *et al*., 2011). Nakamura *et al*. (2004) indicated that high circulating levels of adiponectin might be protective against the development of coronary artery disease. Adiponectin secretion is often diminished in obese and overweight subjects and during metabolic syndrome. Obesity is associated with low antioxidant levels in tissue and plasma compared with normal weight (Vincent *et al*., 2009), low antioxidant defense and imbalance favors systemic oxidative stress (Vincent and Taylor, 2006). Plant foods rich in bioactive phytochemicals and functional ingredients have received a growing interest for protection from incidence of chronic non communicable diseases including CVD and diabetes (Nicolle *et al*., 2004; Vincent *et al*., 2009). In previous studies, two plant food mixtures as well as their extracts rich in bioactive constituents and nutrients such as phenolic compounds, dietary fibers, carotenoids, tocopherols, unsaturated fatty acids and phytosterols showed promising cardiovascular protective effect in hypercholesterolemic rats (Al-Okbi *et al*., 2010; Mohamed *et al*., 2010). Mixture I was thyme herb, wheat germ and dry carrot, while mixture II included celery seeds, rice bran and dry tomato. So, the present work was postulated to study the cardiovascular protective effect of two balady bread containing these two plant food mixtures in type 2 diabetic patients. Also one of the main aims was to study the correlation between adiponectin concentration and testosterone with body mass index, waist circumference and lipid profile in type 2 diabetic patients which are the main components of metabolic syndrome. The aim also includes assessment of proximate composition, rheological properties, sensory attributes and color quality of the produced balady bread.

Materials And Methods

Materials:

- **Plants' foods.** Fresh tomato (*Lycopersicum esculentum*), carrot (*Daucus carota*), thyme herb (*Thymus vulgaris* L.) and celery seeds (*Apium graveolens*) were purchased from local markets, Giza, Egypt. Wheat germ (*Triticum aestivum*) was obtained from North Cairo and Giza Milling Co., Egypt. Rice bran was stabilized instantaneously after milling.
- **Wheat flour** (82%) was obtained from North Cairo Flour Mills Company, Egypt. Salt (sodium chloride) and active dry yeast (*Saccharomyces cerevisiae*) were purchased from the local market, Cairo, Egypt.
- **Patients:** Patients under study were thirteen men with type 2 diabetes. They were recruited at the outpatient of the Internal Medicine Department, Al-Zahraa Hospital, Al-Azhar University, Cairo, Egypt. Their age ranged from 40 to 59 years old (average: 56.8±6.465) as mean ± SD. Patients were receiving oral hypoglycemic drugs (metformin and sulphonylureas).

Methods:

- **Preparation of plant materials.** Fresh tomato and carrot were washed by tap water and cut into small pieces. All plants materials (tomato,carrot, thyme herb, celery seeds, wheat germ and rice bran) were dried separately in an air-circulated oven at 40 °C till complete dryness, and then they were reduced into powder form.
- **Preparation of powder mixture.** Thyme herb, wheat germ and carrot powder constituted mixture (I). Celery seeds, rice bran and tomato powder were mixed to give mixture (II).
- **Preparation of flour blends.** Wheat flour (82% extraction) was well blended with 10% of mixture I or II. All samples were stored in airtight containers and kept at 5-7°C until used.
- **Rheological properties.** Rheological properties of different blends were evaluated using farinograph and extensograph according to AACC methods No. 54-10 and 54-21 (2000), respectively.
- **Preparation sensory and evaluation of balady bread.** Balady bread was prepared by mixing 1000g of wheat flour of 82% extraction (control, or blend with mixture I or II), 5 g of active dry yeast, 15 g of sodium chloride, and 750-800 mL of water by hand for about 6 min to form the dough. The dough was left to ferment for 1 h at 30ºC and 85% relative humidity and was then divided into 100 g pieces. The pieces were arranged on a wooden board that had been sprinkled with a fine layer of bran and were left to ferment for about 45 min at the same temperature and relative humidity. The pieces of fermented dough were flattened to be about 20 cm in diameter. The flattened loaves were proofed at 30-35ºC and 85% relative humidity for 15 min and then were baked at 400-500ºC for 1-2 min. The loaves of bread were allowed to cool on racks for about 1 h before evaluation. Balady bread loaves were evaluated organoleptically by 15 trained panelists according to El-Farra *et al*. (1982). The tested characteristics were general appearance, separation of layers, roundness, distribution of crumb, crust color, taste and odor.

