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Research Article

Chemical, Technological and Biological Evaluation of Raw and Germinated Flax and Pumpkin Seed Mixtures

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ABSTRACT

Background: Flax seeds are rich in linolenic acid (omega-3) which possess antioxidant, anti-inflammatory, and anti-carcinogenic effects. Pumpkin seeds are good sources of Omega 6 fatty acids which have antioxidant, anti-inflammatory and hypolipidemic effects. Seed germination, as a method of food processing, improves its nutrient value.

Objectives: to investigate some biological and biological effects of complementary foods of mixed raw and/or germinated flax and pumpkin seeds on rats.

Methods: In a 35-day experimental study, five groups (5 rats/ each) of male albino rats were fed on basal control diet [control group] and 4 combined mixtures of raw flax and pumpkin seeds (FNPN) and/or germinated flax and pumpkin seeds (FGPG) [experimental groups]. The (FNPN)/(FGPG) mixtures were chemically analyzed. Rats were biologically and histologically investigated as well.

Results: Crude ether extract, protein & fibers; nitrogen free extract; minerals (except Mg); β-carotene and riboflavin contents were significantly higher in FGPG mixture compared to others. The sensory properties of the biscuits and cakes products in terms of colour, texture, taste, odour and overall acceptability were not significantly different in biscuits compared to the control. However, in case of cakes, there were slight significant differences but still accepted by the panelist. Additionally, FNPN was the best in LDL-ch, TG and Tch values, respectively (2.67, 37.00 and 50.00 mg/dl). There was no significant difference between different groups in the ratio of HDL-ch/LDL-ch. But the FGPN and FNPN, respectively recorded the lowest values of AIP (31.67 and 39.33 IU/L, respectively) and ALT (228.00 and 232.33 IU/L, respectively), as liver function indicators, whereas, the FNPG and FGPG groups were the best in creatinine (0.427 and 0.440 mg/dl, respectively) and urea (32.00 and 46.33 mg/dl, respectively) parameters compared to others. Finally, the histobathological effects on the liver tissues of rats revealed that FGPG had beneficial effects for the liver compared with the other mixtures which showed multiple focal areas of hepatic necrosis, some vacuolations in the intra-cytoplasm and enlargement in some hepatocytes. .

Conclusion: Using germinated mixtures of flax and pumpkin seeds for fortifying some food products seemed to produce good beneficial effects on the nutritional and biological parameters.

Keywords: *Flax seed, pumpkin seed, germination*

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INTODUCTION

Flax seeds (*Linum usitatissimum*) and pumpkin seeds (*Cucurbita pepo*) are becoming new compounds of the traditional healthy food especially in North African countries [(1)] and the knowledge of the chemical composition of seeds is essential because they are considered as an important source of food for both people and animals, source of the useful raw materials for various industrial purposes, and reserve nutrients and growth substances for seed germination.[(2)] Flax seeds contain high levels of Omega-3 fatty acid, fiber components such as lignans.[(3)] Attributed the main physiological

benefits of flax seeds to their high linolenic acid (omega-3) content lignans and phenolic compounds which contributes to their antioxidant properties against various diseases, including atherosclerosis, diabetes and, hypertension, anti-inflammatory, and anti-carcinogenic effects.[4] They also contain high quality digestible proteins. Therefore flaxseed is emerging as an important functional food. It possess healthy chemo-protective properties in animals and humans.[5]

The pumpkin seeds are excellent sources of protein (25.2–37%), vitamins and oil (37.8–45.4%) especially Omega 6 fatty acids which have a significant antioxidant activity, an anti-inflammatory and hypolipidemic effects.[4] In some Arabian countries pumpkin seeds utilize as a snacks after salting and roasting. The roasting can be used to enhance the utilization of fluted pumpkin seeds flour as a functional ingredient in food system.[6] Raw dried and germinated fluted pumpkin seeds flour at low pH 2.0 or high and alkaline pH 9-10, where protein solubility is desired, it may be useful in preparing vegetable milk and food emulsion. The seeds are also recommended to add in diet low in Zn.[7] Genarally food processing of seeds (soaking or germination) improves their nutritional value.[8] The main physiological benefits of flaxseeds are attributed primarily to the high linolenic acid content which contributes to their antioxidant properties [9], anti-inflammatory, and anti-carcinogenic effects.[10] Also pumpkin has been grown for the purpose of consumption as vegetable or as medicine. Pumpkin seeds are in great demand for their pharmacological effects. Pumpkin seed oil also confers many health benefits and it can prove as a potential source of functional foods.[11]

In Egypt, malnutrition is one of the major nutritional problems. This problem is due to an inappropriate feeding practice, low nutritional quality of traditional foods and high cost of food quality. Therefore this study was undertaken to formulate four mixtures from raw and germinated, these mixtures used as a functional ingredients during preparing biscuit and cake products and to investigate some biological and histological effects of flax and pumpkin seeds on rats.

METHODS

1-Raw materials and chemicals: The dried flax seeds, pumpkin fruits, Wheat flour (72% extraction ratio), sucrose, butter, whole eggs, sodium and ammonnium bicarbonate and dry milk were purchased from local market in Alexandria, Egypt. The pumpkin seeds were obtained from their fruits, the resulted seeds were washed under run tap water, drained and left to dry at room temperature. Chemicals used in this study were analytical grade and purchased from Lab Services Company, Alexandria, Egypt.

Adults male albino rats were obtained from the Institute for Graduate Studies and Research, Alexandria University, Egypt**.**

Casein, corn oil, sucrose and maize starch were obtained from local market in Alexandria, Egypt.

Vitamin mixture and salts were purchased from local Pharmacy (veterinary department), Alexandria, Egypt.

2-Technological methods:

A- Germination of seeds: The dried seeds of each flax and pumpkin were soaked in distilled water a ratio of 1:5 w/ v seed/ water for 12 hrs at room temperature. The resulted soaked seeds were rinsed twice with distilled water before germination on wet cotton layers and covered with wet cotton cloth at room temperature (22±3°C) for 7 and 14 days for flax and pumpkin soaked seeds respectively, while access to hung seeds to germination case. [12] The germinated seeds were rinsed with distilled water then dried at 50°C in hot air oven for 12 hrs ,then ground to 60 mesh using electric grinder .

B-Preparation of seed mixtures: Four mixtures of raw and germinated seeds of flax and pumpkin at 1:1 (w/w) ratio as following; (1) raw flax and pumpkin seeds (FNPN), (2) germinated flax and pumpkin seeds (FGPG), (3) germinated flax and raw pumpkin seeds (FGPN) and (4) raw flax and germinated pumpkin seeds (FNPG). The formulated mixtures were packed in glass jars and stored at refrigerator temperature (4±1°C) until used.

