Quality Characteristics of Pan Bread Enriched With Defatted Germinated Pumpkin Seed Flour
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ABSTRACT
The present study was carried out to utilize different levels of germinated pumpkin seed flour (GPSF), as a source of protein and minerals in fortification of wheat flour for making pan bread. Organoleptic properties of prepared pan bread and its biological effect on lipids profile of rats fed on hypercholesterolemic diet were studied. Pan bread was prepared with GPSF at different levels. The results showed a significant decrease in fat content and an increase in protein, fiber contents and caloric value by increasing defatted GPSF replacement level. Biological assay indicated that food intake, protein intake, feed efficiency ratio and protein efficiency ratio of rats were not significantly affected by defatted GPSF replacement. Serum TC, TG and LDL-C were significantly decreased by replacement with 20% defatted GPSF while HDL-C increased and caused a beneficial effect in improving the lipids profile of rats fed on hypercholesterolemic diet.

Key words: Germinated pumpkin seeds, pan bread, enrichment, lipid profile, hypercholesterolemic rats.

INTRODUCTION
Germination has been shown to increase crude protein content and the levels of the protein fractions albumin and globulin of fluted pumpkin seed (Giami et al., 1999). Therefore, fluted pumpkin seed flour would be expected to be valuable for enhancement wheat flour protein, which is deficient in lysine and thus supply relatively inexpensive protein–enriched and nutritionally adequate bread. Pumpkin seeds are good source of essential fatty acids, protein, potassium, calcium, manganese and magnesium. These seeds in addition to their importance as an oil seeds (45% fat), they are a valuable source of protein (Akwaowo et al., 2000, Seo et al., 2005). Germination, a natural process which leads to the birth or evolution of a new plant is characterized by a lot of biochemical changes which have been widely hard-done by for modifying the taste, appearance and physico-chemical properties of grains and seeds (Grosch et al., 2001). It is known to supplement the release of bioactive compounds, to enhance antioxidant activity and bioavailability of essential minerals of such seeds (El-Refai et al., 2012).

Wheat flour bread represents the main source of carbohydrate for most of Egyptians. Increasing protein content of wheat flour by the addition of legumes and oil seeds (soybeans, cotton seeds, sesame, sunflower, and peanut flour) can get well the nutritional quality of bread, especially lysine content and baking characteristics, such as loaf volume and dough mixing (Mansour et al., 1999). Pumpkin seeds have high contents of dietary fiber, protein and polyunsaturated oils (Younis et al., 2000). Recently, pumpkin (Cucurbita pepo) has received great attention mainly due to the nutritional and health protective values of its seeds which are a good source of zinc (Abd El-Majied et al., 2008).

Cerqueira et al. (2008) reported that the pumpkin seed flour caused a significant decrease in the serum glucose and triacylglycerol levels of rats (Sprague Dawley). “However, fiber intake in our diet is commonly lower than recommended; therefore, the development of food with high fiber content should be eligible. The potential use of various fiber sources, as fiber enriching agents in bread making formulate, lead food scientists to believe that breads could be consumed as therapeutic for various targeted population such as hypercholesterolemia (Jinshui et al., 2002).

Pumpkin (Cucurbita moschata, variety Dickinson) seeds could be utilized successfully as good sources of edible protein (320 g/kg) and oil (450 g/kg) for human consumption, as well as animal feed. At the same time, they minimize waste pollution (Lazos, 1986). There is a need to find other food applications for fluted pumpkin seed flour. One such application could include its use in composite flours, thereby animating growth and use of this indigenous crop for the production of high protein bread.
The present study was conducted to evaluate the effect of partial replacement of wheat flour by germinated pumpkin seed flour on the organoleptic properties, chemical composition and mineral contents of pan bread, as well as lipids profile and other tested parameters in hypercholesterolemic rats (Sprague Dawley).

MATERIALS AND METHODS

Materials:

Pumpkin seeds (*Cucurbita moschata*, variety Dickinson) were obtained from the Agricultural Research Center, Giza, Egypt.