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- **Baking quality of bread.** Loaf volume was measured by rapeseed displacement. Both loaf weight and loaf volume were determined according to the method described by Kulp *et al*., (1985). Specific volume =loaf volume/loaf weight
- **Freshness of bread.** Balady bread loaves freshness was tested after wrapping using polyethylene bags and storage at room temperature (1, 3 and 5 days) using Alkaline Water Retention Capacity test (AWRC) according to the method of Yamazaki (1953), as modified by Kitterman and Rubenthaler (1971).
- **Chemical analysis.** Protein, fat, ash, and crude fiber contents were determined according to A.O.A.C. (2000). Carbohydrates were calculated by difference.
- **Color determination.** Objective evaluation of surface color of crust of balady bread samples was measured. Hunter a, b and L parameters were measured with a color difference meter using a spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 [L= lightness (100= white; 0= black), a= redness (+ 100) to green (-80), b= yellowness (70) to blue (-80)] (Sapers and Douglas, 1987). The Hue (H), and Chroma (C) were calculated according to the method of Palou *et al*. (1999) as follows:

H = tan-1 (b/a) (1)

C = square root of $(a^2 + b^2)$ (2)

$\Delta E= (\Delta a^2 - \Delta b^2 + \Delta L^2)^{1/2}$.

- **Design of the clinical study (intervention study):** This study has been carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt. Consent was taken from each subject enrolled in the present study. Type 2 diabetic patients were divided into two groups. Group one comprised seven patients; each patient was given daily quantity of 125g from balady bread containing mixture I. Group two of six patients; each patient received daily quantity of 125g from balady bread containing mixture II in replacement of their dietary carbohydrate. The study continued for a month. Nutritional status of all patients was assessed before and after dietary intervention through anthropometric measurements and food intake. Biochemical analysis of blood was carried out at the start and end of the study.
- **Anthropometric Measurements:** Measurements of anthropometric parameters; body weight, height and waist circumference. The ratio of waist circumference to height was calculated. Body mass index (BMI) was computed according to Bray (1998), where BMI (kg/m^2) = Weight / Squared Height.
- **Food Intake:** Diabetic patients were subjected to questionnaire for one-day dietary recall, in addition to frequency of food items consumed to determine the daily nutrient intake. Analysis of intake of protein, fat, carbohydrates, calories, cholesterol, dietary fiber, magnesium, zinc, iron, vitamin C and vitamin E per day was carried out using the computer program (Nutrisurvey for Windows, 2007). The adequacy of nutrient intake was evaluated as percent of recommended dietary allowance RDA (FAO/WHO, 1989). After taking questionnaire, patients were advised to reduce calories and carbohydrates from their diets, substitute saturated fats, purified flour and full milk products and full milk and milk products by unsaturated fats, whole cereals and cereal products, and skimmed milk and low fat milk products, respectively and to take daily specific amount of fresh vegetables and fresh fruits (low in total sugar). At the end of the study another questionnaire for one-day dietary recall was taken from all patients. The nutrient contents of the tested balady bread were among the calculated nutrients' intake. Different nutrient intake of patients at the end of the study was compared with that at the start. Questionnaire concerning daily physical activity of patients was recorded.
- **Biochemical analysis of blood:** Blood samples were obtained from fasted patients. The blood samples were mixed with heparin for separation of plasma and determination of plasma glucose (Trinder, 1969), T-Ch (Watson, 1960), HDL-Ch (Burstein *et al*., 1980), LDL-Ch (Schriewer *et al*., 1984) and TGs (Megraw *et al*., 1979). T-Ch/HDL-Ch and TGs/HDL-Ch ratios were calculated. Plasma adiponectin (Ryan *et al*., 2003) was analyzed in all samples as antidiabetic, anti-inflammatory and antiatherogenic parameter using ELISA kit (human Adiponectin/Arcp30 Immunoassay; R&D systems, USA). Plasma testosterone was determined according to Chen *et al*. (1991). Plasma MDA was assessed (Satoh, 1978) as indicator of lipid peroxidation and oxidative stress. Plasma level of creatinine (Houot, 1985) and urea (Fawcett and Scott, 1960) were determined as kidney function tests. Postprandial plasma glucose was determined in both groups at the start and end of the study, where normal meal was consumed at the start while a meal containing the prepared balady bread was given at the end of study. The biochemical parameters of patients were compared before and after dietary supplementation.
- **Statistical analysis** The results of organoleptic evaluations were evaluated by an analysis of variance and least significant difference (LSD) according to McClave and Benson (1991). Data from blood and food intake were analyzed using Student's t-test (2-tailed). For associations of adiponectin and testosterone with plasma lipid profile, lipid peroxidation and anthropometric measurements (BMI and waist circumference) Person correlation test was used.