C-Preparation biscuits and cakes: 15% of the wheat flour required for preparing biscuits and cakes were replaced by each of above seeds mixtures .Biscuits and cakes free and containing each seeds of above seeds mixtures were prepared.[13]The biscuit dough was shaped into round pieces then baked at 190 °C for 12 min in an electrical baking oven, cake was baked in aluminum foil cups at 180°C for 30 min at electrical baking oven .

3-Chemical analysis:

The standard procedures of Association of Official Analytical Chemists were used in triplicate to estimate moisture, crude ether extract, crude protein, total ash, crude fibers and the nitrogen free extract content was calculated by the differences (summing all the

previous values and the result subtracted from one hundred). Minerals (Fe, Ca, Mg, Mn, Cu and Zn) were determined by atomic absorption spectrophotometer (Perkin –Elmer, 2380).[14] Fatty acids were estimate using Shimadzu gas chromatograph (GC-4 cm, PFE) and a standard mixture of methyl esters.[15] The ratio of saturated to unsaturated fatty acids and omega-6 to omega-3 to omega-9 were calculated from the results of fatty acids analysis.

Protein of each seed mixture was extracted from their defatted meals with 0.025 M triglycine buffer of pH8.3using 1:20w/v sample to buffer solution ratio. After hydration overnight at refrigerator temperature(4±1°C) ,the mixture was centrifuged at 4000 rpm for 30 min, the volume of supernatant was performed on a discontinuous buffered system using 12% separating gel and 4% stacking gel. The protein samples were solubilized in 0.5M Tris–HCl buffer (pH 6.8), containing 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol (2-ME), 10% (v/v) glycerol and 0.002% (w/v) bromophenol blue, and heated for 3 min in boiling water before electrophoresis. For each sample, 10 µL was applied to each lane. After the electrophoresis, solution of 1% Coomassie blue (R-250) in water was used for staining the gel and then mixture from methanol: acetic acid: water, 2:3:5 v/v/v, respectively for distaining.[16]

Both β-carotene and riboflavin (vitamin B_2) were analyzed using reversed phase HPLC reverse-phase HPLC technique, A Hewlett Packard HPLC Series 1100, USA equipped with degasser, quaternary pump, auto-sampler and diode array detector was used.[17]

4-Sensory evaluation

The colour, consistency, flavour, appearance and overall acceptability of the prepared biscuits and cakes were subjected to sensory evaluation. Using ten trained panelists from Food and Science Technology Department, faculty of Agriculture, Alexandria, Egypt. Those were asked to evaluate the samples using numerical scoring test.[18]

5-Biological evaluation

A- Experimental design: Twenty five of male Albino rats (weighing 90–110 g) were maintained under standard laboratory conditions (22±3°C, 12-h light/dark cycle), during 35days of experimental period. This study was conducted on five groups (each group 5 rats): control group was fed on basal control diet (10% casein, 10% corn oil, 23.5% sucrose, 47% maize starch, 5% fiber, 2% salt mixture and 1% vitamin mixture).[19-20] FNPN ,FGPN ,FNPG and FGPG rat groups were fed on basal diet with 15% substitution of prepared raw and germinated flax and pumpkin seed mixtures (this ratio of substitution was the best ratio used for obtaining accepted biscuits and cakes).

B- Blood sampling and animal organs weight: The blood samples were taken once at the end of experiment after sacrificed. Each sample was placed in a dry and clean centrifuge tube, half of samples allowed to clot (undisturbed) for 1-2 hr at 37°C .Serum was then removed using a Pasteur pipette and centrifuged for 10 min. at 300 r.p.m.to remove any suspended red blood cells.

The clean non haemolysed supernatant serum was then pipetted into 1.5 ml tube and kept frozen until analysis .The other half of the samples was mixed with heparin as a blood anti-coagulant to determine the blood picture in it. Liver, kidney, heart, tests, lung and spleen were collected from sacrificed rats and recorded the weight of these organs. The liver organs were kept in 10% formalin until histological assay performed.

C- Blood picture analysis: Hemoglobin (Hb), red blood cell count (RBCs), white blood cell count (WBCs), hematocrit (Ht), mean cell volume (M.C..V.), mean corpuscular hemoglobin (M.C.H.), and mean cell hemoglobin concentration (M.C.H.C.) were determined.[21]

D- Lipids in serum analysis: Serum lipid parameters such as the low-density lipoprotein-cholesterol (LDL-C), High-density lipoprotein-cholesterol (HDL-C) triacylglycerol (TG) and total cholesterol (TC), levels were determined by enzymatic colorimetric methods.[22]

F-Determination liver and kidney function parameters: alkaline phosphatase (AIP) and Alanine amino transferase (ALT) activities in serum used as biochemical markers for hepatic damage were determined by enzymatic methods.[23] Also urea and creatinine in serum used as biochemical markers for kidney function were determined.[24]

6- Histopathological examination: The liver intended for histological examination by light microscopy, was removed and immediately fixed in formalin solution, embedded in paraffin, serially sectioned at 5 µm and stained with hematoxylin-eosin (H & E) technique.[25]

Statistical analysis: Data were subjected to analysis of variance (ANOVA) multiple range test to separate

the treatment means. The analysis was computed using the SAS program.[26]

RESULTS

Chemical composition: Experimentally, it was ascertained in Table (1) that the proximate composition was 10.43-10.90% moisture, 35.31-48.49% crude ether extract, 12.16-21.66% crude protein, 4.16-4.90% total ash, 7.48-13.58% crude fibers and 11.37-40.53% nitrogen free extract content.

Mineral content: Mineral contents of Fe, Ca, Mg, Mn, Cu and Zn were 156.53-195.94, 370.26-512.36, 28.55-39.81, 21.95-34.67 and 61.11-70.28 mg/100g dwb, respectively as showed in Table (1).

Vitamin content: As shown in Table (1) the β-Carotene, which is the pro of vitamin A and anti-oxidant bio-component, was ranged from 1272.19 to 4946.22 IU/100 and the riboflavene (B2) was reached to 13.70 mg/100g in FGPG mixtures.

Electrophoretic protein patterns: The major protein bands were ranged from 48 KDa to 180 KDa in germinated samples. In raw seed mixture appeared the highest molecular weight bands (245KDa) and the very low molecular weight bands (25 and 35 KDa). The molecular weight of studied samples ranged from 25 to 245 KDa,the intensity of low molecular weights up to 75KD were more than the high molecular weights (100 to 245KDa) as shown in Fig (1).

