

Nutritive Value of Cookies Containing Wheat Germ Oil

Muhammad Umair Arshad, Surayia Zakir, Faqir Muhammad Anjum¹, Tahir Zahoor¹ and Haq Nawaz²

Department of Food Science and Technology, University of Sargodha, Sargodha, Pakistan

¹National Institute of Food Science & Technology, University of Agriculture, Faisalabad, Pakistan

²Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan

Abstract

Wheat germ, the main fatty component of wheat grain, is characterized by consisting of polyunsaturated fatty acids as well as its elevated content of natural antioxidant i.e. α -tocopherol. Cookies were prepared from wheat germ oil (WGO) with the objective of providing higher antioxidant content in the diet. Normal shortening was replaced with WGO at the levels of 0, 25, 50, 75 and 100% in the cookies formulation. The α -tocopherol content was 1660mg/kg in the WGO. Fatty acid profile of WGO revealed the content of oleic acid (14.69%), linoleic acid (56.99%) and linolenic acid (9.51%). The α -tocopherol content of cookies improved significantly with gradual increase of WGO in cookies formulation, however sensory attributes of the cookies containing WGO up to 50% were as acceptable as control cookies. Above which the sensory characteristics of cookies were affected negatively. The biochemical effects of the cookies were assessed through weanling albino rats by feeding a diet of cookies (10 % protein basis) for three months. The results indicated a significant decrease in serum cholesterol and LDL (Low density lipoprotein) concentration of serum of rats fed over diets containing WGO against the control group. However, non significant differences were observed among diet groups fed over 50, 75 and 100% WGO levels. High density lipoprotein (HDL) concentration was not affected by the treatments during the study. Results indicated that WGO containing cookies lower LDL, without significantly changing the HDL in rats. These findings support the nutritional value of wheat germ as a natural source of edible oil containing essential fatty acids and natural

antioxidant whose combined effects play a crucial role in reducing lipid peroxidation, which is ultimately useful in decreasing the risk of cardiovascular diseases.

Key words: Wheat germ oil, α -tocopherol, cookies; lipid profile

Introduction

Edible vegetable oils are very important component of humane diet. The beneficial effects of vegetable oils are basically due to their high content of unsaturated fatty acids and their valuable bioactive compounds, which have been found to decrease the risk of cardiovascular disease (CVD). Among edible oils, WGO is of the major importance due to presence of highly beneficial polyunsaturated fatty acids (PUFA), octacosanol and the highest tocopherol content (Saito & Yamauchi, 1990). WGO has been shown to reduce plasma and liver cholesterol in animals (Horrobin & Manku, 1983; Kahlon, 1989). Role of WGO to cope with cholesterol content is associated with its octacosanol and tocopherol content. Tocopherol content in WGO is unique, represented mainly as α -tocopherol. Natural α -tocopherol is a superior radical chain-breaking antioxidant as compared to the synthetic one. It is a lipid-soluble antioxidant carried in LDL and inhibits the proliferation of smooth-muscle cells in vitro and when added to plasma, thus increases the resistance of LDL to oxidation. Park & Choi (2002) also investigated that α -tocopherol supplementation was beneficial in lowering the blood-lipid peroxide concentrations without affecting antioxidant activities in patients with type-2 diabetes.

WGO is extracted by two ways from the precious wheat germ (a minor by-product of wheat milling industry and constitutes about 2-3% of the whole wheat kernel); either by mechanical pressing or solvent extraction method. Wheat germ contains about 10-12% oil (Singh & Rice, 1979). In recent years; with the improvement in food industry sector

Corresponding author: M. Umair Arshad
Department of Food Science & Technology,
University of Sargodha, Sargodha, Pakistan

there is now a rich annual production of wheat germ in the world, most of which are generally used with animal feed formulations. Hitherto, the overall level of wheat germ utilization has not yet progressed beyond an initial stage and the beneficial wheat germ source has not been fully and efficiently utilized. Therefore, oil extraction from wheat germ can open new ways to improve the utilization of wheat germ.