Results:

Chemical composition of wheat flour and balady bread samples:

Table (1) summarizes the chemical composition of wheat flour and balady bread samples. Balady bread containing mixture I and II were found to contain the highest protein, fat, ash and crude fiber and the lowest total carbohydrate compared to control or wheat flour.

Table 1: Chemical composition of wheat flour and balady bread samples (on dry weight basis).

Samples	Protein	Fat	Ash	Crude fiber	Carbohydrate*
Wheat flour (82%)	11.82	2.45	1.50	2.32	81.91
Control balady bread	11.90	2.52	2.65	2.86	80.07
Balady bread with Mixture I	13.80	3.01	3.35	3.56	76.28
Balady bread with Mixture II	14.40	3.68	3.44	3.48	75.00

* Calculated by differences

Rheological properties of dough:

Rheological properties of three blended flours (control, mixture I and II) were evaluated using Farinograph and Extenograph as shown in Table (2). It is clear that the control sample (wheat flour) had the lowest value of water absorption, arrival time, mixing tolerance index and weakening compared to mixture I and II containing dough. Dough development time and dough stability were low in dough containing mixture I or II compared to wheat flour without additions. As shown in Table (2) resistance to extension (R), extensibility (E), proportional number (R/E) and dough energy decreased in dough containing mixture I or II compared to control (wheat flour).

Sensory evaluation:

The influence of blending mixture I or II on the organoleptic properties of balady bread was evaluated and illustrated in Fig. (1) and Table (3). The obtained results indicated that, addition of mixture I and II decreased sensory scores of balady bread for crust colour, and taste compared to control. Separation of layer, distribution of crumb and roundness of balady bread samples were not affected by the additions. Mixture I and II increased sensory scores of balady bread for odor non significantly compared to control. Though there is decline in some sensory attributes however the products are considered acceptable from the organolepticaly and technological properties, specially because they may possess functional health benefits.

Table 2: Effect of adding mixture I and II on Wheat flours (82% extraction) on rheological properties of doughs.

Parameters	Control balady bread	Balady bread with Mixture I	Balady bread with Mixture II
Farinograph properties			
Water absorption (%)	64	67.5	69
Arrival time (min)	1.1	1.25	1.50
Dough development time (min)	6	5	5
Dough stability (min)	8.5	7	6.5
Weakening (BU)	80	100	110
Mixing tolerance index(BU)	30	60	80
Extensograph properties			
Resistance to Extension (R) (BU)	450	380	360
Extensibility(E)(mm)	120	110	105
Ratio (R /E)	3.75	3.45	3.42
Energy $(Cm)^2$	80	70	65

Where: B U: brabender units

Table 3: Statistical parameters of sensory evaluation of different balady bread

Sample	General appearance (20)	Separation of layers(20)	Roundness (15)	Distribution of crumb (15)	Crust color(10)	Taste (10)	Odor (10)
Control balady bread	18.71 [a]	18.66 [a]	14.45 [a]	12.80 [a]	8.79 [a]	8.41 [a]	8.58 [a]
Balady bread with Mixture I	18.06 [a]	18.50 [a]	14.35 [a]	12.63 [a]	7.02 [c]	6.80 [c]	9.02 [a]
Balady bread with Mixture II	17.00 [b]	18.22 [a]	14.50 [a]	12.60 [a]	5.80 [d]	5.20 [d]	9.13 [a]
LSD at 0.05%	1.36	1.11	1.00	1.006	0.96	0.77	0.93

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Control balady bread   Bread containing Mixture I   Bread containing Mixture II

Fig. 1: Balady bread photos of control, mixture I and II.

Baking Quality of Bread:

The physical characteristics of the produced balady bread, such as weight, volume and specific volume are presented in Table (4). Loaf volume and weight in case of bread containing mixture I or II were high compared to control.

Table 4: Effect of replacement with mixture I or II on the baking quality of balady bread

Samples	Weight(g)	Volume(cm)	Specific volume
Control balady bread	100	320	3.2
Balady bread containing mixture I	116	335	2.88
Balady bread containing mixture II	120	340	2.83

Freshness of balady bread:

The effect of storage period (1-5 days) at room temperature on freshness of balady bread was evaluated. Table (5) showed that, the control bread sample had the lowest values of alkaline water retention capacity. Alkaline water retention capacity was noticed to decline on storge for the three samples during 1, 3 and 5 days.

Table 5: Freshness properties of stored balady bread at room temperature as determined by alkaline water retention capacity (%).

