كلية التربية النوعية

بحوث فى العلوم و الفنون النوعية

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Blood Sugar; Lipid Profile and Bone Health of Obese Diabetic Rats fed on Bread Supplemented with Chia and Quinoa Flours

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Abstract :

The association of certain foods and dietary habits with several chronic health conditions, as osteoporosis, obesity and diabetes, has boosted the search for novel and healthier food options. Among a diversity of plant foods with high nutritional interest, chia and quinoa seeds have recently been highlighted. This study aims to identify the effect of supplemented bread with chia (C) and quinoa (Q) flours on blood sugar, lipid profile, bone mineral density (BMD) and bone mineral concentration (BMC) of obese diabetic rats. The bread was made by adding different proportions of chia and quinoa flours. Regarding sensory evaluation, it was found that the overall acceptability of bread supplemented with chia and quinoa flours were acceptable for the panelists mostly bread supplemented with 10% chia and 10% quinoa. Sixty-six male albino rats were distributed into two main groups: The first main group (1) (n=6 rats) was fed on standard diet. The second group was fed on high fat diet for duration of six weeks to activate obesity in rats, and then rats were injected with alloxan to induce diabetes. Rats of second group divided into 10 groups fed on bread supplemented with different levels of chia, quinoa flour and their mixtures. The results of biochemical analyzes indicate an improvement in the level of lipids, glucose, BMD, and BMC in all groups that were fed on bread containing chia, quinoa and their mixture together. In conclusion, all the prepared bread might be considered useful for overweight and people that safer from obesity and diabetes.

Key words: Chia; quinoa; obesity; diabetes; bone mineral density; bone mineral concentration.

سكر الدم؛ نسبة الدهون وصحة العظام لدى الجرذان البدينة المصابة بالسكري و التي تتغذى على الخبز المدعم بدقيق الشيا والكينوا

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المستخلص:

إن ربط بعض الأطعمة والعادات الغذائية بالعديد من الحالات الصحية المزمنة، مثل هشاشة العظام والسمنة والسكري قد عزز البحث عن خيارات غذائية جديدة وأكثر صحة. ومن بين

مجموعة متنوعة من الأطعمة النباتية ذات القيمة الغذائية العالية، تم تسليط الضوء مؤخرًا على بذور الشيا والكينوا. تهدف هذه الدراسة إلى التعرف على تأثير الخبز المدعم بدقيق الشيا (C) والكينوا (Q) على نسبة السكر في الدم، ومستوى الدهون، وكثافة المعادن في العظام (BMD) وتركيز المعادن في العظام (BMC) لدى الجرذان المصابة بالسمنة و السكري . تم عمل الخبز بإضافة نسب مختلفة من دقيق الشيا والكينوا. فيما يتعلق بالتقييم الحسي، وجد أن القبول العام للخبز المدعم بدقيق الشيا والكينوا كان مقبولاً و خاصة الخبز المدعم بـ 10% شيا و10% كينوا. تم توزيع ستة وستين ذكراً من الجرذان البيضاء إلى مجموعتين رئيسيتين: المجموعة الرئيسية الأولى (1) (عددها = 6 فئران) تم تغذيتها على نظام غذائي قياسي. تم تغذية المجموعة الثانية على نظام غذائي عالي الدهون لمدة ستة أسابيع لتنشيط السمنة لدى الجرذان، ومن ثم تم حقن الجرذان بمادة الألوكسان لتحفيز مرض السكري. تم تقسيم فئران المجموعة الثانية إلى 10 مجاميع تم تغذيتها على الخبز المضاف إليه مستويات مختلفة من دقيق الشيا والكينوا وخلطاتهما. اظهرت نتائج التحاليل الكيموحيوية تحسن مستوى الدهون والجلوكوز وكثافة وتركيز المعادن في العظام في جميع المجموعات التي تغذت على خبز يحتوي على الشيا والكينوا وخليطهما معاً. في الختام، فإن جميع أنواع الخبز المحضر قد تعتبر مفيدة لأصحاب الوزن الزائد والأشخاص التى تعانى من السمنة ومرض السكري.

الكلمات المفتاحية: الشيا؛ الكينوا؛ البدانة؛ السكري؛ كثافة المعادن في العظام؛ تركيز المعادن في العظام.

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Introduction:

Obesity can be defined as a composite disorder including an abnormal or extreme amount of body fat. This imbalance raises the risk related with different diseases such as diabetes, heart disease and high blood pressure **(Collins et al., 2018).**

Diabetes millets (DM) is a metabolic illness considered by hyperglycemia, consequential damage, dysfunction or failure of various organs. The common subjects with DM can be divided into two types, where type-1 DM is produced by a shortage of insulin secretion, and type-2 DM is the consequence of resistance to insulin and an insufficient response of insulin to glucose **(Moestafa, 2021).**

Besides the insulin role in the metabolism of glucose, it is believed that insulin has an anabolic effect on bone, resultant an increase in bone mineral density (BMD). Consequently, diabetes mellitus has become a subject of interest in the research of bone mineral density because both of these disorders are often associated with each other **(Lee et al., 2021).** In addition, diabetes is now concerned with increased bone fragility and fracture risk **(Wintermeyer et al., 2016).**

The occurrence of mortality and morbidity over the world are strictly related with the obesity and osteoporosis diseases **(López-Gómez et al., 2016)**. Several studies have shown protective role of plant foods against obesity and osteoporosis. Obesity may show risk factors for decreased bone density and fractures **(Kim et al., 2010).** Osteoporosis can be well-defined as a skeletal disorder characterized by compromised bone strength which causes an increase in the risk of fracture. Bone strength reflects the combination of two main features: bone quality and bone density **(Sözen et al., 2017).** This bone disorder is defined on the basis of BMD assessment. According to the criteria of WHO, osteoporosis is defined as a state in which BMD lies 2.5 SD or more under the average value for young healthy **(Akkawi and Zmerly, 2018).** The bone mass is measured by bone mineral density (BMD) or bone mineral content (BMC). The dual energy X-ray absorptiometry used to measure the BMD of lumbar spine, femoral neck **(Ulivieri and Rinaudo, 2021)**.