Bread ingredients including wheat flour (72% extraction), compressed baker’s yeast, salt, sugar, vegetable shortening) were purchased from the local market in Kafrelsheikh Governorate, Egypt.

Male albino rats of Sprague Dawley strain were obtained from the Agricultural Research Center, Giza, Egypt. Cholesterol powder was obtained from Morgan Co. Cairo, Egypt.

Methods:

Preparation of germinated pumpkin seed flour (GPSF):

Pumpkin seeds were cleaned to remove broken seeds and foreign materials and were germinated on wet cotton wool at room temperature (28 ± 1°C) for 72 hr. The cotton wool was watered with distilled water at regular intervals of 12 hr (Giami et al., 1999). Germinated seeds, with intact pericarp, were boiled in tap water in a covered stainless steel pot for 1 hr to soften the seed coats. The seed coats were removed by using hand pressure and the cotyledons were dried (60°C, 24 hr) in a hot-air fan oven, then ground using a laboratory mill and screened through a 0.25 mm sieve. Flours obtained from germinated fluted pumpkin seeds (GPSF) were defatted by using n-hexane in a Soxhlet apparatus (Tecator Inc., Colorado, USA) for 8 hr. The defatted GPSF were spread on aluminum trays and dried in a hot-air fan oven (70°C, 30 min) to remove hexane residue and stored in air-tight plastic containers at 4°C until used.

Preparation of pan bread:

Blends containing 0, 5, 10, 15 and 20% of defatted GPSF replacing wheat flour (WHF) were prepared by gradual mixing of defatted GPSF with wheat flour in rotary mix dough. Flour blends were prepared using the straight-dough method according to Chauhan *et al.* (1992) with minor modifications. The dough formula was 500g of flour blend, 9g of compressed baker’s yeast, 5g of NaCl, 13g of cane sugar, 10g of vegetable shortening and approximately 280 ml of water or (as required). All the ingredients were mixed in a Kenwood mixer (Model A 907 D) for 3.5 min. The dough was fermented for 90 min at 28 ± 1°C, and then punched, scaled to 250 g dough pieces, proofed for 90 min at 30°C, 85% relative humidity and baked at 250°C for 30 min.

Organoleptic properties of pan bread:

Organoleptic properties were performed by 20 graduate students and staff members in Home Economic Dept. Fac. of Specific Education, Kafrelsheikh Univ., Egypt. Pan bread was evaluated for appearance, colour, flavour, texture and overall acceptability according to Peter (2004).

Analytical methods:

Gross chemical composition of pan bread:

Moisture, crude protein, ash and ether extract contents were determined according to the method of AOAC (2000). Crude fiber content was determined as described by Kirk & Sawyer (1991). N-Free extract content was calculated by difference.

Caloric value was calculated from the sum of the percentages of crude protein and total carbohydrates multiplied by a factor of 4 (kcal.g⁻¹) plus the crude fat content multiplied by 9 (kcal. g⁻¹), according to Zambrano *et al.* (2004).

Minerals content was determined by dry ashing according to procedure of the (AOAC, 2000). Potassium, calcium, sodium, magnesium, iron and zinc were determined using an atomic absorption (Model 2380, USA). Phosphorus was determined by the molybdo vanadate method using the procedure of the AOAC (2000).

Biological Assay:

Animals and experimental design:

Male albino rats of Sprague Dawley strain (n =9), weighing (100-110g) were kept under hygienic conditions for one week acclimation period. Rats were fed on a standard casein diet according to
Reeves et al. (1993) the water was supplied at libidum under hygienic conditions.

Hypercholesterolemic rats:

For inducing hypercholesterolemia, the diet was prepared from the following ingredients per 100g according to Rashwan (1998): Fat (10%) as corn oil, sucrose (10%), mineral mixture (4%), vitamin mixture (1%), choline chloride (0.2%) cholesterol powder (1.5%), natural casein 16.28 g (protein content 12%) and corn starch up to 100g (Campbell, 1963). After the adaptation period, the preliminary body weights were initially recorded and rats were then divided into 5 experimental groups (9 rats each):

G1: Negative control, rats fed on basal diet;
G2: Positive control, rats fed on hypercholesterolemic diet;
G3: Rats fed on the control pan bread;
G4: Rats fed on hypercholesterolemic diet + pan bread with defatted GPSF (15%);
G5: Rats fed on hypercholesterolemic diet + pan bread with defatted GPSF (20%)

During the experimental period (42 days), rats were weekly weighed. Food intake, body weight gain (BWG) and food efficiency ratio (FER) were calculated at the end of the experiment according to Chapman et al. (1959).