Sample	Storage period		
	1 day	3 days	5 days
Control balady bread	302.2	287.6	275.7
Balady bread containing Mixture I	320.6	300.8	280.3
Balady bread containing Mixture II	330.3	315.7	295.2

Color characteristics:

Color characteristic is a major criterion that affects the quality of the final product. The substituted flours blends showed a difference in color in relation to the control (100% wheat flour). Mean color values of balady bread of different treatments are recorded in table 6 that shows Hunter values of whiteness (L), redness (a) and Yellowness (b) for crumb and crust colors. Bread containing mixture I or II had lower crust and crumb L values than the control, indicating darker color. Both samples also showed less red (a-values) and yellow (b-values) values compared to control. The results also showed that the saturation decreased in the substituted bread samples compared to control.

Table 6: Hunter color values of balady bread containing mixture I or II.

samples	L*	a*	b*	a/b	Chroma	Hue	ΔE*
Control balady bread							
Crust	56.17	10.62	24.48	0.43	26.68	66.55	-
Crump	56.02	6.97	20.48	0.34	21.63	71.02	-
Balady bread containing mixture I							
Crust	50.89	9.37	21.75	0.43	23.68	66.69	7.87
Crump	48.40	7.1	19.31	0.37	20.57	69.81	7.71
Balady bread containing mixture II							
Crust	50.47	7.87	23.76	0.33	25.03	71.67	8.21
Crump	43.22	5.01	13.58	0.37	14.47	69.75	14.67

[L= lightness (100= white; 0= black), a= redness (+ 100) to green (-80), b= yellowness (70) to blue (-80)]. $\Delta E= (\Delta a^2 – \Delta b^2+ \Delta L^2)^{1/2}$. Where: ΔL= L of control- L of sample, Δa= a of control - a of sample, Δb= b of control – b of sample

Nutritional status and biochemical parameters of type 2 diabetic patients:

Assessment of nutritional status was carried out through anthropometric parameters (Table 7) and food intake (Table 8). BMI in the start of the study revealed that patients of both groups were overweight. There was significant reduction in body weight after receiving bread containing mixture I, while non-significant reduction was observed after consumption of bread containing mixture II. Waist circumference, a reliable indicator of visceral obesity, and the ratio of waist circumference to height were reduced significantly at the end of the study in patients of both groups.

Table 7: Anthropometric parameters (mean±SE) of patients before and after a month of dietary supplement with balady bread containing mixture I or II.

Parameters	Balady bread containing mixture I (n= 7)		Balady bread containing mixture II (n= 6)	
	Before intervention	After intervention	Before intervention	After intervention
Age (years)	56±1.673	56±1.673	57.8±3.246	57.8±3.246
Weight (Kg)	86.6±1.673	80.3*±2.086	87.3±2.400	82.7±2.361
Height (m)	1.6±0.017	1.6±0.017	1.7±0.031	1.7±0.031
BMI (kg/m^2)	26.6±1.126	24.7±0.640	25.8±0.600	24.4±0.578
Waist circumference (cm)	106.7±3.715	92.1**±1.905	101±3.870	88.7*±2.556
Waist/Height	0.657±0.022	0.567**±0.013	0.597±0.025	0.525*±0.018

Values statistically significant when data after intervention were compared with that before intervention:
*: $p < 0.05$, **: $p < 0.005$

Table 8: Mean dietary intake of different nutrients (mean±SE) at the start and end of the clinical study.

	Balady bread containing mixture I (n= 7)				Balady bread containing mixture II (n= 6)			
Parameters	Before intervention	% RDA	After intervention	% RDA	Before intervention	% RDA	After intervention	% RDA
Energy (Kcal)	2399.1±89.024	104	1484.30±74.540	65	2282.6±105.626	99	1660±34.229	72
Carbohydrate (g)	388.14±7.9516	-	196±5.5154	-	303.5±23.277	-	219±8.864	-
Protein (g)	66.2±10.572	105	74.3±9.140	118	74±6.887	116	75.7±2.746	120
Fat(g)	64.8±4.149	-	41.8±2.840	-	83.9±6.701	-	51.1±2.263	-
Saturated Fat (g)	25.7±2.058	-	6.9±1.449	-	24±2.178	-	11.8±0.689	-
Polyunsaturated Fat (g)	13.3±1.996	-	17.2±1.479	-	32.9±4.612	-	20.0±1.088	-
Cholesterol (mg)	253.2±84.168	-	117.3±36.445	-	322.9±17.292	-	122.2±12.096	-
Dietary fiber (g)	32.7±2.035	-	43.1±1.894	-	34.5±5.081	-	47.7±1.656	-
Vit.E (mg α-tocopherol)	9.9±1.655	99	9.2±1.338	92	11.1±4.468	114	10.3±0.714	103
Vit.C (mg)	42±15.356	70	110.0±20.751	183	44.7±11.948	75	107±2.106	178
Magnesium (mg)	269.8±14.321	64	465.8±12.069	111	357.6±30.459	85	463.7±11.554	110
Iron (mg)	7.9±1.739	79	15.4±0.898	154	7.1±2.869	71	15.8±0.559	158
Zinc (mg)	11.5±1.542	77	15.3±1.203	102	10.4±1.795	69	15.1±0.900	101