Chia and quinoa seeds have recently been highlighted for its potential nutritional value and chemical composition **(Fernández-López et al., 2020).** Chia (*Salvia hispanica* L.) seed is an herbaceous plant that belongs genus Salvia and order Lamiales **(Iolascon et al., 2017).** Chia contains important quantities of protein, minerals, fiber, polyphenols, and polyunsaturated fatty acids (PUFA) and is currently known as one of the best plant sources of the omega-3 (n-3) fatty acid **(Kulczyński et al., 2019)**. The chia seed is considered an important source of plant that contains n-3 PUFA to be explored in different research models for human health and disease prevention **(Katunzi Kilewela et al., 2021).**

Quinoa **(***Chenopodium quinoa*) is a pseudo-cereal and belongs genus *Chenopodium*, family Chenopodiacea **(Multari et al., 2018)**, quinoa seeds are rich in proteins whose essential amino acids content are well-balanced and higher compared with that in common cereals. It is an excellent source of protein, carbohydrates, lipids, and macro- and microelements (K, Ca, Mg, P, and Fe). Quinoa seeds contain high levels of phenolics **(Park et al., 2021).** Quinoa represents an excellent example of functional food that aims to reduce the risk of various diseases. Its beneficial effects are associated to its nutritional composition: minerals, vitamins and fatty acids give an important contribution to human nutrition and physiology, particularly for cell membranes’ protection and brain neuronal functions **(Aguilar et al., 2019).**

Bread among all the bakery products is of precise interest for today's food industry due to its vital role as a main food across the world **(Gao et al., 2018)**. In this study, bread was supplemented with different level of chia and quinoa flour. This study aims to evaluate the effects of bread supplemented with chia and quinoa flour on blood sugar, lipid profile and bone mineral content.

Materials and Methods

Materials:

Chia and quinoa seeds, wheat flour extracted 72%, sugar, salt and yeast were obtained from local markets in Alexandria, Egypt.

- Normal male albino rats of Sprague Dawley Strain (n=66 weighed 200±10g) were obtained from animal house of Institute of Graduate Studies and Research, Alexandria University, Egypt.
- All kits for determination of the biochemical parameters were obtained from Sigma chemical Company, Egypt.

Methods:

Preparation of chia flour:

Chia seeds were milled by using the electric grinder (Moulinex, France) to obtain powder form and kept stored at 4±1°C in clean bags until used according to (**Demin et al., 2013).**

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Preparation of quinoa flour:

Quinoa seeds were washed in potable water, using fine frame sieves. This process aimed to remove saponins and conducted until there were no more foaming then dried and were milled by using the electric grinder (Moulinex, France) to obtain powder form and kept stored at 4±1°C in clean bags until used according to (**Demin et al., 2013).**

Preparation of bread:

Formulation:

The formulation used for the bread preparation is shown in the following **Table (1)** according to **(Juarez-Garcia, et al. 2006).**

Table (1): Ingredients used in preparation of chia, quinoa and wheat bread

Samples	Chia flour	Quinoa flour	Wheat flour	Yeast	Salt	Sugar
	(g)					
Control bread (100%) WF	-	-	100	2.5	0.7	2
Bread (10% CF + 90% WF)	10	-	90	2.5	0.7	2
Bread (20% CF + 80% WF)	20	-	80	2.5	0.7	2
Bread (30% CF + 70% WF)	30	-	70	2.5	0.7	2
Bread (10% QF + 90% WF)	-	10	90	2.5	0.7	2
Bread (20% QF + 80%WF)	-	20	80	2.5	0.7	2
Bread (30% QF + 70% WF)	-	30	70	2.5	0.7	2
Bread mixture 10% (5% CF+ 5% QF+ 90% WF)	5	5	90	2.5	0.7	2
Bread mixture 20% (10% CF+10% QF + 80% WF)	10	10	80	2.5	0.7	2
Bread mixture 30% (15% CF+15% QF+70 % WF)	15	15	70	2.5	0.7	2

CF: chia flour QF: quinoa flour WF: wheat flour

Bread-Making Procedure:

Wheat flour or its blends were mixed with salt, sugar, yeast and the adequate amount of water. The ingredients were mixed for 7 min, left for 10 min, divided into (100 g), kneaded, and then left again for 15 min. After fermentation, dough was manually rolled and baked in an electric oven at 200 ^{O}C for 29 min and cooled at room temperature for 60 min for subsequent analyses **(American Association of Cereal Chemists [AACC], 2000).**

Chemical composition of chia and quinoa flours and bread samples:

Crude protein, crude fat, ash, crude fiber and moisture contents were determined using the methods mentioned in Association of Official Analytical Chemists **(AOAC 2007)**.

Carbohydrate content was estimated as a difference by calculation as described in American Association of Cereal Chemists [AACC] (2000) as follows:

Nitrogen free extract (NFE) =100- (protein % + ash% + fat% + fiber %).

Sensory evaluation of chia, quinoa and wheat bread samples

The sensory characteristics members were evaluated according to **Hooda and Jood (2005)** by 50 persons from staff and students of Faculty of Specific Education, Alexandria University. Parameters were taste, color, texture, odor and the overall acceptability which was determined as the total mean score of all the sensory parameters. The 9-point hedonic scale with a scale ranging from 1(representing extreme dislike) to 9 (representing extreme like) was used to evaluate the sensory attributes.

Ethical Approval

Study experiments in rats were ethically approved by the Scientific Research Ethics Committee from the University of Alexandria, Animal Ethics Committee, Faculty of Medicine (on 5 December, 2023, with serial number 019). The sensory evaluation was ethically approved by the Scientific Research Ethics Committee from the Faculty of Specific Education at Alexandria University (approval date: 22 October, 2023; Serial Number: AU 21012).

Biological study:

Preparation of diets:

The basal and experimental diets were prepared according to the method of **Reeves et al. (1994).**

Experimental animal design:

A total of 66 male albino rats of Sprague Dawley strain (200±10gm) were obtained from animal house of Institute of Graduate

Studies and Research; Alexandria University. Rats were housed in wire cages in a room temperature 25°C and kept under normal healthy conditions for one week. After a period of adaptation on standard diet, the rats (n=66) were distributed into two main groups:

The first main group (G1) (n=6 rats) was fed on standard diet and they were kept as a negative control group (-ve).

The second group (n=60 rats) was fed on high fat diet (19% saturated fat {lamb fat}+ 1% sun flower oil) for a duration of six weeks to activate obesity in rats. Serum was used to confirm that obesity occurs through determine lipid profile ratio of triglycerides and cholesterol on the 10 groups. The control group rat (-ve) recorded 64.67 mg/dl triglycerides and 78.67 mg/dl cholesterol, while high fat diet (HFD) group recorded 116.0 mg/dl triglycerides and 155.33 mg/dl cholesterol. Then rats were injected with alloxan (120mg/kg body weight) to induce diabetes **(Dhanabal et al., 2005).** After four days serum glucose was determined in all rats and the weight of all rats was estimated to insure the induction. The mean of serum glucose in the negative control group was approximately (73 mg/dl) and the positive control was approximately (160 mg/dl). Then rats of second group divided into 10 sub-groups (each 6 rats) as follows:

Group 2 (first sub-group): Obese diabetic rats fed on high fat diet containing bread (100% wheat flour) as positive control group (+).

Groups 3, 4, 5: Obese diabetic rats fed on high fat diet containing bread (90, 80, 70 % wheat flour supplemented with 10, 20,30% chia flour, respectively).

Groups 6, 7, 8: Obese diabetic rats fed on high fat diet containing bread (90, 80, 70 % wheat flour supplemented with 10, 20, 30 % quinoa flour, respectively).