Wheat bread and cookies are widely consumed in many developing countries, and, therefore, present a valuable supplementation vehicle for nutritional enhancement however; cookies have been recommended as a better use than bread due to their ready-to-eat form, wide consumption and long shelf-life (Lorens *et al.* 1979). A few studies have been reported in the literature about the use of synthetic forms of tocopherol in baked products. Investigations of Hix *et al.* (1997) have recommended the utilization of these synthetic antioxidants for cookies production. From the instrumental texture analysis and consumer acceptance, the researchers recommended the use of these natural antioxidants for effectively substituting BHA in cookies. The importance of these natural antioxidants (tocopherols) in our diets and their huge presence in WGO, led us to trial WGO suitability for the production of cookies and to further investigate the role of its antioxidants in the balance of lipid profile of albino rats.

Materials and Methods

Procurement of Raw materials

Raw wheat germ was procured from Sunny Flour Mills, Lahore, Pakistan. Wheat flour was purchased from local market for the production of cookies.

Oil extraction

The oil of wheat germ was extracted with hexane in a Soxhlet extractor (Quickfit, England) for several hours. The organic phase was then removed using a rotary evaporator under reduced pressure and the oil was flushed with a stream of nitrogen and stored at -20 °C in sealed dark color tubes.

Characterization of extracted WGO

The amount of oil extracted by solvent was gravimetrically determined. Refractive index, density, viscosity, free fatty acid, iodine value, saponification value, unsaponifiable fraction and peroxide value of the oil were determined according to the methods given by American Oil Chemists Society (AOCS, 1998).

Fatty acids profile of WGO was estimated by the Method No. Ce 1f-96 as described in AOCS (1998). The fatty acids were converted to their respective methyl esters prior to analysis by Gas Chromatograph (Model 3900 Varian USA). The oil samples 50 µL was methylated in 4 mL KOH for one hour at room

temperature. The resultant fatty acid methyl ester were extracted with HPLC grade hexane and analyzed by GC immediately using fused capillary column (Silica 30m x 0.25), a flame ionization detector (FID) and nitrogen gas carrier (3.5 mL/min). GC split ratio was 100% Injector and Detector temperature was 260°C and column oven temperature was 222°C for 7.5 minutes. The fatty acid methyl esters (FAMES) were injected manually. The fatty acids were identified by chromatography retention time by comparison with standard fatty acids provided by sigma. α -tocopherol content of WGO were analyzed according to the Method Ce 8-89 (explained below, under the heading of “determination of α -tocopherol content of cookies”) as given by American Oil Chemists Society (AOCS, 1998).

Formulation and preparation of cookies

WGO was used in the formulation of cookies at 0, 25, 50, 75 and 100% on a replacement basis with normal shortening. The cookies were then packed in polyethylene bags, sealed and stored in a freezer (0–5 °C) until required.

Cookies were prepared according to the procedure described by McWatters *et al.* (2003) with slight modifications. The basic ingredients used were 380 g of flour blend, 100 g vegetable shortening, 225 g of granulated cane sugar, 21 g of beaten whole egg, 3.75 g of salt, and 1.8 g of baking powder. The dry ingredients were weighed and mixed thoroughly in a bowl by hand for 4 to 5 minutes. Shortening (as prepared above by replacements with WGO at different levels) was added and rubbed-in until uniform. The egg was added and dough thoroughly kneaded in a mixer for 5 minutes. The dough was rolled thinly on a sheeting board to a uniform thickness (8 mm) and cut out using a round scorn cutter to a diameter of 35 mm. The cut out dough pieces were baked on greased pans at 160 °C for 15 minutes in baking oven. The prepared cookies were cooled at room temperature (30 ± 2 °C) and packed in high density polyethylene bags.

Chemical composition of cookies

The moisture, crude protein, crude fat, total ash and crude fiber contents of cookies were determined by AACC (2000). Nitrogen free extract (NFE) was calculated by difference. The factors, n=5.70 (for wheat flour), and n=6.25 (for cookies) were used for conversion of nitrogen to crude protein. All reagents used were of analytical grade (BDH Chemicals, Poole, UK). The TBA value of the cookies was recorded using the method of Pokorny and Dieffenbacher (1989). It was measured by the absorbance at 530 nm using an ultra violet (UV) spectrophotometer (Model: Aquarius CE-7200).