Mean Dietary intake of patients (Table 8) in the beginning of the study revealed that all diabetic patients were more or less normo-caloric. Dietary intake of protein was higher than RDA, while dietary intake of vitamin C, magnesium, iron and zinc were lower than RDA. Dietary intake of vitamin E was ranging from 99% to 114% of RDA. Mean Dietary intake of patient (Table 8) after a month of intervention study revealed that all diabetic patients reduced their caloric intake. Protein intake was increased to 118% and 120 % of RDA in patients taking balady bread containing mixture I and II, respectively. Saturated fat and cholesterol intake of diabetic patients of both groups was reduced as a result of dietary advice, while polyunsaturated fat was increased in group given bread with mixture I when compared to the intake in the start of the study. Patients in both groups showed increase in minerals, vitamins and dietary fibers intake as a result of dietary advice except for vitamin E. Questionnaire showed that daily physical activity of patients was low.

Biochemical parameters of diabetic patients before and after supplementation with balady bread containing mixture I or II are present in table (9). The administration of balady bread containing mixture I or II to diabetic patients resulted in significant decrease of fasting glucose level by 11% and 14%, respectively and significant reduction of postprandial glucose by 13% and 19%, respectively.

The basal line of diabetic patients showed them to be hypercholesterolemic compared with normal laboratory values that reported to be 150-220 mg/dl (Murray *et al*., 1996). All diabetic patients showed an increase of coronary artery disease risk in the starting of the study due to the elevated ratio of T-Ch to HDL-Ch which is >5 as reported by Kinosian *et al*. (1994). Also, the studied diabetic patients showed elevated plasma triglycerides (> 150 mg/dl), reduced HDL-Ch (< 40 mg/dl).

The present study showed significant positive correlation between plasma levels of adiponectin and testosterone (r= 0.556). Plasma levels of adiponectin and testosterone have significant negative correlation with the levels of T-Ch (r= -0.511 & -0.716, respectively), TGs (r= -0.505 & -0.698, respectively), MDA (r= -0.209 & -0.336, respectively), LDL-Ch (r= -0.312 & -0.385, respectively), BMI (r= -0.250 & -0.254, respectively) and waist circumference (r= -0.331 & -0.352, respectively), while have positive correlation with HDL-Ch level (r= 0.502 & 0.506, respectively).

Table 9: Plasma parameters (mean±SE) of diabetic patients before and after a month of dietary intervention with balady bread containing mixture I or II.

Parameters	Balady bread containing mixture I (n= 7)		Balady bread containing mixture II (n= 6)	
	Before intervention	After intervention	Before intervention	After intervention
Fasting Glucose (mg/dl)	179.9 ± 4.288	159.3**** ± 2.404	179.3 ± 5.043	154.2**** ± 5.068
% Change		-11		-14
Postprandial Glucose (mg/dl)	273.3 ± 9.363	237.9**** ± 4.199	277.2 ± 10.099	225**** ± 7.636
% Change		-13		-19
Total cholesterol (mg/dl)	227.6 ± 5.821	180.4**** ± 3.999	228.2 ± 6.584	162.7**** ± 4.332
% Change		-21		-29
HDL-Ch (mg/dl)	39.7 ± 0.746	51.4** ± 0.921	39.5 ± 0.846	52.5**** ± 0.957
% Change		29		33
LDL-Ch (mg/dl)	131.7 ± 4.899	114.4* ± 4.958	141.3 ± 3.929	107.2**** ± 4.315
% Change		-13		-24
T-Ch/ HDL-Ch	5.8 ± 0.243	3.5**** ± 0.115	5.800 ± 0.259	3.1**** ± 0.114
% Change		-40		-47
Triglycerides (mg/dl)	152.1 ± 1.841	118.1**** ± 3.661	153.3 ± 3. 073	113.3**** ± 5.109
% Change		-22		-26
TGs/HDL-Ch ratio	3.8 ± 0.102	2.3****±0.066	3.9 ± 0.113	2.2****±0.126
% Change		-39		-44
MDA (mmol/l)	12.4 ± 0.648	10.3** ± 0.605	13.1 ± 0.583	9.3**** ± 0.309
% Change		-17		-29
Adiponectin (ug//ml)	6.6 ± 0.514	8.3* ± 0.354	6.4 ± 0.180	8.5**** ± 0.213
% Change		26		33
Testosterone (ng/ml)	5.6 ± 0.396	6.5 ± 0.427	5.8 ±0.394	7.3** ± 0.381
% Change		-16		26
Creatinine (mg/dl)	0.998 ± 0.043	0.938 ± 0.023	1.0 ± 0.046	1.0 ± 0.021
% Change		-6		0
Urea (mg/dl)	42 ± 1.673	41.6 ± 1.523	40.3 ± 1.282	38.8 ± 0.792
% Change		-1		-4