Groups 9, 10, 11 : Obese diabetic rats fed on high fat diet containing bread (90, 80,70 % wheat flour supplemented with mixture (5% chia +5%quinoa, 10% chia +10%quinoa, 15% chia +15%quinoa, respectively) flours.

Biochemical measurement in serum:

Determination of glucose and insulin:

Quantitative determination of glucose was carried out according to the method of **(Lott and Turner, 1975).** Insulin was determined using Monobind kit and Enzyme Linked Immuno Sorbant Assay (ELISA) method **(Turkington et.al. 1982).**

Determination of Lipid profile:

Total cholesterol (TC) was determined according to **Richmond (1973).** Enzymatic colorimetric GPO-PAP kit was used for the measurement of triglycerides (TG) as described by **Fossati and Prencipe (1982).** High density lipoprotein **(**HDL-c) fraction-cholesterol present in the serum was determined according to **Lopes-Virella et al. (1977).** Low density lipoprotein (LDL-c) was carried out according to **(Jaye et al., 2009),** and calculated using the following equation

LDL-c (mg/dl) = Total cholesterol – (Triglycerides /5 + HDL-c)

Very Low density lipoprotein LDL-cholesterol was carried out according to **(Jaye et al., 2009),** and calculated using the following equation

VLDL-c (mg/dl) = Triglycerides /5

Determination of bone mineral density and bone mineral concentration

Bone mineral density (BMD) and bone mineral concentration (BMC) of left tibia are measured using technique called dual-energy X-radiographic analysis (DEXA). Femur bones were taken and fixed, immediately after separation, cleaning and drying by a filter paper, in neutral buffered formalin. Then, BMD and BMC were measured in rats of the all experimental groups by DEXA **(Dhonukshe et al., 2003).**

Statistical analysis:

The data were analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**)**. Quantitative data was described using mean, standard deviation. Significance of the obtained results was judged at the 5% level**. F-test (ANOVA)** used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (LSD) for pairwise comparisons **(Kirkpatrick, 2015).**

Results and discussion

Chemical composition of chia, quinoa and wheat flours

The data in **Table (2)** showed significant differences ($P \leq 0.05$) in the amount of protein, fat, fiber, ash and NFE between chia, quinoa and wheat flours based on a dry weight. The highest protein content was presented in chia flour (17.78 g/100 g) followed with quinoa (15.1 g/100 g) and wheat flour (9.45 g/100 g). The wheat flour contains low percentage of protein compared to chia and quinoa.

The data also showed a significant difference ($P \leq 0.05$) in the percentage of fat between chia, quinoa and wheat flours. The wheat flour contains low percentage of fat compared with chia or quinoa. The highest percentage of fat was presented in chia flour (32.55 g/100 g) followed with quinoa (7.11 g/100 g) and wheat flour (1.28 g/100 g). It could be observed that, The highest fiber content was presented in chia flour (30.09 g/100 g) followed with quinoa (4.97 g/100 g) and wheat flour (0.73 g/100 g). Further, The same trend was in the percentage of ash.

In contrast, a significant difference ($P \leq 0.05$) was recorded in NFE between chia, quinoa and wheat flours. The highest percentage of NFE was reported in wheat flour (87.75 g/100g) followed with quinoa (69.74 g/100g) and chia flour (15.26 g/100g). Meanwhile, the data of moisture content showed a significant difference ($P \leq 0.05$) between chia and quinoa flour, while non-significant differences ($P \leq 0.05$) were noticed between wheat flour, chia and quinoa flour in the percentage of moisture content. These results were on line with **Miranda-Ramos and Haros (2020)** who reported that wheat flour (72% extraction) contained 11.54% protein, 1.00% fat, 0.58% ash and 83.3% carbohydrates.

The current results were in agreement with **Miranda-Ramos et al. (2020)** who reported that the amount of dietary fiber in chia seeds varied between 30.9% and 36.2% depending on the variety of chia. The obtained results also, agreed with that reported by **Fernandes and Salas-Mellado (2017)** in which chia contained 19.55% protein, 35.2% fat and 4.93% ash. In the meantime, **Puri et al. (2020)** reported that quinoa contained 15.06% protein, 5.96% fiber, 5.62% fat and 2.47% ash. Moreover, **Preedy and Watson (2019)** reported that the high lysine content in the amino acid profile is present in quinoa and chia grains while is deficient in cereals. **Ghafoor et al. (2022)** reported that the content of selected chemical components in the flour of chia were approximately 2 folds higher protein content, 20 folds higher total fat content, 13 folds higher total dietary fiber content, compared with that in wheat flour. The chia seed and chia flours had up to two times more essential amino acids than wheat flour **(Kulczyński et al., 2019).**

Table (2): Chemical composition of chia, quinoa and wheat flours (g/100 g dry weight basis)

Treatment	Crude protein	Crude Fat	Crude Fiber	Ash	NFE	Moisture
	g/100g dry weight					
Wheat flour	$9.45^{c} \pm 0.05$	$1.28^{c} \pm 0.03$	$0.73^{c} \pm 0.04$	$0.79^{c} \pm 0.02$	$87.75^{a} \pm 0.03$	$5.06^{ab} \pm 0.12$
Chia flour	$17.78^{a} \pm 0.02$	$32.55^{a} \pm 0.05$	$30.09^{a} \pm 0.02$	$4.32^{a} \pm 0.03$	$15.26^{c} \pm 0.09$	$4.81^{b} \pm 0.01$
Quinoa flour	$15.10^{b} \pm 0.02$	$7.11^{b} \pm 0.02$	$4.97^{b} \pm 0.02$	$3.07^{b} \pm 0.03$	$69.74^{b} \pm 0.04$	$5.43^{a} \pm 0.50$
F	652.17^{*}	858.069^{*}	109.62^{*}	163.358^{*}	117.704^{*}	3.307
P	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	0.108
LSD 5%	0.058	0.062	0.052	0.049	0.121	0.597

Data was expressed using Mean ± SD.
F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**
Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$) (NFE) Nitrogen free extract
p: p value for comparing between the studied groups

Chemical composition of bread supplemented with different levels of chia, quinoa flours and their mixtures

The data in **Table (3)** revealed the chemical composition of wheat bread (control), bread supplemented with 10, 20 and 30% chia, quinoa flours and their mixtures. The mean values of protein, fat, fiber, ash, NFE and moisture in control bread were 9.93, 1.28, 0.54, 0.96, 87.29 and 4.45 g/100 g respectively. The data showed significant differences ($P \leq 0.05$) between wheat bread and bread which supplemented with chia, quinoa flours and their mixtures. The percentage of protein increased with increasing the percentage of chia, quinoa and their mixtures. The protein content ranged from 10.38 to 11.97% in bread supplemented with 10 - 30% chia flour, while protein content ranged from 10.2 to 11.63 g/100 g in bread supplemented with 10 and 30% of quinoa flour. Moreover, the range of protein content was 11.47 and 12.52 g/100 g in bread supplemented with mixture of (5% C + 5% Q) and mixture of (15% C +15% Q), respectively.