Sensory properties: In Table (3) the organoleptic evaluation scores reported that the biscuits and cakes made from mixtures were recorded 6.3 to 6.7 for over all acceptability of biscuits but the overall acceptability of cakes were ranged from 5.6 to 6.3. And the ground seed colour after germination became darker than the raw seeds.

Table 1: Chemical composition (%), mineral (mg/100g) and vitamin contents of flax and pumpkin seed mixtures.

Components	Seed mixtures				
	FNPN	FGPN	FNPG	FGPG	LSD
Moisture*	10.43^{a} ±1.50	10.60^{a} ±0.153	10.80^{a} ±0.100	10.90^{a} ±0.600	1.53
Crude ether extract*	35.31^{b} ±1.72	36.24^{b} ±0.819	37.42^{b} ±1.48	48.49^{a} ±0.600	2.34
Crude protein* (N×6.25)	12.16^{d} ±1.12	17.34^{b} ±0.400	14.54^{c} ±0.252	21.66^{a} ±0.200	1.16
Total ash*	4.16^{c} ±0.120	4.64^{b} ±0.025	4.74^{b} ±0.030	4.90^{a} ±0.055	0.129
Crude fiber*	7.84^{c} ±0.599	10.82^{b} ±0.630	8.50^{c} ±0.385	13.58^{a} ±0.900	1.23
Nitogen* Free extract*	40.53^{a} ±1.76	30.96^{c} ±0.611	34.80^{b} ±1.47	11.37^{d} ±0.551	2.30
Fe	156.53	165.12	178.39	195.94	-
Ca	370.26	466.38	432.07	512.36	-
Mg	39.16	28.55	39.81	29.66	-
Mn	51.53	58.13	88.60	89.96	-
Cu	21.95	32.45	22.41	34.67	-
Zn	61.11	68.08	69.80	70.28	-
β-Carotene (IU/100g)	1272.19	3157.38	4283.74	4946.22	-
Vitamin B2 (mg/100g)	0.368	5.62	2.38	13.70	-

Values not sharing a common superscript within row are statistically significant at $p < 0.05$. * mean of values ± SD.

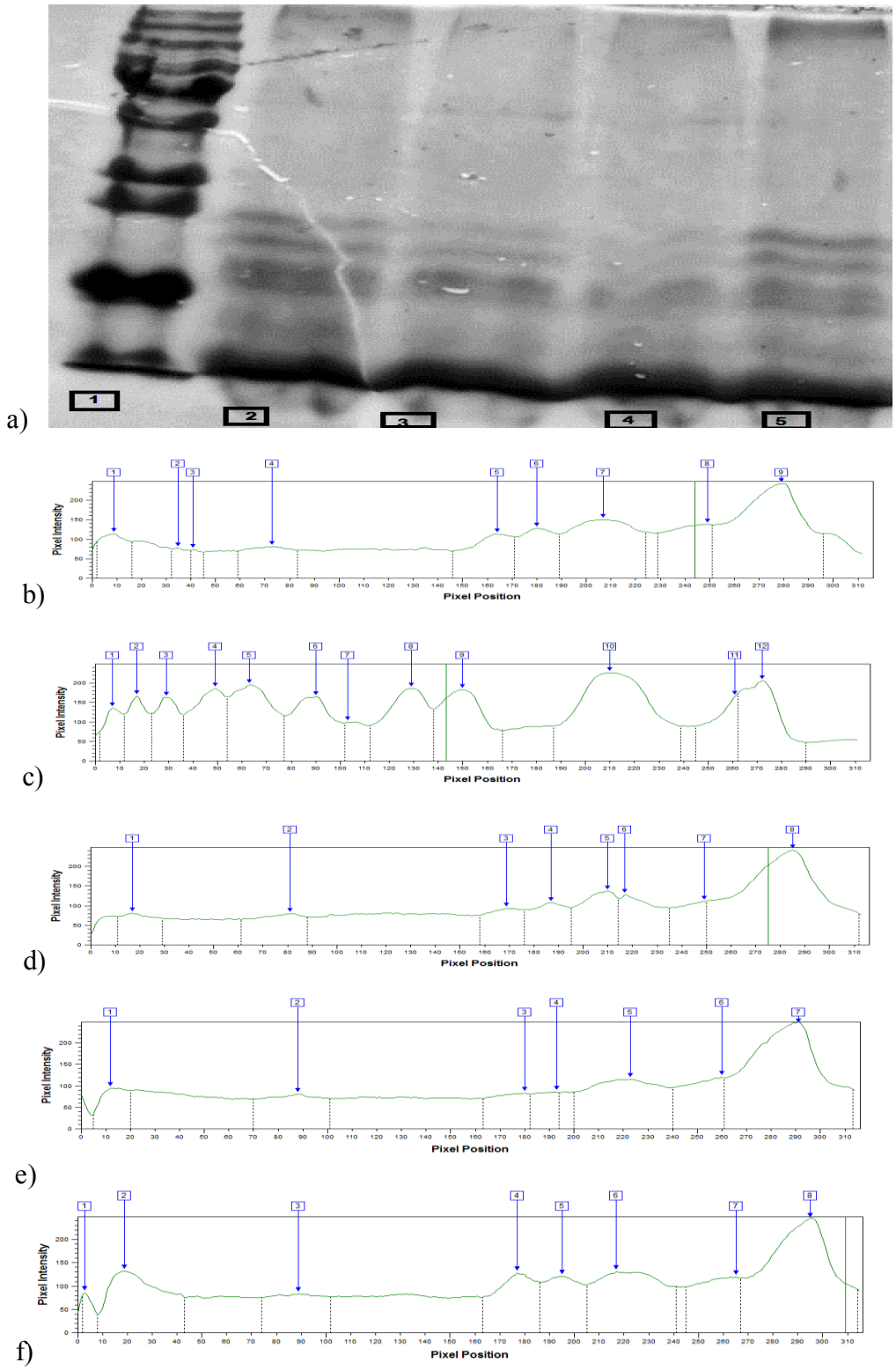

Figure 1: a) SDS-PAGE of molecular weight marker (lane 1), FNPN (lane 2), FGPG (lane 3), FGPN (lane4) and FNPG (lane5), b-f) Pixcel intensity and position of marker, FNPN, FGPG, FGPN and FNPG protein sample bands.