Hematological and biochemical analysis:

At the end of the experiment periods, all rats were fasted overnight, anaesthetized by diethyl ether and sacrificed. Blood samples were collected from the medial canthus of the eye according to the technique of Sanford (1954). The 1st blood sample (1 ml) was collected in a dry clean tube containing heparin as anticoagulant and used for hematological parameters. The 2nd one (5 ml) was drawn and left to clot in a clean dry test tube then centrifuged at 3000 xg for 20 min to separate serum which kept at -20°C for biochemical analysis. Hemoglobin concentration (HB), hematocrit level (HT), red blood cell (RBCS) and white blood cell (WBCS) count were determined using animal blood counter hematology analyzer (Roche Diagnostic system, ABX Hematology, COBAS Microso OTAutomated Hematology Analyzer, RAE 013A Instrument) for the in vitro diagnostic testing of whole blood specimens. Collected sera were used for the determination of total cholesterol (TC), triacylglycerols (TG) and high-density lipoprotein cholesterol (HDL-C) (Fassati & Prencipe, 1982).

Low density lipoprotein cholesterol (LDLC) was calculated as described by Lee & Nieman (1996) as follows:-

\[
LDL = (TC) - (HDL-C+VLDLC)
\]

while \[VLDLC = TG/5\].

Atherogenic index (AI) was calculated using the following equation as described by Kawase et al. (2000).

\[
AI = (TC - HDL-C) / HDL-C.
\]

Statistical analysis:

Statistical analysis was performed with V12 (IBM Corp.; Armonk, NY, USA) software. Statistical significance was determined in (ANOVA). A significant difference was assumed at a P value of < 0.05. Data are expressed as mean ± standard deviations (SD)

RESULTS AND DISCUSSION

Organoleptic properties

The effect of wheat flour replacing with different levels (5, 10, 15 and 20%) of defatted GPSF on the quality of pan bread is shown in Table (1). The addition of 10, 15 and 20% defatted GPSF showed no significant (P>0.05) difference among the prepared bread for taste, colour, texture, appearance and overall acceptability. On the other hand, there was a significant (P<0.05) difference between the control bread and that containing 5% defatted GPSF for all properties except odour, this may be due to the presence of high percentage of defatted GPSF and consequently raising the flavour components present in defatted GPSF. Evaluation of the functionality of the seed flour in bread making showed that up to 10% of wheat flour could be replaced with fluted pumpkin seed flour to get acceptable bread (Giami, 2000).

The panelists considered that pan bread prepared with addition of 15 and 20% defatted GPSF were acceptable because they have achieved higher grades in taste, appearance and overall acceptability, and no significant differences were found between the three mentioned properties. Therefore, the produced pan bread containing 15 and 20% defatted GPSF were subjected to chemical and biological evaluation in the current study.
Gross chemical composition of pan bread containing defatted GPSF.

Chemical composition of pan bread containing different levels of defatted germinated pumpkin seed flour is presented in Table (2). Protein content of pan bread containing defatted GPSF was significantly (P<0.05) higher than that of the control, and it increased markedly with increasing the replacement ratio (from 10.42% in the control to 18.76% in pan bread containing 20% defatted GPSF). In addition, the crude fiber and ash contents of pan bread containing defatted GPSF were significantly (P<0.05) higher than that of the control pan bread. In consistence, previous studies have also shown that pumpkin seed has a high content of dietary fiber, and protein (Esuoso et al., 1998, Younis et al., 2000).