The results in **Table (3)** showed significant ($P \leq 0.05$) differences in the fat content between wheat bread and bread which supplemented with chia, quinoa flours and their mixtures, except bread supplemented with 10% quinoa. The percentage of fat increased with increasing the percentage of chia, quinoa and their mixtures. The fat content ranged from 3.30 to 7.89 g/100 g in bread supplemented with 10 and 30% of chia flour, while the range of fat content was 1.56 and 3.03 g/100 g in bread supplemented with 10 and 30% of quinoa flour. Moreover, the range of fat content was 1.74 and 4.74 g/100 g in bread supplemented with mixture of (5% C + 5% Q) and mixture of (15% C + 15% Q), respectively.

On the other hand, the percentage of fiber increased with increasing the levels of chia, quinoa and their mixtures. The fiber content increased from 2.93 to 7.48 g/100 g in bread supplemented with 10 and 30% of chia flour. While, increased from 1.25 to 2.10 g/100 g in bread supplemented with 10 and 30% of quinoa flour. Moreover, increased from 1.31 to 3.10 g/100 g in bread supplemented with mixture of (5% C+ 5% Q) and mixture of (15% C + 15% Q), respectively compared with 0.54 g/100 g in control bread.

The same trend was observed in the ash content whereas significant differences ($P \leq 0.05$) was observed between wheat bread and bread supplemented with chia, quinoa flours and their mixtures except bread supplemented with 10% chia. The percentage of ash increased with increasing the levels of chia, quinoa and their mixtures.

The data presented in **Table (3)** showed that NFE reduced from 87.29 in control bread to 82.33 and 70.89% in bread supplemented with 10 and 30% chia and from 86.02 to 81.91% in bread supplemented with 10 and 30% quinoa. Moreover, the range of NFE content was 84.50 and 78.03% in bread supplemented with mixture of (5% C+5% Q) and mixture of (15% C+15% Q), respectively.

The low levels of moisture content were noticed in bread supplemented with chia compared with control bread, quinoa bread and mixtures of chia and quinoa. The percentage of moisture content was 2.63 and 2.48 g/100 g in bread supplemented with 10 and 30% chia flour, while it was 4.45 g/100 g in the control bread. The range of moisture content was 3.52 and 4.42 g/100 g in bread supplemented with 10 and 30% quinoa flour. Moreover, the range of moisture content was 3.59 and 3.79 g/100 g in bread supplemented with mixture of (5% C + 5% Q) and mixture (15% chia + 15% quinoa), respectively.

The current data in **Table (3)** revealed that protein and ash content increased gradually with increasing the level of chia and quinoa in preparing the supplemented bread, while carbohydrate decreased gradually, these data are in line with that reported by **(Ali et al., 2012).**

Also, **Miranda and Haros (2020)** found that the addition of quinoa, chia, and their mixture flours significantly increased the total fiber content compared to control bread. This increase might due to refined flours is lack in germ fraction and outer cereal layers. Moreover, **Câmara et al. (2020)** demonstrated that chia flour and chia-by products showed significant increase in the levels of ash, total dietary fiber (TDF), lipids, and proteins, while a significant decrease in the starch content in comparison with control wheat flour. **Further more, Mu et al. (2023)** conducted an investigation on quinoa and other pseudo-cereals and suggested a hypo-cholesterolemic effect of its seeds that rich in fibers, saponins, and squalene. Mainly, the action of these ingredients appears to lead to the absorption of dietary cholesterol and to the reduction of cholesterol synthesis in liver.

The breads with chia provide higher contributions to intake of calcium, iron, and zinc than wheat bread. Accordingly, bread with chia seed or chia flour meets almost all the daily requirement of these minerals in women and men, which is not the case with wheat bread.

Table (3): Chemical composition of bread supplemented with different levels of chia, quinoa flour and their mixtures

Treatment	Crude protein	Crude Fat	Crude Fiber	Ash	NFE	Moisture
	g/100 g dry weight					
Control bread	$9.93^{f} \pm 0.06$	$1.28^{g} \pm 0.03$	$0.54^{i} \pm 0.04$	$0.96^{e} \pm 0.03$	$87.29^{a} \pm 0.09$	$4.45^{b} \pm 0.04$
Bread supplemented with 10%chia	$10.38^{e} \pm 0.02$	$3.30^{d} \pm 0.03$	$2.93^{d} \pm 0.03$	$1.06^{e} \pm 0.03$	$82.33^{e} \pm 0.09$	$2.63^{g} \pm 0.03$
Bread supplemented with 20%chia	$10.86^{d} \pm 0.02$	$5.86^{b} \pm 0.02$	$3.68^{b} \pm 0.02$	$1.64^{b} \pm 0.03$	$77.96^{g} \pm 0.05$	$2.48^{h} \pm 0.03$
Bread supplemented with 30%chia	$11.97^{b} \pm 0.02$	$7.89^{a} \pm 0.01$	$7.48^{a} \pm 0.03$	$1.77^{a} \pm 0.02$	$70.89^{h} \pm 0.01$	$2.47^{h} \pm 0.03$
Bread supplemented with 10%quinoa	$10.20^{e} \pm 0.02$	$1.56^{fg} \pm 0.66$	$1.25^{h} \pm 0.05$	$0.97^{f} \pm 0.08$	$86.02^{b} \pm 1.12$	$3.52^{f} \pm 0.02$
Bread supplemented with 20%quinoa	$11.58^{c} \pm 0.03$	$2.48^{e} \pm 0.03$	$1.77^{f} \pm 0.03$	$1.18^{d} \pm 0.03$	$82.99^{d} \pm 0.06$	$5.63^{a} \pm 0.03$
Bread supplemented with 30%quinoa	$11.63^{c} \pm 0.03$	$3.03^{d} \pm 0.03$	$2.10^{e} \pm 0.02$	$1.33^{c} \pm 0.03$	$81.91^{e} \pm 0.07$	$4.42^{b} \pm 0.02$
Bread supplemented with mixture(5%C+5%Q)	$11.47^{c} \pm 0.43$	$1.74^{f} \pm 0.03$	$1.31^{g} \pm 0.02$	$0.98^{f} \pm 0.02$	$84.50^{c} \pm 0.04$	$3.59^{e} \pm 0.01$
Bread supplemented with mixture(10%C+10%Q)	$12.14^{b} \pm 0.04$	$3.16^{d} \pm 0.02$	$2.15^{e} \pm 0.03$	$1.36^{c} \pm 0.03$	$81.19^{f} \pm 0.03$	$3.86^{c} \pm 0.02$
Bread supplemented with mixture(15%C+15%Q)	$12.52^{a} \pm 0.02$	$4.74^{c} \pm 0.03$	$3.10^{c} \pm 0.02$	$1.61^{b} \pm 0.03$	$78.03^{g} \pm 0.07$	$3.79^{d} \pm 0.02$
F	122.38^{*}	29.56^{*}	14.72^{*}	21.57^{*}	645.778^{*}	50.21^{*}
P	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$
LSD 5%	0.235	0.360	0.047	0.061	0.551	0.042