Table 2: Fatty acid composition of flax and pumpkin seed mixtures

Fatty acid (%)	FNPN	FGPN	FNPG	FGPG
Caproic C6:0	0.071	0.279	0.692	0.098
Caprylic C8:0	0.263	0.177	0.369	0.236
Capric C10:0	0.084	0.034	-	-
Lauric C12:0	0.153	0.107	-	-
Myristic C14:0	0.261	0.142	0.232	0.610
Pentadecanoic C15:0	0.166	0.122	0.239	0.056
Palmitic C16:0	11.69	13.04	0.229	0.457
Stearic C18:0	5.22	5.69	0.336	0.232
Arachidic C 20:0	0.174	0.209	0.112	0.117
Heneicosanoic C21:0vvcv4r	-	0.083	9.66	8.06
Saturated fatty acids	18.08	19.88	11.87	9.87
Myristoleic C14:1	0.081	0.102	0.134	0.073
Pentadecaenoic C15:1	0.132	0.197	0.170	0.109
Palmitoleic C16:1	-	0.097	0.144	0.106
Oleic C18:1	16.04	16.41	8.10	7.20
Linoleic C18:2	10.11	18.33	7.57	13.68
Linolenic C18:3	48.36	39.01	58.57	56.95
Eicosenic C20:1	0.525	0.484	0.331	0.903
Unsaturated fatty acids	75.25	74.63	75.02	79.02
Others	6.67	5.49	13.11	11.11
S:U ratio	1:4.16	1:3.75	1:6.32	1:8.01
n 3:	4.78:	2.38:	7.74:	7:.91:
n 6:	1:	1.12:	1:	1.90:
n 9 ratio	1.59	1	1.07	1

Table 3: Sensory evaluation of biscuits and cakes (substitution 15% flax and pumpkin seed mixtures

Samples		Organoleptic properties				
		Colour	Odour	Taste	Texture	Overall acceptability
Biscuit	Control	$7.2^{a}\pm0.994$	$7.3^{a}\pm0.949$	$7.3^{a}\pm0.669$	$7.2^{a}\pm1.23$	$7.2^{a}\pm1.64$
	FNPN	$6.5^{a}\pm1.27$	$6.7^{a}\pm1.06$	$6.7^{a}\pm0.850$	$6.7^{a}\pm0.675$	$5.7^{b}\pm0.949$
	FGPN	$5.9^{a}\pm1.20$	$6.7^{a}\pm0.949$	$6.7^{a}\pm0.949$	$6.9^{a}\pm0.876$	$5.3^{b}\pm1.16$
	FNPG	$5.8^{a}\pm1.93$	$6.3^{a}\pm1.27$	$6.5^{a}\pm1.35$	$6.2^{a}\pm1.55$	$6.6^{ab}\pm1.77$
	FGPG	$6.2^{a}\pm2.39$	$6.6^{a}\pm1.90$	$6.6^{a}\pm1.89$	$6.6^{a}\pm1.65$	$6.1^{ab}\pm2.27$
	LSD	1.41	1.46	1.15	1.13	1.48
Cake	Control	$8.1^{a}\pm1.85$	$7.4^{a}\pm1.75$	$7.9^{a}\pm1.75$	$6.9^{a}\pm1.85$	$7.3^{a}\pm1.64$
	FNPN	$5.3^{b}\pm1.83$	$6.50^{a}\pm1.91$	$6.5^{ab}\pm2.06$	$5.1^{b}\pm1.79$	$5.3^{b}\pm1.57$
	FGPN	$5.2^{b}\pm2.10$	$6.7^{a}\pm1.55$	$5.9^{b}\pm1.34$	$5.4^{ab}\pm1.78$	$5.2^{b}\pm1.55$
	FNPG	$6.7^{b}\pm1.16$	$6.4^{a}\pm1.43$	$5.8^{b}\pm1.26$	$6.4^{ab}\pm1.65$	$6.3^{ab}\pm1.57$
	FGPG	$6.5^{b}\pm1.27$	$6.3^{a}\pm1.03$	$6.2^{b}\pm1.66$	$6.1^{ab}\pm1.66$	$6.0^{ab}\pm1.41$
	LSD	1.51	1.10	1.48	1.58	1.40

Values not sharing a common superscript within column are statistically significant at $p < 0.05$.

Feed efficiency: According to the data listed in Table (4), the amount of the daily food intake (7.025 - 8.795 g/d) was followed by increase insignificantly in the body weight gain values (0.749 -1.03 g/d). On the other hand, the feed efficiency ratio of basal diet and experimented diet groups ranged from 0.100 to 0.200.

The relative weight of internal organs: Results in Table (5) showed that the weight of liver, lung, kidney, spleen, heart and tests ranged from 2.66 to 4.17g, 0.690 to 1.00g, 0.714 to 0.870g, 0.320 to 0.500g, 0.427 to 0.586g and from 1.49 to 2.82g, respectively among the five rat groups.

The Blood picture: The different blood parameters of the five rat groups, such as hemoglobin (10.68 -13.90 gm/dl), red blood cells count (6.9-8.54 millions /mm^3), white blood cell count (5.00 -10.88 hundred/mm^3), platelets count (468.00-562.00 millions/mm^3), haematocrit (34.60-45.40%), mean cell volume (50.36-57.57FL), mean corpuscular haemoglobin (15.45-16.73Pg) and mean corpuscular haemoglobin concentration (29.17-31.23%) were listed in Table (6).

The lipid profile: The lipid profile comprises LDL-C, HDL-C, HDL/LDL ratio, total triglycerides and total cholesterol. The data in Table (7) showed that serum LDL-C and LDL-C ranged from 2.67 to 5.20 mg/dl and 40.00 to 58.33 mg/dl, respectively in the five rat groups, the ratios of HDL-C: LDL-C ranged from 12.17 to 27.97, the total triglycerides and the total cholesterol ranged from 37.00 to 61.67 mg/dl and 50.00 to 74.00 mg/dl, respectively between the same rat groups.

Table 4: Changes in body weight gain in grams of rats feeding on basal diet or basal diet with 15% substitution flax and pumpkin seed mixtures

Groups	Initial body weight (g)	Final body weight (g)	Total food intake (g/day)	Body weight gain (g/day)	Feed efficiency ratio
Control	97.50^{a}	123.00^{a}	8.80^{a}	0.983^{a}	0.110^{a}
	±19.37	±14.14	±1.48	±0.516	±0.043
FNPN	100.00^{a}	130.00^{a}	7.72^{a}	0.821^{a}	0.120^{a}
	±27.99	±10.80	±2.76	±1.44	±0.171
FGPN	100.00^{a}	124.00^{a}	7.75^{a}	0.893^{a}	0.150^{a}
	±13.69	±5.02	±3.23	±3.29	±0.597
FNPG	87.00^{a}	116.00^{a}	7.03^{a}	1.03^{a}	0.200^{a}
	±22.53	±21.62	±2.39	±1.20	±1.11
FGPG	97.00^{a}	118.00^{a}	8.40^{a}	0.749^{a}	0.100^{a}
	±18.57	±9.75	±1.57	±0.793	±0.191
LSD	28.70	18.87	3.44	2.63	27.72

Values not sharing a common superscript within column are statistically significant at $p < 0.05$.