Determination of α -tocopherol content of cookies

The α -tocopherol content of the WGO was analyzed by using high performance liquid chromatography (HPLC) method (Katsanidis and Addis, 1999). The oil samples were dissolved in hexane (0.025 mg oil/mL) and filtered through a 0.21 mm filter. A normal phase HPLC column was used for separation of tocopherol isomers. Analytical separation of oil components on the column was achieved by using a mobile phase consisting of hexane: isopropyl alcohol (99:1 v/v) on isocratic mode. Total run time and flow rate were 15 minutes and 1.3 mL/min, respectively. The HPLC system (Perkin Elmer-series 200) consisted of a separations module and fluorescence detector. The detector was set at 290 nm excitation wavelength and 400 nm emission wavelengths. The column temperature was 35°C. The injection volumes of the both, individual standards and the oil sample were 2 μ L. A standard calibration curve was prepared

for tocopherol standard to calculate the amount of this tocopherol isomer present in the oil sample.

Physical and organoleptic evaluation of cookies

Physical parameters including, diameter, height, weight and spread factor of cookies were made on three replicates and mean values were reported. Cookie diameters and heights were measured with a vernier caliper. Weights were determined using a Mettler digital top loading balance (PC 400; Mettler, Buchi Switzerland). Spread factor is measured as the ratio between weight and height of cookies.

Cookies were evaluated for color, flavor, taste, texture, crispiness and overall acceptability according to a preference method of Land and Shepherd (1988). Ten (trained) judges participated in the sensory evaluation of the cookies on a 5-point hedonic scale. The coded cookie samples were randomized and presented to the judges in the midmorning in white plates in sensory evaluation laboratory, Institute of Food Science and Technology, University of Agriculture, Faisalabad.

Composition of experimental diets (WGO based cookies)

Diets	Ingredients							
	Cookies (g)	Corn starch (g)	Corn oil (g)	Glucose (g)	Min. mix (g)	Vit. mix (g)	Total (g)	Protein (%)
A	84.67	0.33	-	5.0	5.0	5.0	100	10
B	84.67	0.33	-	5.0	5.0	5.0	100	10
C	84.67	0.33	-	5.0	5.0	5.0	100	10
D	84.67	0.33	-	5.0	5.0	5.0	100	10
E	84.67	0.33	-	5.0	5.0	5.0	100	10

A = Diet prepared from cookies (0% WGO)

B = Diet prepared from cookies (25% WGO)

C = Diet prepared from cookies (50% WGO)

D = Diet prepared from cookies (75% WGO)

E = Diet prepared from cookies (100% WGO)

Table 1: Fatty acid composition of WGO

Fatty acid	No. of C-atoms	%age
Palmitic	16:0	18.09
Palmitoleic	16:1	0.22
Stearic	18:0	0.50
Oleic	18:1	14.69
Linoleic	18:2	56.99
Linolenic	18:3	9.51

Table 2: Physico-chemical parameters of WGO

Parameters	Values
<i>Physical</i>	
Refractive index	1.48
Density (g/mL)	0.91
Viscosity (cP)	32
Absorbance at 290 nm (log ϵ)	0.97
<i>Chemical</i>	
Free fatty acid (%)	27.3
Iodine value (g/100g)	107
Saponification value (mg KOH/g)	207
Unsaponifiable fraction	2.76
Peroxide value (mmol/kg)	1.47
α -tocopherol (mg/g)	166