Data was expressed using Mean ± SD (g/100 g dry weight basis)
F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**
Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$)
p: p value for comparing between the studied groups
(NFE) Nitrogen free extract

Sensory evaluation of bread supplements with different levels of chia, quinoa flours and their mixtures

The data in Table (4) showed the evaluation of the sensory parameters which was determined as mean score values of all the sensory parameters out of ten points. Sensory parameters of bread supplements with different levels of chia, quinoa flours and their mixtures were compared with the control bread (wheat flour). The mean values of color of treatment groups showed that the control bread had the highest score value of color acceptance (8.4) followed by treatment of bread supplemented with 10% quinoa and 20% (8.4 and 8.28) while treatment of bread supplemented with chia 30% had the lowest values of the color acceptance. Also, the bread supplemented with chia and quinoa (20% and 30%) had low values of the color acceptance (5.42 and 5.34). The statistical analysis showed non-significant differences ($P \leq 0.05$) between the values of the color acceptance of control bread and bread supplemented with 10% or 20% of quinoa, while a significant difference ($P \leq 0.05$) of the values of the color acceptance was observed between the color of control bread and bread supplemented with chia and quinoa mixtures.

In the case of taste, the mean values of acceptance showed that the control bread (wheat flour) had the highest value of taste acceptance (8.52) followed by bread supplemented with 10% quinoa and 10% chia (8.4 and 8.26), while bread supplemented with 30% quinoa and mixture (15% C+15% Q) had the lowest values of the taste acceptance (3.2 and 3.26). Moreover, non-significant differences ($P \leq 0.05$) were observed between the values of the taste acceptance of control bread and bread supplemented with 10% of chia or quinoa, while a significant difference ($P \leq 0.05$) in the taste acceptance values was observed between control bread and bread supplemented with chia and quinoa mixtures.

According to the data showed in **Table (4)** there were non-significant differences ($P \leq 0.05$) in the values of odor, texture and general acceptance between control bread and bread supplemented bread with 10% chia or quinoa. While, there were significant difference ($P \leq 0.05$) between control bread and bread supplemented with chia and quinoa mixture.

In general, the overall acceptability revealed that bread supplemented with chia and quinoa flours were acceptable for the panelists mostly bread supplemented with 10% chia and 10% quinoa. Moreover, all the prepared bread might be considered useful for overweight and people that safer from obesity and diabetes. This knowledge was achieved in our studies on rats.

The current results were matched with that reported by **Pizarro et al. (2015)** in which pound cake produced from 15% of chia powder with flour showed good sensory acceptance.

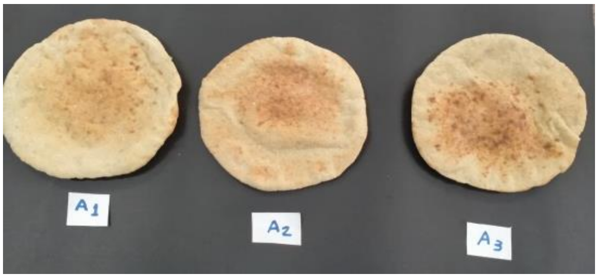
Romankiewicz et al. (2017) Showed that addition of chia seeds caused darkening of the bread crumb, but it was generally not rejected by the panelists. Chia seeds flour at 8% had significantly lower notes for crumb color compared with control bread. **Levent (2018)** reported that the use of chia flour and quinoa flour improved the taste-odor score of gluten-free cakes compared to control. Cakes with 10% chia flour and 10% quinoa flour received the highest appearance, texture, mouth feel and overall acceptability score in all cake samples. Usage of 25% chia flour and 25% quinoa flour decreased the appearance, mouth feel and overall acceptability scores of gluten-free cakes. However, **Turkut et al. (2016)** reported that quinoa flour can be successfully used in gluten-free bread formulation and 25% quinoa bread gained higher sensory scores with its softer texture. Moreover, **Steffolani et al. (2014)** found that usage of chia flour at 15% (rice flour basis) in gluten-free biscuits formulation did not reduce sensorial acceptability of these products.

Table (4): Sensory evaluation of bread supplements with different levels of chia, quinoa flour and their mixtures

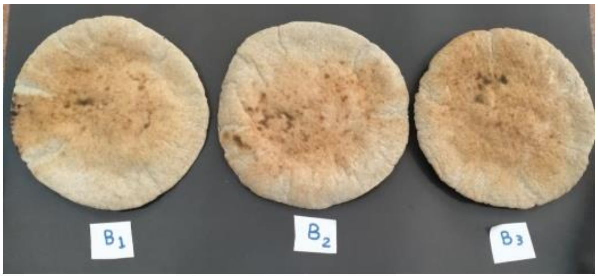
Treatment groups	Color	Taste	Odor	Texture	General acceptance
Control bread	8.48 ^{a} ±0.61	8.52^{a}±0.74	8.46^{a}±0.91	8.46^{a}±0.86	8.36^{a}±0.53
Bread supplemented with 10% chia	7.22^{b}±0.89	8.26^{a}±0.92	7.58^{b}±0.50	7.20^{b}±1.07	8.14^{a}±1.16
Bread supplemented with 20% chia	5.34^{c}±0.89	7.32^{b}±0.74	7.48^{bc}±0.61	5.64^{c}±0.90	7.24^{b}±1.10
Bread supplemented with 30% chia	3.58^{d}±0.73	5.18^{d}±0.80	7.30^{c}±0.46	5.46^{c}±1.07	5.38^{c}±0.92
Bread supplemented with 10% quinoa	8.40 ^{a} ±0.57	8.40^{a}±0.53	8.40^{a}±0.49	8.12^{a}±1.53	8.16^{a}±1.17
Bread supplemented with 20% quinoa	8.28^{a}±0.90	7.22^{b}±0.62	7.48^{bc}±0.50	7.18^{b}±1.44	7.46^{b}±0.89
Bread supplemented with 30% quinoa	7.42^{b}±0.61	3.20^{e}±0.73	5.42^{d}±0.50	5.48^{c}±0.86	3.42^{d}±0.86
HFD bread supplemented with mixture(5% C+5% Q)	7.34^{b}±1.06	7.14^{b}±0.73	7.26^{c}±0.83	7.44^{b}±1.25	7.46^{b}±0.81
HFD bread supplemented with mixture(10% C+10% Q)	5.42^{c}±0.73	5.50^{c}±0.91	7.34^{bc}±0.89	5.46^{c}±1.01	5.52^{c}±0.74
HFD Bread supplemented with mixture(15% C+15% Q)	5.34^{c}±0.85	3.26^{e}±0.90	7.48^{bc}±0.93	5.46^{c}±1.15	3.60^{d}±0.86
F	**322.750***	**472.081***	**137.123***	**93.138***	**287.315***
P	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
LSD 5%	**0.314**	**0.303**	**0.271**	**0.447**	**0.362**