Values statistically significant when data after intervention were compared with that before intervention: *: $p<0.025$, **:$p<0.01$, ***:$p<0.005$, ****:$p<0.001$.

After a month of dietary intervention with balady bread containing mixture I or II plasma levels of total cholesterol, triglycerides, LDL-Ch, TG/HDL-Ch and T-Ch/HDL-Ch showed significant reduction compared to basal values. Plasma level of HDL-Ch showed significant elevation in diabetic patients of both groups when compared with the starting level. A significant reduction in plasma level of MDA as indicator of lipid peroxidation was observed at the end of the study in the diabetic patients of both groups compared to the starting level.

Plasma levels of testosterone and adiponectin in both groups of diabetic patients showed significant increase after supplementation with balady bread containing either mixture I or II compared to their starting values.

Kidney function tests showed non-significant change in diabetic patients at the end of the study when compared with the starting level.

Discussion:

Previously we have proved the cardio protective effect of two plant food mixtures in rats (Al-Okbi *et al*., 2010; Mohamed *et al*., 2010). These two mixtures contained rice bran or wheat germ as source of tocopherols, phytosterols and unsaturated fatty acids; carrot or tomato as source of carotenoids; and thyme or celery seeds as source of phenolic compounds. All plant foods used are rich sources of dietary fibers. So, in the present study the two mixtures were incorporated separately into balady bread to produce two types of bread as functional foods to be evaluated as cardio protective in type 2 diabetic patients.

Addition of either mixture I or II to wheat flours certainly affects chemical analysis, physical, rheological and sensory properties of bread. Elevation of proximate composition parameters (protein, ash, fibers and fat) of bread may be due to the fact that both mixtures have high percentage of protein, fat, ash and dietary fiber as previously determined by Al-Okbi *et al*. (2010). These results are in agreement with those reported by Yaseen *et al*. (2007). The increased water absorption, arrival time, mixing tolerance index and weakening on incorporation of the mixtures may be due to their high protein and fibers content, where protein and fibers tend to bind more water. Proteins and fibers in the mixtures may interact with wheat flour ingredients and added water, consequently mixing tolerance index of dough increased. In this respect, Shouk and Ramadan (2007) reported that water absorption and mixing tolerance index of dough increased with increasing percent of rice dietary fibers. The decrease in dough development time and stability in mixture I and II bread may be due to dilution of gluten protein from wheat flour with the increased fiber content in the bread containing mixtures. This may also be due to the interaction between fibrous materials and gluten, which affects the dough mixing properties as reported by Hussein *et al*. (2010). Reduction in resistance to extension, extensibility, proportional number and dough energy as a result of addition of plant food mixtures to wheat flour may be attributed to high fiber in these plant food mixtures that dilute the gluten content of dough. It is well known that, viscoelastic property of wheat dough depends on gluten quality and quantity, so, as gluten content increased viscoelastic property is improved. Such findings agreed with the results of Naeem *et al*. (2002). Incorporation of either mixtures decreased sensory scores of balady bread for crust colour, and taste compared to control which, might be due to the taste of spices in the mixtures (thyme and celery) and the color of carrot and tomato which render the bread different taste and color from that of the bread that panalest are accustomed to consume. Loaf volume and weight increased in mixture I and mixture II compared to control. These effects may be due to high fiber and protein contents in mixture I and mixture II compared to control. Fibers and proteins are characterized by its higher water holding capacity that may lead to increased loaf weight. Hussein and Hegazy (2007) reported that bread volume and weight increased with the addition of different levels of aleurone layer to wheat flour. Addition of mixture I or II caused a noticeable increase in alkaline water retention capacity values at the same storage period. Such effect might be related to the difference in quantitative distribution of protein fractions and physicochemical properties of fiber of the mixtures. Such increase can be related to a higher hydrophilic nature of proteins and fiber (Barron and Espinoza, 1993). Color characteristic is a major criterion that affects the quality of the final product. The substituted flour blends showed a difference in color in relation to the control (100% wheat flour). It was not considered to be a real disadvantage since even the commercial control bread varies in color intensity according to the flour from which it is produced. Bread containing mixture I or II had lower crust L values than the control, indicating darker color. These results are similar to those obtained by Kenny *et al*. (2000) when mixing wheat flour with some functional ingredients. Subjective evaluations confirmed that the bread samples were darker, less red (a-values) and yellow (b-values) compared to control. The results showed that saturation decreased in the substituted bread samples. These results are similar with those obtained by Ahmed (1999) and Eisa *et al*. (2007).