Table 3 : Chemical composition and α -Tocopherol content of WGO based cookies

Components	WGO level in cookies (%)				
	0	25	50	75	100
Moisture (%)	7.96 \pm 0.13 ^a	7.98 \pm 0.14 ^a	8.01 \pm 0.15 ^a	7.97 \pm 0.13 ^a	8.03 \pm 0.15 ^a
Crude protein (%)	9.96 \pm 0.15 ^{ab}	10.01 \pm 0.18 ^a	9.97 \pm 0.16 ^{ab}	9.97 \pm 0.16 ^{ab}	9.93 \pm 0.14 ^b
Crude fat (%)	16.76 \pm 0.25 ^a	16.79 \pm 0.26 ^a	16.78 \pm 0.25 ^a	16.79 \pm 0.26 ^a	16.80 \pm 0.27 ^a
Crude fiber (%)	1.86 \pm 0.06 ^a	1.84 \pm 0.05 ^a	1.83 \pm 0.05 ^a	1.86 \pm 0.07 ^a	1.84 \pm 0.06 ^a
Ash (%)	0.87 \pm 0.02 ^{ab}	0.90 \pm 0.03 ^a	0.88 \pm 0.04 ^{ab}	0.89 \pm 0.05 ^{ab}	0.86 \pm 0.05 ^b
NFE (%)	62.43 \pm 1.90 ^a	62.31 \pm 1.50 ^a	62.38 \pm 1.60 ^a	62.36 \pm 1.60 ^a	62.38 \pm 1.60 ^a
TBA value (malenaldehyde/Kg)	0.09 \pm 0.004 ^a	0.07 \pm 0.003 ^b	0.06 \pm 0.001 ^c	0.06 \pm 0.002 ^d	0.04 \pm 0.001 ^c
α -Tocopherol (mg/kg fat)	22.846 \pm 1.05 ^e	192.378 \pm 2.06 ^d	370.200 \pm 5.06 ^c	526.468 \pm 7.06 ^b	633.120 \pm 9.07 ^a

Means in the same row bearing the same letter are not significantly different ($p \leq 0.05$).

Table 4: Physical and organoleptic evaluation of WGO based cookies

Components	WGO level in cookies (%)				
	0	25	50	75	100
Weight (g/cookie)	13.834 \pm 0.13 ^a	13.870 \pm 0.15 ^a	13.838 \pm 0.13 ^a	13.714 \pm 0.10 ^b	13.848 \pm 0.14 ^a
Height (cm)	1.304 \pm 0.05 ^{bc}	1.296 \pm 0.03 ^{cd}	1.330 \pm 0.06 ^a	1.290 \pm 0.03 ^d	1.310 \pm 0.04 ^b
Diameter (cm)	9.120 \pm 0.15 ^a	9.126 \pm 0.16 ^a	9.162 \pm 0.18 ^a	9.106 \pm 0.14 ^a	9.136 \pm 0.17 ^a
Spread factor (D/H)	6.990 \pm 0.08 ^b	7.041 \pm 0.11 ^{ab}	6.891 \pm 0.07 ^c	7.062 \pm 0.12 ^a	6.980 \pm 0.08 ^b
Color	6.90 \pm 0.07 ^a	6.90 \pm 0.06 ^a	6.9 \pm 0.08 ^a	6.4 \pm 0.04 ^b	6.3 \pm 0.04 ^b
Flavor	6.5 \pm 0.05 ^a	6.2 \pm 0.03 ^b	6.6 \pm 0.05 ^a	6.0 \pm 0.02 ^c	5.8 \pm 0.02 ^d
Taste	6.0 \pm 0.02 ^a	5.8 \pm 0.01 ^{ab}	5.9 \pm 0.02 ^b	5.6 \pm 0.01 ^c	5.4 \pm 0.01 ^d
Texture	7.0 \pm 0.08 ^a	6.8 \pm 0.07 ^{ab}	6.8 \pm 0.07 ^{ab}	6.7 \pm 0.07 ^b	6.5 \pm 0.06 ^c
Crispiness	7.2 \pm 0.09 ^a	7.2 \pm 0.09 ^a	7.2 \pm 0.09 ^a	7.1 \pm 0.08 ^{ab}	7.1 \pm 0.07 ^{ab}
Overall acceptability	6.3 \pm 0.05 ^{ab}	6.2 \pm 0.05 ^b	6.3 \pm 0.06 ^a	6.2 \pm 0.04 ^{bc}	6.0 \pm 0.03 ^c

Means in the same row bearing the same letter are not significantly different ($p \leq 0.05$). D/H: Diameter/Height

Table 5: Effect of WGO based cookie diet on biochemical parameters of rats

Components (mg/dl)	WGO level in cookies (%)		
	0	25	50
Total Cholesterol	103.450 \pm 1.83 ^a	99.530 \pm 1.75 ^b	96.973 \pm 1.73 ^c
Triglycerides	77.333 \pm 1.55 ^a	76.863 \pm 1.52 ^b	77.267 \pm 1.55 ^a
LDL-C	46.433 \pm 1.15 ^a	39.610 \pm 1.05 ^b	35.040 \pm 0.98 ^c
HDL-C	41.900 \pm 1.07 ^b	42.290 \pm 1.10 ^{ab}	41.540 \pm 1.05 ^b
Total protein	6.317 \pm 0.05 ^b	6.315 \pm 0.05 ^b	6.347 \pm 0.06 ^{ab}
Serum Albumin	2.940 \pm 0.03 ^{ab}	2.877 \pm 0.03 ^c	2.947 \pm 0.04 ^a

Means in the same row bearing the same letter are not significantly different ($p \leq 0.05$).