Data was expressed using Mean ± SD.
F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**
Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant at $p \leq 0.05$)
p: p value for comparing between the studied groups
HFD: high fat diet

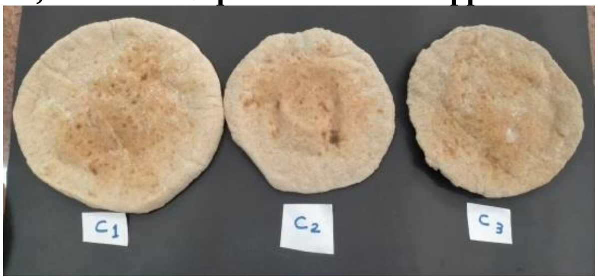
Control bread



Bread supplemented with 10, 20 and 30% quinoa



Bread supplemented with 10, 20 and 30% chia



Bread supplemented with 10, 20 and 30% mixture (chia +quinoa)

Fig (1): Bread supplemented with different levels of chia and quinoa flours and their mixtures

Serum glucose and insulin levels of obese diabetic rats :

The data presented in **Table (5)** revealed the effect of bread supplemented with different levels of chia, quinoa flours and their mixtures on serum glucose and insulin levels of obese diabetic rats.

The data indicated that the obese diabetic rats HFD (control +) had significantly higher ($P \leq 0.05$) serum glucose (126 mg/dl) and lower insulin (0.45 mIU/L) than the healthy rats (control -), where the serum glucose and insulin levels were as follows 75 mg/dl and 2.43 mIU/L, respectively. Moreover, the level of insulin increased in HFD fed on bread supplemented with the three levels of chia (from 1.43 to 1.87 %), quinoa (from 1.10 to 1.56 %) and their mixtures (from 1.73 to 2.0 %), whereas the increase of insulin is concentrated dependent.

The mixtures of chia and quinoa together exhibited the highest levels of insulin compared with the other treatment of HFD which fed on bread supplemented with chia or quinoa alone. The levels of insulin in the chia and quinoa mixtures were closes to the level of insulin in the healthy rats (control -). The data also indicated that the level of serum glucose was reduced with increasing the levels of chia or quinoa, whereas the level reduced from 105 to 93.33 mg/dl at 10% and 30% chia, while it reduced from 104.7 to 85.67 mg/dl at10% and 30% quinoa. Further, the reduction of serum glucose was 92.67 and 83.67 mg/dl at the two mixtures (5% C+5% Q) and (15% C+15% Q), respectively.

The current results matched with that reported by **da Silva et al. (2016)** who found that both, chia seed and chia flour, reduced serum glucose in normal wistar rats after 14 days of treatment. Also, **Motyka et al. (2023)** reported that the consumption of chia seeds had improved glucose and insulin tolerance in HFD group with chia. Also, **Helal et al. (2023)** found that feeding on chia seeds powder caused significant ($P \leq 0.05$) decreases in serum glucose as compared with control (+ve) group.

Our results also, in agreement with **Lopes et al. (2019)** who observed that quinoa seeds significantly decreased the level of glucose. Moreover, **Oliva et al. (2013)** demonstrated an improvement in insulin resistance, adiposity, and dyslipidemia of wistar rats by administering 362 g of chia seed per kg of food in a sucrose-rich diet. Also, **da Silva et al. (2015)** found that the consumption of 13.3% chia seed in the rat’s diet showed an improvement in glucose and insulin resistance.

Table (5): Serum glucose and insulin levels of obese diabetic rats.

Treatment groups	Insulin (mIU/L)	Glucose (mg/dl)
Control(-)	2.43[a] ± 0.31	75.0[g] ± 2.0
HFD containing bread control (+)	0.45[g] ± 0.05	126.0[a] ± 1.0
HFD fed on bread supplemented with 10%chia	1.43[e] ± 0.21	105.0[b] ± 2.0
HFD bread supplemented with 20%chia	1.69[cd] ± 0.08	99.67[bc] ± 3.06
HFD bread supplemented with 30%chia	1.87[bc] ± 0.02	93.33[d] ± 3.79
HFD bread supplemented with 10%quinoa	1.10[f] ± 0.18	104.7[b] ± 5.51
HFD bread supplemented with 20%quinoa	1.45[e] ± 0.05	94.33[cd] ± 3.06
HFD bread supplemented with 30%quinoa	1.56[de] ± 0.04	85.67[f] ± 4.16
HFD bread supplemented with mixture(5% C+5% Q)	1.73[cd] ± 0.09	92.67[de] ± 2.52
HFD bread supplemented with mixture(10% C+10% Q)	1.88[bc] ± 0.02	87.33[ef] ± 2.08
HFD bread supplemented with mixture(15%C+15%Q)	2.00[b] ± 0.10	83.67[f] ± 3.21
F	**44.389***	**55.426***
p	**<0.001***	**<0.001***
LSD 5%	**0.227**	**5.371**

Data was expressed using Mean ± SD.
F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**
Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant at $p \le 0.05$)
p: p value for comparing between the studied groups

Lipid profile of obese diabetic rats:

The data presented in **Table (6)** showed the effect of bread supplemented with different levels of chia and quinoa flour on lipid profile of obese diabetic rats. The data indicate that the obese diabetic rats HFD (control +) had significantly ($P \le 0.05$) higher total cholesterol (160 mg/dl) compared with the healthy rats (control -) (78.67 mg/dl).

The data also showed a decrease in total cholesterol with increasing the levels of chia, whereas the total cholesterol values reduced from 129 mg/dl to 115.7 mg/dl at 10% and 30% chia. Same trend of reduction was observed for quinoa levels and chia plus quinoa mixtures, whereas cholesterol values reduced from 125 mg/dl to 116 mg/dl at 10% and 30% quinoa, while reduced from 102 mg/dl to 88.67 mg/dl at (5% C+5% Q) and (15% C+15% Q) mixtures. The data revealed that the lowest value of total cholesterol was recorded in (control -) and the highest value in HFD (control +). Chia and quinoa levels reduced the total cholesterol and the reduction concentration dependent.

The same trend was observed for triglycerides (TG), the data indicated that the obese diabetic rats HFD (control +) had significantly ($P \leq 0.05$) higher TG (122 mg/dl) compared with the healthy rats (control -) (64.67 mg/dl). The data also showed a decrease in TG with increasing the levels of chia, whereas the TG values reduced from 92 mg/dl to 83.67 mg/dl at 10% and 30% chia. In addition to that, same reduction was observed for quinoa levels and chia plus quinoa mixtures, whereas TG values reduced from 98 mg/dl to 87 mg/dl at 10% and 30% quinoa, while reduced from 77 mg/dl to 69 mg/dl at (5% C+5% Q) and (15% C+15% Q) mixtures. The data revealed that the lowest value of TG was recorded in control (-) and the highest value in HFD control (+). Chia and quinoa levels reduced TG and the reduction in chia levels was more than quinoa levels.