Table 5: Changes in relative weight in grams of some internal organs of rats feeding on basal diet or with 15% substitution flax and pumpkin seed mixtures

Samples	Relative organs weights in grams					
	Liver	Lung	Kidney	Spleen	Heart	Tests
Control	3.69^{ab}	1.00^{a}	0.87^{a}	0.346^{c}	0.586^{a}	2.36^{a}
	±0.102	±0.138	±0.066	±0.069	±0.051	±0.302
FNPN	4.09^{a}	0.860^{ab}	0.800^{ab}	0.320^{c}	0.440^{b}	1.51^{b}
	±0.600	±0.168	±0.065	±0.065	±0.041	±0.450
FGPN	2.66^{c}	0.870^{ab}	0.763^{ab}	0.500^{a}	0.450^{b}	1.56^{b}
	±0.068	±0.132	±0.045	±0.050	±0.086	±0.153
FNPG	3.33^{b}	0.690^{c}	0.830^{a}	0.400^{bc}	0.437^{b}	2.82^{a}
	±0.750	±0.032	±0.155	±0.050	±0.111	±1.09
FGPG	4.17^{a}	0.800^{bc}	0.714^{b}	0.482^{ab}	0.440^{b}	1.49^{b}
	±0.533	±0.090	±0.054	±0.099	±0.138	±0.493
LSD	0.624	0.151	0.110	0.084	0.114	0.752

Values not sharing a common superscript within column are statistically significant at $p < 0.05$.

Table 6: Changes in blood picture of rats feeding on basal diet or with 15% substitution flax and pumpkin seed mixtures

Samples	Blood picture parameters							
	Hb (gm /dl)	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Platelets (10^6/mm^3)	Haemotocrit (%)	M.C.V (FL)	M.C.H (Pg.)	M.C.H.C (%)
Control	13.90^{a}	8.54^{a}	10.88^{a}	562.00^{a}	45.40^{a}	52.10^{bc}	16.26^{ab}	30.84^{a}
	±0.758	±0.486	±1.44	±40.84	±2.51	±0.328	±0.207	±0.288
FNPN	11.47^{b}	7.29^{bc}	5.00^{c}	513.67^{ab}	37.33^{b}	51.30^{bc}	15.70^{bc}	30.60^{a}
	±1.45	±0.691	±0.934	±46.65	3.62	±0.498	±0.620	±0.853
FGPN	11.70^{b}	8.19^{ab}	5.70^{c}	552.33^{ab}	37.33^{b}	52.10^{bc}	16.27^{ab}	31.23^{a}
	±1.86	±1.49	±0.626	±1.03	±5.16	±3.10	±0.671	±0.671
FNPG	11.60^{b}	6.93^{c}	7.37^{bc}	468.00^{b}	39.67^{b}	57.57^{a}	16.73^{a}	29.17^{b}
	±0.410	±0.163	±2.98	±31.19	±1.37	±2.07	±0.539	±0.052
FGPG	10.68^{b}	6.90^{c}	8.58^{ab}	553.40^{ab}	34.60^{b}	50.36^{c}	15.45^{c}	30.60^{a}
	±2.32	±1.31	±2.70	±49.88	±7.67	±1.81	±0.451	±0.458
LSD	1.88	1.20	2.43	86.72	5.60	2.38	0.665	0.689

Values not sharing a common superscript within column are statistically significant at p <0.05.

Table 7: Changes in lipid profile of rats feeding on basal diet or with 15% substitution flax and pumpkin seed mixtures

Samples	Lipid profile parameters				
	LDL-ch mg/dl	HDL-ch mg/dl	HDL:LDL ratio	Total triglyceride mg/dl	Total cholesterol mg/dl
Control	3.60^{abc}	48.40^{bc}	27.97^{a}	38.00^{b}	59.60^{b}
	±2.51	±10.48	±26.67	±3.67	±7.89
FNPN	2.67^{c}	40.00^{c}	21.31^{a}	37.00^{b}	50.00^{c}
	±1.37	±1.55	±15.29	±1.79	±1.55
FGPN	3.00^{bc}	51.33^{ab}	20.37^{a}	61.67^{a}	66.67^{ab}
	±1.55	±6.83	±8.28	±25.88	±2.25
FNPG	5.00^{ab}	58.33^{a}	12.17^{a}	54.00^{ab}	74.00^{a}
	±0.894	±9.40	±3.93	±10.55	±9.96
FGPG	5.20^{a}	45.80^{bc}	13.59^{a}	57.80^{a}	63.50^{b}
	±2.05	±6.54	±14.04	±10.01	±10.97
LSD	2.12	9.32	18.70	17.12	9.22

Values not sharing a common superscript within column are statistically significant at p <0.05.

The liver function: The liver function enzymes (AIP and ALT) were determined for different experimented groups, the alkaline phosphatase (API) and the alanine amino transferase (ALT) activities in serum were ranged from 31.67 to 70.67 U/L and 228.00 to 262.30 U/L, respectively as shown in Table (8).

The kidney function: The creatinine and the urea were used to estimate the kidney function as shown in Table (8) of different experimented groups, the creatinine value was varied from 0.427 to 0.533 mg/dl and the urea value ranged from 32.00 to 74.33 mg/dl.

Histopathological examination: The microscopical examination of the liver (Fig 2) showed morphological differences between the different liver tissues of the experimented rat groups. The microscopical examination of liver in FNPN, FGPN and FNPG were revealed presence of multiple focal areas of hepatic necrosis, some vacuolation in the intra-cytoplasm and enlargement in some hepatocytes.

Table 8: Changes in liver and kidney functions of rats feeding on basal diet or with 15% substitution flax and pumpkin seed mixtures

Samples	Liver function enzymes		Kidney function	
	AIP(GPT) U/L	ALT(GOT) U/L	Creatinine mg/dl	Urea mg/dl
Control	70.67[a] ±18.90	262.30[a] ± 36.86	0.497[ab] ± 0.04	55.67[ab] ± 25.48
FNPN	39.33[bc] ±10.69	232.33[a] ± 34.65	0.493[ab] ± 0.023	70.33[a] ± 32.81
FGPN	31.67[c] ± 3.22	228.00[a] ± 23.90	0.533[a] ± 0.071	74.00[a] ± 12.49
FNPG	53.00[ab] ± 17.44	261.00[a] ± 36.04	0.427[b] ± 0.021	32.00[b] ± 4.58
FGPG	59.00[ab] ± 14.93	237.30[a] ± 3.79	0.440[b] ± 0.044	46.33[b] ± 10.26
LSD	25.84	54.23	0.079	36.46

Values not sharing a common superscript within column are statistically significant at p <0.05.