Efficacy studies

Albino rats

Rat diets were formulated using flours from cookie samples containing WGO, 0% (diet A, control), 25% (diet B), 50% (diet C), 75% (diet D), and 100% (diet E). Diets were prepared according to AOAC (1995) formulation. Forty weanling male albino rats, 28 days old and weighing between 42 and 45 g, were grouped by randomized block design into five groups on the basis of weight, such that mean initial weights did not differ by more than ± 0.5 g. Each group consisted of eight rats and was housed in individual wire-bottom cages that allowed for easy faecal collection. Rats were given free access to diet and water. The temperature of the animal room was 27 ± 1 °C with alternate 12 hr. periods of light and dark. The diets were supplemented with vitamins and minerals to target requirements (Grant *et al.* 1995). The diet formulation is given in the table below:

Rat bioassay

After two months of study, to avoid the stress on rats, blood samples were withdrawn without heparin by decapitation. Blood samples were then centrifuged at 3000 g for 15 min and kept at -20 °C until analysis. Triglycerides, cholesterol and HDL-C were determined using commercial kits from Human (Wiesbaden, Germany) Ref. 10724, 10028 and 10084 respectively. LDL-C was calculated as the difference between the serum cholesterol value and HDL-C (McNamara *et al.* 1990).

Statistical Analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance according to the methods described by Steel *et al.* (1997). Multiple comparisons of the various means were carried out by LSD (Least Significant Difference) test at $p \leq 0.05$.

Results and Discussion

Extraction and characterization of WGO

The extraction rate of oil through soxhlet was found 12%, which was in close agreement with the results found in the study of Dunford & Zhang (2003) who reported that 11-13% oil can be extracted from wheat germ by soxhlet extraction method. The results of physico-chemical parameters in Table 1 indicate that the characteristics of extracted WGO are in agreement with current published values for these indices in literature (O'Brien, 2004 & Przybylski, 2004). The density or specific gravity of oil at any given temperature compared to water at a specified temperature is known to increase as the degree of unsaturation increases (i.e. with higher iodine

value). The density values (g/mL) found for extracted WGO are 0.905. The iodine value (g Iodine/100g sample) for WGO was found to be 107, which is also within the limits of literature (O'Brien, 2004 & Przybylski, 2004). Saponification value (mg KOH/g sample) of WGO was 207, varying significantly from that of 191.4 for corn germ oil and 193.9 for olive oil (Rudan-Tasic & Klofutar, 1999), show that the fatty acids present in this oil have a high number of carbon atoms. Quality evaluation through unsaponifiable fraction (2.76) and peroxide value (1.47 mmol/kg sample) confirmed that the quality of this oil is satisfactory. Freshly deodorized oil should have zero peroxide value, but in most cases, for the product to have acceptable storage stability the peroxide value of oils used should be less than 5 (Rudan-Tasic & Klofutar, 1999). Regarding the parameters of absorbance in the ultraviolet region and the peroxide index, the degree of oxidation of the lipids present in the WGO is minimal, and the slight degradation in the oil is due to the oxidation of tocopherols, exhibited by the peroxide value (1.47 mmol/Kg). The results for the physico-chemical parameters of WGO are found to be in agreement with those found by Dunford & Zhang (2003).

The results for fatty acid profile of WGO are presented in (Table 2). According to these results, extracted WGO consisted of about 57% linoleic acid (18:2, n6), which is an essential fatty acid (Table 2). WGO has very high unsaturated and polyunsaturated fatty acid content and an excellent n-3/n-6 fatty acid ratio i.e. 1/6 (Table 2). Total unsaturated and polyunsaturated fatty acid (PUFA) content of WGO was about 82 and 66%, respectively. It has been suggested that unsaturated fatty acid, especially PUFA intake reduces cardiovascular heart disease (CHD) (Simopoulos, 1999). Several scientific studies have shown that n-3 fatty acids have benefits for lowering CHD risk (Hu, 2001). It has also been suggested that n-3/n-6 ratio of 10 or less results in reduction in fatal CHD risk (Hu, 2001). Keeping in view this recommended ratio WGO has excellent profile.