Therefore, fluted pumpkin seed flour would be expected to be valuable for supplementing wheat flour protein, which is deficient in lysine and thus provide relatively inexpensive protein–enriched and nutritionally adequate bread (Akwaowo et al., 2000, Seo et al., 2005). In contrast to protein and fiber, the fat and N-free contents were significantly (P<0.05) lower than the control. The lowest level of N-free extract was observed in pan bread containing 20% defatted GPSF. Importantly, both fat and carbohydrate content decreased with increasing the levels of defatted GPSF. Also, all pan bread containing defatted GPSF had higher values of protein, ash and fiber, but lowest values of fat, N-free extract and energy than that of the control.

The results also showed that elevating the defatted GPSF replacement level significantly (P>0.05) decreases the caloric value of pan bread from 399.12 Kcal/100g for the control to 365.07 Kcal /100g for 20 % GPSF replacement level. The low caloric constituents may be due to the increase protein and decrease fat and carbohydrates level. The results obtained in the present study are in agreement with Giami et al. (2003) who found that fluted pumpkin seed flour is favourable for fortification of wheat flour to increase the nutrient content of the resultant bread.

Mineral contents of pan bread containing defatted GPSF

The results in Table (3) show that pan bread prepared with defatted GPSF has a significantly (P<0.05) higher levels of all minerals as compared to the control; except for magnesium and phosphorus. Moreover the minerals contents of pan bread were found to increase, markedly with increasing the substitutions ratio. A similar increasing in calcium, sodium, potassium and phosphorus contents of bread were reported by Kailasapatty et al. (1985) when wheat flour was fortified with 10% winged bean flour. Pumpkin seeds are good source of potassium, calcium, manganese and magnesium.

**Table 1: Organoleptic properties of pan bread containing defatted GPSF**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Pan bread</th>
<th>Taste</th>
<th>Colour</th>
<th>Odour</th>
<th>Texture</th>
<th>Appearance</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control bread</td>
<td></td>
<td>3.00 ± 1.17 ab</td>
<td>4.00 ± 1.12 a</td>
<td>3.20 ± 1.20 c</td>
<td>4.10 ± 0.97</td>
<td>3.55 ± 1.47 a</td>
<td>3.40 ± 0.88 c</td>
</tr>
<tr>
<td>Bread containing 5% defatted GPSF</td>
<td></td>
<td>2.75 ± 1.21 b</td>
<td>3.80 ± 1.15 ab</td>
<td>3.55 ± 0.89 b</td>
<td>3.70 ± 1.17 ab</td>
<td>3.35 ± 1.31 a</td>
<td>3.60 ± 1.05 bc</td>
</tr>
<tr>
<td>Bread containing 10% defatted GPSF</td>
<td></td>
<td>3.30 ± 1.13 ab</td>
<td>3.60 ± 0.99 bc</td>
<td>4.00 ± 0.92 a</td>
<td>3.75 ± 1.12 ab</td>
<td>3.65 ± 1.04 a</td>
<td>4.20 ± 0.77 ab</td>
</tr>
<tr>
<td>Bread containing 15% defatted GPSF</td>
<td></td>
<td>3.80 ± 1.15 a</td>
<td>3.15 ± 0.99 c</td>
<td>3.60 ± 1.14 b</td>
<td>3.95 ± 0.94 a</td>
<td>4.00 ± 1.38 a</td>
<td>4.30 ± 1.08 a</td>
</tr>
<tr>
<td>Bread containing 20% defatted GPSF</td>
<td></td>
<td>3.30 ± 1.34 ab</td>
<td>3.10 ± 1.02 c</td>
<td>3.25 ± 1.52 c</td>
<td>3.55 ± 1.23 b</td>
<td>4.00 ± 1.21 a</td>
<td>4.15 ± 0.81 ab</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD

Mean values in each column having different superscript (a, b, c, d…..) are significantly different at P<0.05.
Table 2: Gross chemical composition of pan bread containing defatted GPSF (on dry weight basis g/100g)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Control pan bread</th>
<th>Pan bread containing 5% defatted GPSF</th>
<th>Pan bread containing 10% defatted GPSF</th>
<th>Pan bread containing 15% defatted GPSF</th>
<th>Pan bread containing 20% defatted GPSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>12.97±0.90a</td>
<td>12.36±0.50a</td>
<td>12.35±1.00a</td>
<td>11.84±0.80a</td>
<td>11.72±0.60a</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>10.42±1.00d</td>
<td>11.91±0.97cd</td>
<td>13.98±0.89c</td>
<td>15.85±0.78</td>
<td>18.76±0.67a</td>
</tr>
<tr>
<td>Fat</td>
<td>11.12±0.30d</td>
<td>9.07±0.50b</td>
<td>8.80±0.70b</td>
<td>7.22±0.40b</td>
<td>6.43±0.25c</td>
</tr>
<tr>
<td>Ash</td>
<td>2.12±0.12d</td>
<td>2.36±0.10d</td>
<td>2.47±0.20e</td>
<td>3.21±0.19b</td>
<td>3.58±0.15c</td>
</tr>
<tr>
<td>Fiber</td>
<td>1.15±0.05d</td>
<td>1.26±0.07c</td>
<td>1.34±0.12bc</td>
<td>1.39±0.11ab</td>
<td>1.47±0.12a</td>
</tr>
<tr>
<td>N-free extract**</td>
<td>64.34±4.00b</td>
<td>63.04±6.00ab</td>
<td>61.06±5.00ab</td>
<td>60.49±3.00ab</td>
<td>58.04±5.00a</td>
</tr>
<tr>
<td>Kcal/100g</td>
<td>399.12±46.00a</td>
<td>379.80±54.00c</td>
<td>379.80±54.00c</td>
<td>370.34±38.00d</td>
<td>365.07±51.00c</td>
</tr>
</tbody>
</table>

**By difference

Each value is the mean ± SD

Mean values in each column having different superscript (a, b, c, d…..) are significantly different at P<0.05.

Table 3: Mineral composition of pan bread containing defatted GPSF (On dry weight basis mg/100g)

<table>
<thead>
<tr>
<th>Minerals (mg/100g)</th>
<th>Pan bread containing 5% defatted GPSF</th>
<th>Pan bread containing 10% defatted GPSF</th>
<th>Pan bread containing 15% defatted GPSF</th>
<th>Pan bread containing 20% defatted GPSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>105.24±12 c</td>
<td>113.68±20c</td>
<td>140.91±15b</td>
<td>176.58±16a</td>
</tr>
<tr>
<td>Ca</td>
<td>36.22±6.00b</td>
<td>39.16±3.00a</td>
<td>56.53±4.00a</td>
<td>58.07±2.00a</td>
</tr>
<tr>
<td>Na</td>
<td>285.40±40.00c</td>
<td>403.13±50.00b</td>
<td>469.20±45.00b</td>
<td>502.61±36.00a</td>
</tr>
<tr>
<td>Mg</td>
<td>89.31±7.00a</td>
<td>92.34±9.00a</td>
<td>96.08±5.00a</td>
<td>97.18±6.00a</td>
</tr>
<tr>
<td>Fe</td>
<td>1.56±0.80c</td>
<td>1.98±0.60b</td>
<td>2.16±1.00b</td>
<td>2.9±0.40b</td>
</tr>
<tr>
<td>Zn</td>
<td>1.79±0.11c</td>
<td>2.02±0.15c</td>
<td>2.34±0.20b</td>
<td>2.65±0.14a</td>
</tr>
<tr>
<td>P</td>
<td>237.08±25.00c</td>
<td>256.32±30.00c</td>
<td>274.12±32.00c</td>
<td>281.53±27.00c</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD

Mean values in each column having different superscript (a, b, c, d…..) are significantly different at P<0.05.