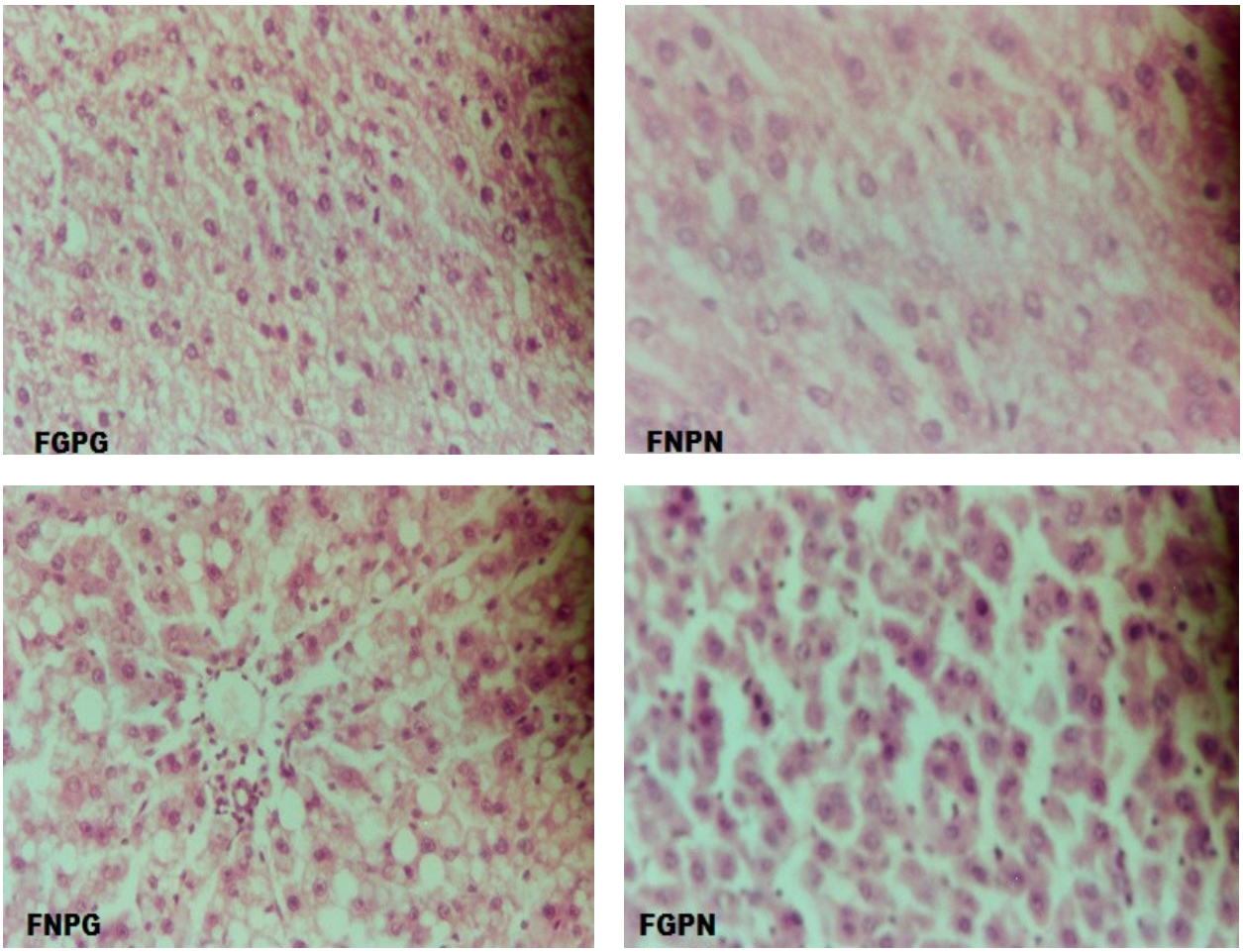

Fig2: Light microscopic photograph of rat liver tissues (X40) feeding on in FNPN, FGPN, FNPG and FGPG seed mixtures.

DISSCUSSION

Chemical composition:- As showed in Table (1) The germination increased the moisture, the crude ether extract, crude protein and total ash contents by 0.05, 0.37, 0.78 and 0.18 folds, respectively. Also, the crude fiber content increased by germination. The raw pumpkin seed flour contained high level of crude protein 38.75% and crude oil 47.44% and the germination increased the protein by 2.8% but the lipid content was increased by germination (4.62%) [27]. The protein, fiber, fat and moisture levels of pumpkin seeds on dry basis were 33.92%, 21.97%, 31.57% and 8.46%, respectively. Sprouting grains causes increased activities of hydrolytic enzymes, improvements in the contents of total proteins, fat, certain essential amino acids, total sugars, B-group vitamins, and a decrease in dry matter, starch and anti-nutrients. The increased contents of protein, fat, dietary fibers and total ash are only apparent and attributable to the disappearance of starch. However, improvements in amino acid composition, B-group vitamins, sugars and protein, and decrease in phytates and protease inhibitors are due to the metabolic effects of the sprouting process. [28]

Mineral content: The content of mineral of experimental samples illustrated increased in the level of minerals especially iron, manganese and zinc in samples by germination. On the other hand, the magnesium level was high as possible in samples containing raw flax seeds. The calcium in raw pumpkin seed flour was 112.80 mg/100g. [27] The mineral contents in pumpkin seeds of copper, zinc, iron, manganese, magnesium and calcium were 36.66, 25.19, 15.37, 3.42, 146.13 and 271.89 mg/100g respectively. [28]Also, the germination increases the level of some minerals. [29] On the other view, when seeds are sprouted, minerals chelate or merge with protein, in a way that increases their function.[30] It is important to note that while these changes may sound impressive, the comparisons are between dormant non-sprouted seed to sprouted seed rather than comparisons of sprouts to mature vegetables. Compared to dry seeds there are very large increases in nutrients whereas compared with mature vegetables the increase is less. However, a sprout, just starting out in life, is likely to need and thus have more nutrients (percentage wise) than a mature vegetable.