Chemical analysis of cookies

Results regarding the chemical parameters of cookies are presented in Table 3. The α -tocopherol content in cookies made with varying levels of replacement with WGO ranged from 192.378 to 633.120 mg/kg fat compared with 22.846 mg/kg fat in the control (0% WGO) cookies (Table 3). These results explicated that the cookies made with 100% replacement with WGO were higher in total α -tocopherol content (633.120 mg/kg fat) than the cookies made with 100% normal shortening

(22.846 mg/kg fat) and all other prepared cookies. About 2 thirds of α -tocopherol was found to be retained in cookies from ingredients after dough making and baking process. Stabilization of α -tocopherol is attributed to acetylation of the phenolic hydroxyl group on α -tocopherol. One serving cookie (28 g) incorporated with 50% WGO would provide about 120–150% of the adult daily RDA of vitamin E (8–10 mg α -TE) (NAS 1989). Synthetic antioxidants such as BHA, butylated hydroxytoluene (BHT), propyl gallate (PG) and t-butylhydroquinone (TBHQ) are being widely used by the baking industry to retard rancidity and preserve freshness in cookies (Hix *et al.* 1997). But utilization of this natural anti oxidant was found to be more effective regarding both; stability and nutritional effects.

Analysis of variance (Table 2) showed that WGO incorporation has significant effect on TBA value of cookies. TBA value for control cookies was maximum 0.094 mg of malenaldehyde/Kg in comparison to the lowest value of the cookies prepared with 100% WGO i.e. 0.044 mg of malenaldehyde/Kg. It is evident from the results that by increasing the percentage of WGO, the TBA no. decreases and the on set of rancidity are delayed as shown in Figure 1. It is certainly due to the tocopherols and tocotrienols present in WGO that act as natural antioxidants (Lloyd *et al.* 2000). Molero Gómez & Martínez de la Ossa (2000) also reported the levels of these nutritionally significant components (tocopherols and tocotrienols) in wheat germ oil. WGO based products have extended shelf life since WGO is extremely stable against the onset of rancidity and oxidative deterioration.

Physical and Organoleptic Evaluation of Cookies

Data on the physical characteristics of cookies are presented in Table 4. There were no significant ($p \leq 0.05$) differences between the values obtained for physical parameters of cookies prepared from WGO and the control (100% Normal shortening) cookies. These results were similar to those reported for cookies prepared from wheat-cowpea (McWatters *et al.* 2003).

The sensory evaluation is very important criterion in food industry. Sensory evaluation is usually performed towards the end of product development. The cookies prepared from commercial wheat flour with different levels of WGO as shortening were evaluated for various sensory attributes. Data presented in Table 4 shows the sensory properties of cookies prepared with different concentrations of WGO. Analysis of variance explicit that cookies differed significantly

($p \leq 0.05$) regarding various sensory attributes like taste, texture and overall acceptability but crispness, color and flavor found to be non significant ($p > 0.05$) in this case. WGO is not only rich in natural tocopherols, but was also found to produce cookies with good sensory characteristics and consumer acceptability. The cookies prepared with more than 50% WGO had lower rating scores than the control for all attributes except crispness, which was similar ($p \leq 0.05$) to the control. On the other hand, cookies prepared with WGO replacement levels upto 50% had similar ($p \leq 0.05$) sensory scores as the control for all attributes except appearance and color in cookies prepared with WGO replacement levels upto 50% which had higher scores than the control. Ratings of the sensory panel showed that cookies prepared with WGO replacement levels upto 50% were preferred for all attributes. Therefore, these treatments were selected for the shortening replacement in further bioassay study.