Biological evaluation of hypercholesterolemic rats

Data presented in Table (4) show the effect of feeding and growth parameters of hypercholesterolemic rats. The initial weight, final weight, body weight gain (g), food intake and feed efficiency ratio of rats fed on diets containing 15 and 20% defatted GPSF were estimated to follow up the healthy...
feed parameters during the experimental period. The results in Table (4) show that the weight gain (g) of rats ranged from 43.65 g to 55.35 g for G1 to G5. while the body weight gain decreased for the positive control G2 -19.43 g. The rat group fed on defatted GPSF 20% had the highest body weight gain (55.35 g). The feed consumption was unchanged in case of cholesteromic rats as well as the normal control group rats. For feed daily intake, protein intake, feed efficiency ratio and protein efficiency ratio, it was clear that it increased in all treated groups compared with the control positive group. The results show a significant (P≤0.05) different between the two control groups and rat fed on defatted germinated pumpkin seed flour diets.

### Table 4: Feeding and growth parameters of hypercholesterolaemic rats fed on pan bread containing defatted GPSF

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Variables</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight Gain (g)</th>
<th>Food Intake (g)</th>
<th>Protein Intake</th>
<th>Food efficiency ratio (FER)</th>
<th>Protein efficiency ratio (PER)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td></td>
<td>102.60± 0.02</td>
<td>146.25± 0.02</td>
<td>43.65± 0.02</td>
<td>4.90± 0.02</td>
<td>1.36± 0.02</td>
<td>2.92± 0.02</td>
<td>32.09± 0.02</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>105.58± 0.02</td>
<td>146.25± 0.02</td>
<td>-19.43± 0.02</td>
<td>9.55± 0.02</td>
<td>1.31± 0.02</td>
<td>1.55± 0.02</td>
<td>-14.35± 0.02</td>
</tr>
<tr>
<td>Control pan bread</td>
<td></td>
<td>103.30± 0.02</td>
<td>146.25± 0.02</td>
<td>51.20± 0.02</td>
<td>15.13± 0.02</td>
<td>1.49± 0.02</td>
<td>3.25± 0.02</td>
<td>34.36± 0.02</td>
</tr>
<tr>
<td>Pan bread containing 15%</td>
<td></td>
<td>103.72± 0.02</td>
<td>146.25± 0.02</td>
<td>52.43± 0.02</td>
<td>16.80± 0.02</td>
<td>1.49± 0.02</td>
<td>3.12± 0.02</td>
<td>35.19± 0.02</td>
</tr>
<tr>
<td>Pan bread containing 20%</td>
<td></td>
<td>104.17± 0.02</td>
<td>146.25± 0.02</td>
<td>55.35± 0.02</td>
<td>17.16± 0.02</td>
<td>1.52± 0.02</td>
<td>3.23± 0.02</td>
<td>36.41± 0.02</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of nine replicates.

Values in the same column with the same letter are not significantly different at P ≤0.05  G1:Negative control fed on basal diet  G2: Positive control fed on basal diet + cholesterol

BWG = Final weight – Initial weight  FER = Body weight gain (g/day) / Food intake (g/day)

Serum lipid profile of rats fed on pan bread containing defatted GPSF.

Serum lipid profile for hypercholesterolemic rats fed on the control and treated pan bread containing defatted GPSF are presented in Table (5). The values of total cholesterol(TC), triacylglycerols (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol VLDL-C, and atherogenic index (AI), were significantly higher in hypercholesterolemic rat positive control as compared with the negative control group. In contrast, the values of high density lipoprotein cholesterol (HDL-C) in the positive control were not significantly lower than the negative control group. Meanwhile, hypercholesterolaemic rats fed on pan bread containing defatted GPSF showed a significant improvement in all the tested lipids parameters as compared with the control positive group. In consistence, Cerqueira et al. (2008) also found a significant reduction in the serum triacylglycerol levels after feeding on pumpkin seed flour. Several pharmacological properties have been reported for different species of pumpkin including anti-oxidant, lipid-lowering, hepatoprotective (Makni et al., 2008).

### Hematological parameters

Hemoglobin (Hb) and red blood cells (RBCs) parameters were significantly lower in the positive control group than the negative control group (Table 6). However, hematocrit (Ht) and white blood cells (WBCs) were not significantly deferent in the two groups. Feeding on the control pan bread exerted a significant (P<0.05) increase in all tested haematological parameters as compared with the positive control.