Vitamin content: The β-carotene and B2 contents were noticeable increased by germination especially the increase in vitamin B2, the increased was by 2.89 and 36.23 folds. The germination increases the level of some vitamins, where germination has an important effect on the water soluble vitamin, composition of legumes, and sprouted legumes usually contain different levels of riboflavin, compared to levels in the corresponding dry seeds. [29]The most reports agree that sprouting treatment of cereal grains generally improves their vitamin value, especially the B-group vitamins. Certain vitamins such as α-to copherol (Vitamin-E) and β-carotene (Vitamin-A precursor) are produced during the growth process. [30] Also the presence of bio-components such as B-Carotene which has the greatest vitamin A activity and is one of the most effective antioxidants of all the known carotenoids, it is a very safe compound because its conversion to retinol is regulated the vitamin A level in the body.[31]Also the effect of different conditions of germination at a semi-pilot scale on the content of available B_2.Vitamin B_2 suffered an important increase after germination whereas vitamin B_1 did not change significantly. [32]

Electrophoretic protein patterns: The numerous protein bands being visualized on a 7.5% polyacrylamide gel. As shown in fig (1-a) FNPN had nine protein bands, which had molecular weights ranged from 25 kDa to 245 kDa. FNPG was the most protein pattern liked to FNPN pattern (without germination) and FGPN was the most pattern different from FNPN. Thus the germination of flaxseed was more effective in intensity and numbers of protein bands than pumpkin seeds germination .According to Fig (1b:1f) in protein pattern of FGPN there were two low molecular weight bands disappeared 25 and 35 KDa and the number of bands in this sample was less than the other samples (7 bands). On the other hand there were development of proteolytic activity and protein degradation in the highest band (245 KDa) by germination accompanied with increased in the intensity of other bands less than 245 KDa, this hydrolysis was reached the maximum in FGPG protein pattern.Very complex qualitative changes are reported to occur during soaking and sprouting of seeds. The conversion of storage proteins of cereal grains into albumins and globulins during sprouting may improve the quality of cereal proteins. [30].Non-uniform disappearance of some proteins and formation of

new proteins were observed during germination of bean seed segments, the increase in proteolytic activity during sprouting is desirable for nutritional improvement of cereals because it leads to hydrolysis of prolamins and the liberated amino acids such as glutamic and proline are converted to limiting amino acids such as lysine. [33]

Fatty acids composition: Gas chromatographic determination of the fatty acids in the Table (5) showed mainly linolenic, linoleic, oleic, palmitic, oleic and stearic acids being the major component. Changes in the distribution of fatty acids were occurred by germination, the fat composition of FGPG had lowest ratios of saturated to unsaturated fatty acids (1: 8.01) and the highest ratio omega-3 to omega-6 (4.16: 1). Also the n-3:n-6:n-9 ratio was the highest in the same sample. Both FNPN and FGPN contain high content of monounsaturated fatty acid (oleic acid), therefore both samples carry a lower risk of peroxidation than others. Increasing in lipase activity has been reported in barley due to an increase in lipolytic activity during germination and sprouting causing hydrolysis of triacylglycerols to glycerol and constituent fatty acids. [33]The changes in structural lipids, as well as changes in their fatty acid composition were accompany seed germination in a number of plant species, the predominant fatty acids of total lipids of neutral lipids as well as glycolipids were linoleic and linolenic acids, while those of phospholipids were linoleic and palmitic acids, the overall changes of lipids seen in borage seeds during germination agree well with results for other oil seeds.[34] These changes in lipid compositions of flax seeds during germination result from the formation of tissues and metabolic interconversion of lipid classes.

Rapid changes in lipid composition during seed germination may enhance the nutritional value of the sprouts. [35] The pumpkin seed oil content is high, ranging from 40-60%, up to 60.8% is contributed from fatty acids oleic acid (up to 46.9%), linolenic acid (up to 40.5%), palmitic and stearic acid up to 17.4%, the ratio of monounsaturated to polyunsaturated acids from 0.60 to 0.75g.[36]

Sensory properties: The sensory properties of bakery products such as biscuits and cakes ,which the wheat flour substituted with15% flour of experimented samples, were ranged from moderately to slightly like for odour, taste ,texture and over all acceptability, except the colour property which reached to neither like nor dislike, this due to the darkening colour of seed mixtures before and after germination. Whereas the control products were recorded moderately like by the panelists.

The organoleptic scores results indicated that it is enough to replace 20% of wheat flour with equal proportion of dried pumpkin to obtain cake with maximum characteristics.[37] Sprouted legumes can be used to produce legume flour, which has a high nutritive value. This flour, either by itself or combined with cereal flour, can be used to prepare easily digestible baby foods.[38] Hassan *et al. (*2012) [39] were formulated functional biscuits by replacing wheat flour in biscuit formula by different plant meals (e.g. barley, mustard, defatted mustard, flaxseed meal and flaxseed oil) as source of active healthy components to a prepare functional prebiotic biscuits for lowering blood lipids at 5, 10, 15, 20, 25 and 30% levels or shortening by flaxseed oil at 25, 50, 75 and 100% levels.

Feed efficiency: The feed efficiency ratio was differed insignificantly between experimental groups, the highest value was in FNPG group followed by FGPN group and it was the lowest in FGPG followed by the control group. It is important to illustrate that feed efficiency is strongly correlated with daily gain in body weight and fed composition. [40]

The relative weight of internal organ: The relative weight of internal organs were differed significantly between the five groups, the relative weights of lung, heart, and test were the highest in the control group. Feeding rats, on the diets containing the mixtures of experimental seeds, led to decrease the relative weight of these organs but on otherwise, it led to increase the relative weight of liver and spleen in some groups but the relative weight of heart had no effect. But they did not exhibit any gross morphological lesions. Administration of flax and pumpkin seed mixtures to hypercholesterolemic rats caused a significant decrease in body weight gain and in the relative organs weight to reach the level of healthy rats. [41]

The blood picture: There were significant differences between the five groups, some of the hematological values such as Hb, RBCs, WBCs, Platelets, Haemotocrit and M.C.H.C parameters of the experimental groups were slightly lower than the control group, However, such values are within the normal ranges, thus indicating the result of normal variation among animal groups.