Lipid profile of rats' serum

Serum lipids (total serum cholesterol, LDL-C and triglycerides) were significantly ($p \leq 0.05$) affected by WGO level in the cookie based diets. Group of rats fed cookie diets prepared with 50% WGO levels had lower ($p \leq 0.05$) total serum cholesterol, LDL-cholesterol and triglycerides than other groups. The lowest values were obtained for total cholesterol and LDL-cholesterol content of rats fed on WGO based cookies. Instead, the WGO diet lowered total and LDL-cholesterol significantly ($p \leq 0.05$) more than did the control diet, but the effect of all diets on HDL- cholesterol was similar (Table 5). These results might be attributed to the higher content of PUFA and α -tocopherol in cookie diets compared to that of the control diet. Although the information available is sparse, previous work established that wheat germ feeding improves plasma lipoproteins in animals and possibly in humans. In rats, the addition of 7% wheat germ to a high-fat, high-cholesterol diet reduced LDL-cholesterol by 37.9% (Borel *et al.*, 1989). The inclusion of wheat germ in a test meal also reduced plasma total cholesterol concentrations by 27.1% over several hours in 6 normolipidemic subjects (Cara *et al.* 1992). These data suggest that WGO may lower circulating cholesterol or at least delay the absorption of cholesterol.

The mechanism of reducing cholesterol by the use of wheat germ is likely to be complex. Because wheat germ contains fiber, it has been thought that some effects on cholesterol metabolism might be mediated by dietary fiber (Cara *et al.* 1992). Therefore, our work extends these studies by showing that bioactive tocopherols are an

additional mechanism by which wheat germ could lower cholesterol absorption and potentially improve cholesterol concentrations. The present study shows that tocopherols intrinsic to WGO are biologically active and have a prominent role in reducing cholesterol absorption. This suggests that natural α -tocopherol in WGO may be as effective as synthetic one in supplements. We have not studied lower doses of WGO, but it is possible that such doses might also have some effect on cholesterol content. Taken together with our present work, these data strongly suggest that α -tocopherol in the normal diet can be nutritionally important with respect to lipid content. Because most of the cholesterol absorbed by humans is recirculating endogenous biliary cholesterol, an effect of natural food tocopherols of reducing cholesterol absorption would be expected to lower serum cholesterol regardless of the amount of dietary cholesterol taken in (Ostlund, 2002).

Conclusions

The principal finding of this study is that value addition of cookies by incorporating WGO in their formulation has a beneficial effect in improving lipid components of rat serum. The results demonstrated the potential beneficiary effect of cookies containing WGO (significantly; upto 50%) reduced total cholesterol, triglycerides and LDL cholesterol levels without effecting HDL cholesterol in serum. This property of WGO may be attributed to the α -tocopherol content present in the oil. The results also confirmed WGO (upto 50%) suitability for the production of cookies and hence similarly needed to be assessed for other baked products. If similar results are obtained for other baked foods, it will be necessary to investigate the possibility that small amounts of naturally occurring tocopherols in the WGO might have important effects on cholesterol metabolism that have been overlooked. In that case, natural dietary tocopherols would be an additional and badly needed tool for reducing LDL-cholesterol concentrations in the population with minimal side effects and risk.

References

AACC. *Approved methods of analysis*. St. Paul, Minnesota: The American Association of Cereal Chemists. 2000.

AOAC. *Official methods of analysis*. 17th edition. Washington, DC: Association of Official Analytical Chemists. 1995.

AOCS. (1998). *Official methods and recommended practices of the American Oil Chemists' Society*. Champaign, IL: AOCS Press.

Bligh, E. G., & Dyer, W. J. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry & Physiology*, 1959.37, 911–917.

Borel, P., Lairon, D., Senft, M., Chautan, M., & Lafont, H. Effect of wheat bran and wheat germ on the digestion and the intestinal absorption of dietary lipids in the rat. *American Journal of Clinical Nutrition*, 1989.49, 1 192-202.

Cara, L., Dubois, C., Borel, P., Armand, M., Senfi, M., Portugal, H., Pauli, A., Bernard, P. & Lairon, D. Effects of oat bran, rice bran, wheat fiber, and wheat germ on postprandial lipemia in healthy adults. *American Journal of Clinical Nutrition*, 1992.55, 81–88.

Dunford, N. T. & Zhang, M. Pressurized solvent extraction of wheat germ oils. *Food Research International*, 2003. 36, 905–909.