Results of clinical study revealed that at the start of the study all diabetic patients were over weight although they were normo-caloric but energy expenditure might be low due to their reduced daily physical activity as noticed from questionnaire. The patients have the main components of metabolic syndrome (visceral obesity, dyslipidemia and a pro-inflammatory and thrombogenic state). In addition to the elevation of the ratios of T-Ch to HDL-Ch and TG to HDL-Ch and oxidative stress which are all risk factors for CVD.

Etiology of metabolic syndrome may be associated with the consumption of high energy-dense foods (Arcari *et al*., 2009). Metabolic syndrome is a combination of interconnected risk factors for insulin resistance, glucose intolerance, dyslipidemia, and hypertension (Shen *et al*., 2012) and is associated with pro-inflammatory and thrombogenic state (Saad, 2009). Excessive intake of dietary saturated fat promotes adipocyte hypertrophy, alters their normal endocrine function to an inflammatory pathologic condition that increases the secretion of tumor necrosis factor-alpha and interleukin-6, among other proinflammatory cytokines, and concomitantly reduces adiponectin secretion (Hotamisligil, 2006; Prada *et al*., 2007). It has been reported by Mah and Wittert (2010) that increased cardiovascular risk might be correlated to visceral obesity metabolic syndrome and reduced plasma testosterone. However, Rodriguez *et al*. (2007) demonstrated that the prevalence of the metabolic syndrome is independently associated with lower androgen levels. Elevation of waist circumference has been reported to be associated with low androgen levels and symptomatic androgen deficiency (Hall *et al*., 2008). A potential association was reported between plasma levels of sex hormones and the risk of developing type 2 diabetes mellitus (Spranger *et al*., 2004).

Diabetes is a major risk factor for CVD and its prevalence has increased dramatically in the past two decades (Shen *et al*., 2012). Type 2 diabetic patients have a 2- to 3-fold higher risk of CVD and premature mortality vs the general population (Almdal *et al*., 2004). Oxidative stress is recognized widely as being associated with various disorders including diabetes, hypertension, and CVD (Kajiyama *et al*., 2008) since oxidative stress stimulates inflammation and promotes cytokine production (Villa-caballero *et al*., 2000).

Dietary intervention with balady bread containing mixture I or II for a month was associated with a reduction in waist circumference which may be due to presence of dietary fiber in mixture I and II that has been reported previously (Al-Okbi *et al*., 2010) to be 24 and 16g/100g dry weight, respectively. The studied mixtures contain two main sources of cereals, wheat germ and rice bran in mixture I and II respectively. These sources of cereals in addition to the other plant foods in the mixture are rich sources of dietary fibers. The results are consistent with a recent report that showed whole grain intake to be inversely associated with abdominal visceral adipose tissue (McKeown *et al*., 2010). BMI is used as an indicator of overall adiposity while waist circumference is a measure of abdominal adiposity and visceral fat deposition (Lee *et al*., 2004). Emerging evidence suggests that different fat compartments may be associated with differential metabolic risk factors (McKeown *et al*., 2010). Visceral adipose tissue compartment is a more “pathogenic” type of fat deposition and is known to be closely associated with increased CVD risk and mortality in type 2 diabetic patients (Fox *et al*., 2007). So the significant reduction in waist circumference as results of dietary intervention in the present study is a good indicator of the reduction of CVD risk in type 2 diabetic patients. Whole grain consumption or cereal fiber intake may reduce glucose levels, and reduce the risk for developing type 2 diabetes mellitus and CVD (Lindstrom *et al*., 2006; Anderson and Conley, 2007).