In the case of HDL, the data indicated that obese diabetic rats HFD control (+) had significantly ($P \leq 0.05$) lower HDL (21 mg/dl) compared with the healthy rats, (control -) (50.33 mg/dl) or with all treatment groups in which bread supplemented with different levels of chia, quinoa flour and their mixtures. The data also showed an increase in HDL with increasing the levels of chia, whereas the HDL values increased from 33.67 mg/dl to 40 mg/dl at 10% and 30% chia. Same trend of increase was observed for quinoa levels and chia plus quinoa mixtures, whereas HDL values increased from 33 mg/dl to 39.33mg/dl at 10% and 30% quinoa, while increased from 38mg/dl to 45 mg/dl at (5% C+5% Q) and (15% C+15% Q) mixtures. The data revealed that the highest value of HDL was recorded in (control -) and the lowest value in HFD (control +). Chia and quinoa levels increased HDL values and the increase in chia and quinoa levels were close and concentration dependent.

As for LDL, the data indicated that obese diabetic rats HFD (control +) had significantly ($P \leq 0.05$) higher LDL (114.6 mg/dl) compared with healthy rats, (control -) (15.42 mg/dl). Data indicated a decrease in LDL with increasing the levels of chia whereas the LDL values reduced from 76.92 mg/dl to 59.92 mg/dl at 10% and 30% chia. The LDL values also reduced from 72.4 mg/dl to 58.28mg/dl at 10% and 30% quinoa, while reduced from 48.7 mg/dl to 29.2 mg/dl at (5% C+5% Q) and (15% C+15% Q) mixtures.

On the other hand, the data indicated that obese diabetic rats HFD (control +) had significantly higher ($P \leq 0.05$) VLDL (24.4 mg/dl) compared with the healthy rats, (control -) (12.93 mg/dl) or with all treatment groups in which bread supplemented with different levels of chia, quinoa flour and their mixtures. The data also showed a decrease in VLDL with increasing the levels of chia whereas the VLDL values reduced from 18.4 mg/dl to 16.73mg/dl at 10% and 30% chia. The same trend of reduction was observed for quinoa levels and chia plus quinoa mixtures, whereas VLDL values reduced from 19.6 mg/dl to 17.4mg/dl at 10% and 30% quinoa, while reduced from 15.4 mg/dl to 13.8 mg/dl at (5% C+5% Q) and (15% C+15% Q) mixtures. Chia and quinoa levels reduced VLDL and the reduction was close and concentration dependent.

The current data showed lowering of lipid in serum of obese diabetic rats that seems to be related to the effect of bread supplemented with different levels of chia, quinoa flour and their mixtures. Chia and quinoa might lead to reduce the cholesterol absorption or decreased serum cholesterol, LDL-c and VLDL, thereby improving the lipoprotein profiles. The results in line with **Tenore et al. (2018)** who reported that the intake of chia seeds by animals reduced the serum triglycerides level, due to their high α-linolenic and ω-3 fatty acids contents. Moreover, **Helal et al., (2023)** found that feeding rats with chia seeds powder caused significant ($P \leq 0.05$) increase in HDL-c, and significant ($P \leq 0.05$) decreases in lipid profile as compared with control (+ve) group. Also, **Agarwal et al. (2023)** mentioned that consumption of chia seeds could avoid the occurrence of cancer, inflammatory and cardiovascular diseases due to their pharmacological effects when consumed within healthy ratios.

Also, **Halaby et al. (2017)** reported that rats fed a high-cholesterol diet with two levels of quinoa seeds (30% and 40%), showed lower values of lipid profile compared to positive control group and that might due to decrease in cholesterol absorption, increase cholesterol excretion and fecal bile acid. **Li et al. (2018)** indicated that quinoa diets significantly reduced cholesterol levels when compared to control. The dietary fiber in quinoa can affect the absorption cholesterol by binding to biliary acid which may increase catabolism of cholesterol and fermentation of dietary fibers in colon which produce short chain fatty acids which assist the inhibition of cholesterol synthesis.

The unsaturated fatty acids and tocopherols in quinoa may also contribute to the reduction in LDL levels **(Ahmed et al. 2020).** The high level of HDL-c was associated with risk reduction of atherosclerosis, since high density lipoprotein in serum facilitate the translocation of excess cholesterol from the peripheral tissues to liver for catabolism, they also seem to equally improve the LDL and HDL levels **(Makni et al., 2010).**

Table (6): Lipid profile of obese diabetic rats

Treatment groups	Cholesterol	T.G	HDL	LDL	VLDL
	(mg/dl)				
Control(-)	$78.67^{i} \pm 1.53$	$64.67^{g} \pm 1.53$	$50.33^{a} \pm 1.53$	$15.42^{k} \pm 0.01$	$12.93^{g} \pm 0.31$
HFD containing bread control(+)	$160.0^{a} \pm 2.0$	$122.0^{a} \pm 2.65$	$21.0^{h} \pm 2.0$	$114.6^{a} \pm 0.30$	$24.40^{a} \pm 0.53$
HFD fed on Bread supplemented with 10% chia	$129.0^{b} \pm 1.0$	$92.0^{c} \pm 4.36$	$33.67^{fg} \pm 1.53$	$76.92^{b} \pm 0.02$	$18.40^{c} \pm 0.87$
HFD Bread supplemented with 20% chia	$123.0^{cd} \pm 2.0$	$85.33^{d} \pm 2.52$	$37.33^{de} \pm 2.08$	$68.60^{d} \pm 0.01$	$17.07^{d} \pm 0.50$
HFD supplemented with 30% chia	115.7e ± 3.21	$83.67^{d} \pm 3.06$	$40.0^{cd} \pm 1.0$	$59.93^{f} \pm 0.02$	$16.73^{d} \pm 0.61$
HFD Bread supplemented with 10% quinoa	$125.0^{c} \pm 3.0$	$98.0^{b} \pm 2.65$	$33.0^{g} \pm 1.0$	$72.40^{c} \pm 0.10$	$19.60^{b} \pm 0.53$
HFD Bread supplemented with 20% quinoa	$120.3^{d} \pm 1.53$	$91.33^{c} \pm 1.53$	$36.33^{ef} \pm 1.53$	$66.04^{e} \pm 0.01$	$18.27^{c} \pm 0.31$
HFD Bread supplemented with 30% quinoa	116.0e± 2.65	$87.0^{d} \pm 2.0$	$39.33^{d} \pm 1.15$	$58.28^{g} \pm 0.02$	$17.40^{d} \pm 0.40$
HFD Bread supplemented with mixture(5% C+5% Q)	$102.0^{f} \pm 3.0$	$77.0^{e} \pm 2.0$	$38.0^{de} \pm 1.0$	$48.70^{h} \pm 0.20$	$15.40^{e} \pm 0.40$
HFD Bread supplemented with mixture(10% C+10% Q)	$95.0^{g} \pm 1.0$	$73.67^{e} \pm 1.53$	$42.33^{bc} \pm 3.21$	$38.30^{i} \pm 0.09$	$14.73^{e} \pm 0.31$
HFD Bread supplemented with mixture(15% C+15% Q)	$88.67^{h} \pm 0.58$	$69.0^{f} \pm 1.0$	$45.0^{b} \pm 1.0$	$29.20^{j} \pm 0.30$	$13.80^{f} \pm 0.20$
F	**326.836***	**126.968***	**59.763***	**973.4***	**126.968***
P	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
LSD 5%	**3.622**	**4.106**	**2.843**	**0.250**	**0.821**

Data was expressed using Mean ± SD.
F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**
Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant at $P \le 0.05$)
p: p value for comparing between the studied groups

Bone mineral density (BMD) and bone mineral content (BMC) of obese diabetic rats:

Table (7) showed the effect of bread supplemented with different levels of chia, quinoa flour and their mixtures on bone mineral density (BMD) and bone mineral content (BMC) of obese diabetic rats.