The lipid profile: There were significant differences in lipid profile parameters such as LDL, HDL, TG and total Cholesterol. There was no significant differences in HDL/LDL ratios of different groups. Administration of Flax and Pumpkin seeds mixture for 30 days normalized lipid profile in diabetic animals. Seed mixtures were not only lowered the TC, TG and LDL but also enhanced the HDL-cholesterol which is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a ‘‘reverse cholesterol transport’’ pathway, and is considered to be a cardio protective lipid. Thus seeds mixture has a significant impact to improve the imbalance in lipoprotein metabolism.[4]

The liver function: There were significant differences in the values of AIP and ALT enzymes between the different groups, whereas the control group was the highest values in both parameters. Also administration of flax and pumpkin seed mixture attenuated the increased levels of ALT enzyme produced by induction of diabetes and caused a subsequent recovery towards normalization comparable to the control group animals.[4]

The kidney function: The FGPN group was higher in creatinine and urea values than the FNPG group. Flax and Pumpkin seeds mixture administration to diabetic rats significantly reversed these changes in levels of urea and creatinine to near normal values.[4]

Histopathological examination: Liver is divided histologically into lobules. The center of the lobule is the central vein. At the periphery of the lobule are portal triads. Functionally, the liver can be divided into three zones, based upon oxygen supply. The first zone encircles the portal tracts where the oxygenated blood from hepatic arteries enters. The third zone is located around central veins, where oxygenation is poor. The second zone is located in between. The microscopical examination of liver in FNPN, FGPN and FNPG were revealed presence of multiple focal areas of hepatic necrosis, some vacuolation in the intra-cytoplasm and enlargement in some hepatocytes. On the other hand, the FGPG card was nearly similar to normal liver. The hepato-protective effect of the mixture of flaxseed and pumpkin seed mixture powder was further accomplished by histopathological examinations.[4]

CONCLUSION

It is concluded that, the mixtures of seeds such as flax and pumpkin seeds have great potentials in the fortification some bakery products such as biscuits and cakes. Thus this study showed that the germination method used to improve the nutrient content of seeds. This study also showed that samples, particularly the mixture of germinated flax and pumpkin seeds sample has the highest content of macronutrients ,minerals (Fe, Ca, Mn, Cu and Zn), vitamins (β-carotene and riboflavin), the electrophoretic protein patterns changed by germination and finally saturated to unsaturated fatty acids ratio decreased and omega-3 to omega-6 to omega-9 ratio increased. The biscuits and cakes which made from mixture of raw and/or germinated seeds have satisfactory scores by the panelists. The germination effected positively on liver function, kidney function and liver tissues of rats.

الملخص العربى

بذور الكتان تساهم في المقام الأول لأرتفاع محتوى حمض اللينولينيك (أوميجا- ٣) في خصائصها المضادة للأكسدة، والمضادة للالتهابات، وتأثيرات مضادة للسرطان. من ناحية أخرى، فان بذور القرع تعتبر مصادر جيدة للبروتين والفيتامينات وخاصة الأحماض الدهنية أوميجا -٦ التي لها نشاطات هامة مضاد للأكسدة ، وهي تأثيرات مضادة للالتهابات وخافض للبيدات الدم. عموما، الإنبات كأسلوب من أساليب التصنيع الغذائي يحسن قيمة المغذيات بالبذور. الهدف من هذه الدراسة صياغة أغذية تكميلية عن طريق استخدام خليط من الكتان واليقطين فى صورة البذور الخام و / أو المنبتة كمكونات وظيفية لتحسين مشكلة سوء التغذية. ولذلك، فإن خليط من بذور الكتان الخام والقرع العسلي (ك غ ق غ)، بذور الكتان واليقطين المنبتة (ك م ق م)،بذور الكتان المنبتة وبذور اليقطين الخام (ك م ق غ) والكتان الخام و بذور اليقطين المنبتة (ك غ ق م). تمت الدراسة لتحديد الخصائص الكيميائية لها ولإستخدمها لإعداد منتجات البسكويت والكيك.ثم تم تقييم الخصائص الحسية لهذه المنتجات. أيضا، تأثيرإستبدال النظام الغذائي للفئران بنسبة ١٥٪ من مخاليط هذه البذور على بعض التغييرات البيولوجية والهستولوجية تم بحثه.وقد أشارت النتائج إلى أن المستخلص الأثيري الخام ، والبروتين الخام، ، ومحتوى الألياف الخام والمستخلص الخالي من النيتروجين و المعادن باستثناء المحتوى المغنيسيوم و بيتا كاروتين والمحتوى الريبوفلافين كانت أعلى في الخليط المصنوع من البذور المنبتة من الكتان و القرع العسلى عن بذورالمخاليط الأخرى.بروتينات مخاليط البذور فصلت بطريقة الفصل الكهربائي إلى ٩ ٨ و ٧ و ٨ حزم على التوالي. هذا الفصل ناشئ عن أختفاء الحزم ذات الوزن الجزيئي المنخفض. نسبة أوميجا- ٣ إلى أويجا- ٦ إلى أوميجا- ٩ كانت (١:١,٩٠:٧,٩١). بالنسبة للخصائص الحسية للبسكويت والكيك التي تحتوي على ١٥ ٪ من مخاليط الأربعة المحتوية على بذور الكتان و القرع العسلي كانت مقبوله من قبل المتذوقين. بالإضافة لدراسة الآثار البيولوجية لأستبدال ١٥ ٪

من وجبة الفئران بواسطة تلك المخاليط المختبرة التي أظهرت أن قياسات الدم كانت الأفضل في المجموعه الكنترول التى لم تحتوي وجبتها الغذائية على المخاليط المختبرة. من ناحية أخرى, كانت الفئران التي تتغذى على وجبة غذائية تحتوي على المخلوط (ك غ ق غ) الأفضل في قيم الليبوبروتين منخفض الكثافة,التراي جلسريد والكلسترول الكلي(٢,٦٧, ٣٧,٠٠ و ٥٠,٠٠مجم/ديسليتر). ولم يكن هناك اختلاف معنوي فى نسب الليبوبروتين عالي الكثافة / الليبوبروتين منخفض الكثافة بين المجموعات المختلفه. من ناحية أخرى (ك م ق غ) و (ك غ ق غ) سجلت على التوالي أقل قيم نشاط للإنزيم الفوسفاتيذيز القاعدي (٣١,٦٧ و ٣٩,٣٣ وحدة دولية / لتر، على التوالي) ونشاط إنزيم الألانين ترانسفيريز (٢٢٨,٠٠ و ٢٣٢,٣٣ وحدة دولية / لتر ، على التوالي) باعتبارها مؤشرات لوظائف الكبد ، في حين كان من أبرز المجموعات المغذاه على وجبات تحتوي على (ك غ ق م) و(ك م ق م) الأفضل في قيم الكرياتينين (٠,٤٢٧ و٠,٤٤٠ وحدة دولية مجم/ ديسيليتر ، على التوالي) و اليوريا (٣٢.٠٠ و ٤٦.٣٣ مجم/ ديسيليتر ، على التوالي) كمؤشرات على وظائف الكلى . أخيرا الآثار الهستوباثولوجية على أنسجة الكبد قررت.من هنا أستخدام خليط من بذور الكتان والقرع العسلي لتدعيم بعض المنتجات الغذائية لإعطاء تأثيرات صحية جيدةعلى المقاييس التغذوية و الصحية .

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