On the other hand, rat groups fed on pan bread
Table 5: Serum lipid profile of rats fed on pan bread containing defatted GPSF

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Variables</th>
<th>Cholesterol (mg/dl)</th>
<th>T.G (mg/dl)</th>
<th>HDLC (mg/dl)</th>
<th>LDLC (mg/dl)</th>
<th>VLDLC (mg/dl)</th>
<th>Athrogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td></td>
<td>75.14±2.07a</td>
<td>103.72±1.00a</td>
<td>30.30±1.40a</td>
<td>24.10±3.80a</td>
<td>20.74±2.00a</td>
<td>1.48±0.27c</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>188.30±1.23a</td>
<td>177.36±0.97a</td>
<td>28.36±1.00a</td>
<td>124.47±4.67a</td>
<td>35.47±1.54a</td>
<td>5.64±0.80c</td>
</tr>
<tr>
<td>Control pan bread</td>
<td></td>
<td>134.43±1.02b</td>
<td>143.34±1.18c</td>
<td>28.41±1.24b</td>
<td>77.35±4.31b</td>
<td>28.67±1.00b</td>
<td>4.05±0.64b</td>
</tr>
<tr>
<td>Pan bread containing 15% defatted GPSF</td>
<td></td>
<td>115.32±1.86c</td>
<td>122.04±1.22c</td>
<td>32.41±1.35c</td>
<td>58.50±3.10c</td>
<td>24.41±1.61c</td>
<td>2.56±0.41c</td>
</tr>
<tr>
<td>Pan bread containing 20% defatted GPSF</td>
<td></td>
<td>91.60±1.96d</td>
<td>103.06±1.14d</td>
<td>38.40±1.20d</td>
<td>32.59±2.86d</td>
<td>20.61±1.30d</td>
<td>1.39±0.30d</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of nine replicates.
Mean values with the same letter are not significantly different (P<0.05).
T.G, triacylglycerols; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein

Table 6: Effect of pan bread containing defatted GPSF on hematological parameters in hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Parameters*</th>
<th>Hb (g/dl)</th>
<th>Ht (%)</th>
<th>RBCs 10^6</th>
<th>WBC 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td></td>
<td>12.510±0.862b</td>
<td>34.89±5.86c</td>
<td>6.792±0.231a</td>
<td>11.470±0.168a</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>11.845±0.898c</td>
<td>35.46±5.32c</td>
<td>5.100±1.122c</td>
<td>10.780±0.165a</td>
</tr>
<tr>
<td>Control pan bread</td>
<td></td>
<td>13.955±0.19ab</td>
<td>41.67±3.41ab</td>
<td>5.911±0.886ab</td>
<td>9.464±0.141bc</td>
</tr>
<tr>
<td>Pan bread containing 15% defatted GPSF</td>
<td></td>
<td>14.817±1.120a</td>
<td>42.97±2.50a</td>
<td>5.786±0.742ab</td>
<td>10.95±0.150b</td>
</tr>
<tr>
<td>Pan bread containing 20% defatted GPSF</td>
<td></td>
<td>14.381±0.793a</td>
<td>43.07±3.84a</td>
<td>5.295±0.164b</td>
<td>8.958±0.128c</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD
Mean values in each column having different subscript (a, b, c, d.) are significantly different at p < 0.05
Hb= Hemoglobin      Ht= Hematocrit      RBC= Red blood cells      WBC= White blood cells

containing defatted GPSF recorded high significant increase in Hb and Ht (%) as compared with the negative and the positive control groups, while there was a significant drop in RBCs and WBCs count as compared with the negative control groups. These results disagreed with those obtained by Akaliane et al. (1986), who reported that rats given cholesterol in the diet for 11 weeks had a decrease in hematocrit level after about 2 weeks of feeding.

CONCLUSION

From the obtained results, it can be concluded that germination process of pumpkin seeds improved the bioactive compounds, and bioavailability of essential minerals which subsequently improve the quality attributes of bread containing defatted germinated pumpkin seed flour up to 15% instead of wheat flour. The addition of defatted GPSF to pan bread...
resulted in an improvement in all nutritional and biological parameters of hypercholesterolemic rats.

REFERENCES