Balady bread containing mixture I or II showed improvement in lipid profile, testosterone, adiponectin, malondialdehyde and fasting and postprandial glucose levels in both studied groups which may be attributed to presence of different functional ingredients in the mixtures. The functional ingredients are represented by phenolic compounds, carotenoids, dietary fibers, unsaturated fatty acids, phytosterols and tocopherols. Mixture I and II contain phenolic compounds as 1.83 and 1.9 g gallic acid equivalent, α-tocopherol as 4.91 and 11.45 mg, γ-tocopherol as 4.01mg and 0, δ-tocopherol as 468.3mg and 13.93mg/100g, respectively as reported previously by Al-Okbi *et al*. (2010).

Antioxidant rich diets can reduce oxidative stress and inflammatory responses (Bakker *et al*., 2010). Diet rich in dietary fiber and phenolics compounds reduce oxidative stress in people with metabolic syndrome (Slavin, 2003). Diets high in fiber, including cereal fibers are associated with a reduction in inflammatory markers (Bullo *et al*., 2007; Herder *et al*., 2009). There are a variety of possible mechanisms for this association. As weight loss in obese subjects improve the low grade inflammation of subcutaneous and visceral adipose tissue depots (Apovian *et al*., 2008), dietary fibers that reduce weight may indirectly reduce inflammation (Koh-Banerjee *et al*., 2004). This anti-inflammatory response has been attributed to the low glycemic impact of cereal fibers (Manning *et al*., 2008). Celery and thyme in the studied mixtures showed previously antioxidant and anti-inflammatory effects due to presence of phenolic compounds (Mohamed and Al-Okbi, 2008; Naemura *et al*., 2008). Also phenolic compounds may also directly reduce lipid accumulation in adipocytes and pre-adipocytes (Hsu and Yen, 2008). Flavonoids elevate the levels of glutathione, the major contributor to the intracellular redox state (Moskaug *et al*., 2005). Therefore, flavonoids can enhance plasma adiponectin profile by influencing the adipocyte redox status (Shabrova *et al*., 2011).

Wheat germ present in mixture I has been shown previously to contain high levels of the antioxidant tocopherols and phytosterol (Wang and Johnson, 2001) thereby it may be capable of reducing lipid peroxidation (Mayes, 1996). Rice bran is rich in bioactive constituents such as γ-oryzanol, polyunsaturated fatty acids, phytosterols, tocopherols and tocotrienols which reported to possess anti-inflammatory (Akihisa *et al*., 2000), antioxidant (Xu *et al*., 2001) and cholesterol lowering activity (Wilson *et al*., 2007). These bioactive constituents have hypolipidemic effect characterized by decreases in serum concentrations of TG, T-Ch, very-low-density lipoprotein cholesterol, and LDL-Ch (Sunitha and Rukmini, 1997). γ-Oryzanol increases adiponectin secretion from adipocyte (Ohara *et al*., 2009). Rice bran also reduces blood sugar in diabetics (Qureshi *et al*., 2002) and reduces the atherogenic index in rats (Chou *et al*., 2009).

The studied mixtures contain tomato and carrot as source of carotenoids including lycopene and β-carotene. It was reported previously that carotenoids such as β-carotene, protect cells from oxidative stress by quenching free radicals and lower risks of diabetes mellitus and CVD (Suzuki *et al*., 2002). Tomato is a rich source of lycopene, β-carotene, potassium, vitamin C, flavonoids, folate and vitamin E that may provide protection against the development of type 2 diabetes and its risks (Wang *et al*., 2006; Shidfar *et al*., 2011).

The elevation of testosterone levels observed in this study after consumption of the studied bread may be a result of improvement in plasma lipid profile, blood glucose, oxidative stress and visceral obesity. These results are in agreement with our previous results in hyperlipidemic rats given the studied dietary mixtures in powder form or as extracts (Al-Okbi *et al*., 2010; Mohamed *et al*., 2010). Overweight may induce oxidative stress and decrease testosterone levels which may alter testicular functions and consequently can be important causative factor in the etiology of the male infertility (Erdemir *et al*., 2011). So reduction in body weight and visceral obesity and the improvement in biochemical parameters are acompanied by increment in testosterone levels in type 2 diabeteic patients as a result of improvement in testicular functions.

Conclusion:

Dietary intervention with functional balady bread beside dietary advice produced increase in plasma adiponectin and testosterone levels in type 2 diabetic patients along with reduction of visceral obesity, dyslipidemia and oxidative stress. The observed elevation in plasma levels of adiponectin and testosterone together with reduction of T-Ch/HDL-Ch and TG/HDL-Ch could be taken as good marker to the reduction of cardiovascular risk in diabetic patients. The functional properties of the studied bread might be attributed to the presence of tocopherol, phytosterols, unsaturated fatty acids, carotenoids, phenolic compound and dietary fibers.

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