The data indicated that the (control +) obese diabetic rats fed on HFD had significantly ($P \leq 0.05$) lower BMD (0.08 g/cm^2) compared with the healthy rats, (control -) (0.24 g/cm^2). The data also showed an increase in BMD with increasing the levels of chia, whereas the BMD values increased from 0.15 to 0.17 g/cm^2 at 10% and 30% chia. Same increase was observed for quinoa levels and chia plus quinoa mixtures, whereas BMD values increased from 0.13 to 0.16 g/cm^2 at 10% and 30% quinoa, while increased from 0.17 to 0.19 g/cm^2 in (5% C+5% Q) and (15% C+15% Q) mixtures.

Concerning the BMC, the data in **Table (6)** indicated that the (control +) obese diabetic rats fed on HFD had lower BMC (0.014 g) compared with the healthy rats, control -) (0.10 g) or with all treatment groups in which bread supplemented with different levels of chia, quinoa flour and their mixtures. The data also showed an increase in BMC with increasing the levels of chia, whereas the BMC values increased from 0.05 to 0.07 g, at 10% and 30% chia. The BMC values also increased from 0.04 to 0.07 g, at 10% and 30% quinoa, while increased from 0.05 to 0.09 g, in (5% C+5% Q) and (15% C+15% Q) mixtures. The data revealed that the highest value of BMC was recorded in (control -) and (15% C+15% Q) mixtures. While, the lowest value in HFD (control +). Chia and quinoa levels increased BMC values and the increase in chia and quinoa levels was non-significant ($P \leq 0.05$). The data showed significant differences ($P \leq 0.05$) between (control +) and all other treatments of chia, quinoa and their mixtures.

According to WHO measures, osteoporosis is defined as a condition in which BMD lies ≥2.5 SD below the average value for young healthy women **(Shuler et al., 2012).** While**, Liberato et al. (2013)** defined osteoporosis as a decline in the bone mineral density

(BMD), which leads to increase the risk of bone fractures mainly in the wrist, hip and spine.

Liu et al. (2010) found that type 2 diabetes may associated with the inefficient redistribution of bone mass and the inadequate compensation for increased body mass, which may result in reduced of bending strength. Also**, Cohen et al. (2013)** reported that the trunk fat, evaluated by DEXA scan, was inversely associated with trabecular bone volume and bone formation rate, observed with a transiliac bone biopsy, even after controlling of age and BMI.

Villa et al. (2016) showed that the rodents BMD and bone quality are impaired when an "obesogenic" diet is administered during growth. Also, **Ionova et al. (2011)** reported that HFD caused greater lean, fat mass and lower biomechanical properties of cortical bone when compared to low-fat diet but these effects vary depending on age. HFD appears to affect bone remodeling leading to decreased femoral trabecular bone mass. Moreover, **Yarrow et al. (2016)** stated that the excessive intake of sugars, such as fructose or glucose, cause damage of BMD, BMC and mechanical strength in rats**.**

da Silva et al. (2019) found that rats fed on chia seeds led to increase bone mineral density, bone mineral content , so chia seeds is a source of functional foods, it is known as a new gold because of its healthy benefits and nutritional values. Also**, Gouda (2023)** explained that chia improvement the levels of P, Ca, bone mineral concentration (BMC) and bone mineral density (BMD), due to the high content of minerals and polyphenolic compounds.

In addition to that, The breads with chia provide higher contributions to intake of calcium, zinc , and iron than wheat bread. Accordingly, bread with chia flour or chia seed meets almost all the daily requirement of these minerals in men and women compared to wheat bread **(Ferreira et al,. 2023).**

According to **Li et al. (2021)** quinoa is particularly rich with the amino acid lysine, which is essential for tissue growth and repair. Quinoa has more calcium than milk and considers a good source of phosphorous, iron, vitamins B and E.

Table (7): Bone mineral density (BMD) and bone mineral content (BMC) of obese diabetic rats.

Treatment groups	BMD (g/cm^2)	BMC (g)
Control (-)	$0.24^{a} \pm 0.06$	$0.10^{a} \pm 0.01$
HFD containing bread control (+)	$0.08^{c} \pm 0.02$	$0.014^{c} \pm 0.002$
HFD fed on Bread supplemented with 10%chia	$0.15^{bc} \pm 0.01$	$0.05^{b} \pm 0.05$
HFD Bread supplemented with 20%chia	$0.16^{b} \pm 0.04$	$0.06^{b} \pm 0.01$
HFD Bread supplemented with 30%chia	$0.17^{ab} \pm 0.03$	$0.07^{b} \pm 0.02$
HFD Bread supplemented with 10%quinoa	$0.13^{bc} \pm 0.03$	$0.06^{b} \pm 0.01$
HFD Bread supplemented with 20%quinoa	$0.14^{bc} \pm 0.02$	$0.05^{b} \pm 0.02$
HFD Bread supplemented with 30%quinoa	$0.16^{b} \pm 0.04$	$0.07^{b} \pm 0.01$
HFD Bread supplemented with mixture(5% C+5% Q)	$0.17^{ab} \pm 0.03$	$0.05^{b} \pm 0.01$
HFD Bread supplemented with mixture(10% C+10% Q)	$0.18^{ab} \pm 0.06$	$0.06^{b} \pm 0.02$
HFD Bread supplemented with mixture(15% C+15% Q)	$0.19^{ab} \pm 0.09$	$0.09^{a} \pm 0.01$
F	**2.548^{*}**	**4.367^{*}**
P	**0.032^{*}**	**0.002^{*}**
LSD 5%	**0.075**	**0.032**

Data was expressed using Mean ± SD.
F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**
Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant at $P \leq 0.05$)
p: p value for comparing between the studied groups

Conclusion:

The results of this study reflect a positive influence of bread supplemented with chia and quinoa flours on lipid profile, glucose, insulin, BMD and BMC. The obtained results recommend that chia, quinoa flour and their mixtures as functional foods could be used to manage obesity, diabetes and improve bone health.

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