Grant, G., Dorward, P., & Pusztai, A. Pancreatic enlargement is evident in rats fed diets containing soybean (*G. max*) or cowpea (*V. unguiculata*) for 800 days but not in those given diets based on kidney bean (*P. vulgaris*) or lupin seed (*L. angustifolius*). *Journal of Nutrition*, 1993.123, 2207–2215.

Hix, D. K., Klopfenstein, C. F., & Walker, C. E. Physical and chemical attributes and consumer acceptance of sugar-snap cookies containing naturally occurring antioxidants. *Cereal Chemistry*, 1997. 74(3), 281–283.

Horrobin, D. & Manku, M. How do polyunsaturated fatty acids lower plasma cholesterol levels? *Lipids*, 1983. 18, 558-562.

Hu, F. B. The balance between omega-6 and omega-3 fatty acids and the risk of coronary heart disease. *Nutrition*, 2001.17(9), 741–742.

Kahlon, T. S. Nutritional implications and uses of wheat and oat kernel oils. *Cereal Foods World*, 1989. 34, 872-875.

Kirk, R. S. & Sawyer, R. *Pearson's Composition and Analysis of Foods*. 9th edition. arlow: AWL. 1991.

Land, D.G. & Shepherd, R. Scaling and ranking methods. In: Piggott, J.R. (Ed.) *Sensory Analysis of Foods*. Elsevier Applied Science, (pp. 155-185), New York.. 1988.

Lloyd, B. J., Siebenmorgen, T. J. & Beers, K. W. Effects of commercial processing on

- antioxidants in rice bran. *Cereal Chemistry*, 2000.77(5), 551-55.
- Lorens, K., Dilsaver, W., & Wolt, M. Fababean flour and protein concentrate in baked goods and in pasta products. *Bakers Digest*, 1979.53, 39-42.
- McNamara, J. R., Cohn, J. S., Wilson, P. W. & Schaefer, E. J. Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. *Clinical Chemistry*, 1990.36, 36-42.
- McWatters, K. H., Ouedraogo, J. B., Resurrection, A. V. A., Hung, Y. C., & Philips, R. D. Physical and sensory characteristics of sugar cookies containing mixtures of wheat, fonio (*Digitaria exilis*) and cowpea (*Vigna unguiculata*) flours. *International Journal of Food Science & Technology*, 2003.38, 403-410.
- Molero Gómez, A. & Martínez de la Ossa, E. Quality of wheat germ oil extracted by liquid and supercritical carbon dioxide. *Journal of American Oil Chemistry Society*, 2000.77(9), 969-74.
- NAS. *Recommended Dietary Allowances*, 10th ed. National Academy Press: Washington, DC. 1989.
- O'Brien, R.D. *Fats and Oils. Formulating and Processing for Applications* (2nd edition), CRC Press, Washington, DC. 2004.
- Ostlund, R. E. Cholesterol absorption. *Current Opinions in Gastroenterology*, 2002.18, 254-58.
- Park, S., & Choi, S. B. Effects of α -tocopherol supplementation and continuous subcutaneous insulin infusion on the oxidative stress in Korean patients with type-2 diabetes. *American Journal of Clinical Nutrition*, 2002.75, 728-733.
- Przybylski, R. Canola oil: physical and chemical properties. <http://www.canola-council.org>, accessed on: April 23, 2007.2004.
- Rudan-Tasic, D. & Klofutar, C. Characteristics of vegetable oils of some slovene manufacturers. *Acta Chim. Slov*, 1999.46(4), 511-521.
- Saito, M., Yamauchi, Y., Inomata, K. & Kottkamp, W. Enrichment of tocopherols in wheat germ by directly coupled supercritical fluid extraction with semipreparative supercritical fluid chromatography. *Journal of Chromatography Science*, 1989.27, 79-85.
- Simopoulos, A. P. Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition*, 1999.70(3), 560-569.
- Singh, L. & Rice, W. K. Method for producing wheat germ lipid product. *U.S. Patent*. 1979.4,298,622.
- Steel, R. G. D., Torrie, J. H., & Dickey, D. A. *Principles and Procedures of Statistics, A biometrical Approach* (3rd Ed.). New York.: McGraw Hill Book Co. Inc. 1997.
- Zwingelberg, H., & Fretzdorff, B. Effect of microwave treatment on the keeping characteristics of food grade wheat germ. *Getredie Mehl und Brot*, 1996. 50, 214-218.