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Effect of Replacing Soybean Flour by Germinated Quinoa Flour on the Physicochemical Characteristics of Vegan Burgers and Biological Activities in Diabetic Rats

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EGYPT has been consuming a growing amount of processed meat, which has had a detrimental impact on several environmental and health issues. The development of vegan burgers grown from germinated quinoa may be promising in Egyptian human foods for its health and nutritional benefits. This study aims to evaluate the physicochemical properties of the vegan burgers and its effect on the biological activities of diabetic rats by using germinated quinoa seed (QS) flour as a partial or full replacement for soybean (SB) flour. Four quinoa seed burger (QSB) treatments (GQB_{20}, GQB_{50}, GQB_{80}, and GQB_{100}) were made by replacing the SB flour, which was used in the control soybean burger (SBB100) with 20, 50, 80, and 100% GQ, respectively. Compared to SBB_{100}, all GQB exhibited a stronger antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ABTS radicals; the activity increased with replacement level.Shrinkage in size and texture attributes, including cohesiveness, hardness, and springiness decreased as GQ flour replacement increased, while chewiness increased.GQB_{50}, GQB_{80}, and GQB_{100} had a pleasant flavor, an acceptable taste, and a juicy texture when compared to SBB_{100}. In the biological study, as the proportion of GQ flour increased, body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER) increased, but serum glucose and insulin decreased. Lipid profiles, kidney, and liver functions were all improved when GQ was added to GQB; the improvement was very comparable to the negative control. GQ content in GQB was also correlated with decreased levels of serum glutathione peroxidase and malondialdehyde. In conclusion, GQ flour can be substituted for SB flour at an 80 or 100% replacement rate to produce a vegan burger with desired sensory qualities and a strong biological effectas it can be classified as functional food.

Keywords: Vegan burgers, Germinated quinoa seeds,Soybean flour, Biological activities.

Introduction

The importance of human health has increased as metabolic diseases have become more prevalent. Food has a greater potential to treat and prevent disease, which is why developing the healthiest ingredients for our food products has received increased attention. Incorporating non-meat ingredients not only lowers costs and enhances consumer health, but also improves the quality of meat products (Abdolghafour and Saghir, 2014). Because soy protein has high biological factors and good functional qualities, it is the most commonly utilized vegetable protein as a meat extender in meat products. This improves the final product's texture and acceptability by increasing its water binding capacity (Passos-Maria and Kuaye, 2002). However, contrary to what Acero (2012) claimed, it was discovered that people with favism (hemolytic anemia), a type of genetic condition, should not eat soy or anything containing soyAccording to research on sex hormones, diets heavy in meat have been shown to have greater levels of testosterone and estradiol than diets high in soy products (Habito et al.,

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2000). Vegan burgers, for example, are no longer considered the health food industry's outlier when it comes to flavor; they are easy to make and offer both vegetarians and non-vegetarians the same low-fat alternative to meat products, especially those high in saturated fats and fiber (Banman, 2008).

Around 90% of the world's calories come from wheat, rice, and corn, so there is a pressing need for crops with better the nutritional value to replace them in order to ensure global food security (Cheng, 2018). Advertised as an alternative agricultural crop, quinoa has been classified as a super grain due to its optimal balance of nutritional properties, gluten-free nature, and high stress tolerance (Kataria et al., 2021; Satheesh and Fanta, 2018). Quinoa has been recognized for its excellent nutritional qualities, including its high content of protein, carbs, fat, minerals, and vitamins. The protein content is actually between 14 and 20 percent, and it is made up of high amounts of important amino acids. The protein is especially rich in essential amino acids like methionine and lysine, which provide high-quality protein. It also goes well with beans, which are frequently lacking in cysteine and methionine. In addition, it is rich in micronutrients, including minerals (Ca, P, Fe, and Z), vitamins (B1, B2, B6, C, and E), and phytochemicals that lower the risk of allergies, cancer, and cardiovascular diseases (Nickel et al., 2016; Ayaşan 2020). Quinoa flour is also used as an alternative ingredient to soybeans in the production of a functional beef burger instead of soybean flour (Shokry, 2016). In a previous study, germinated quinoa showed a greater content of phenol compounds and minerals like Ca, Mg, and Fe, as well as stronger antioxidant activity, than raw, soaked, fermented seeds. It also showed lower levels of anti-nutritional components including saponin, oxalat, and tannins (Ibrahem and Mohamed, 2021).Furthermore, the evaluation of food products' quality is based on their physical and chemical composition, sensory attributes, and low contaminant level. Thus, this study set out to evaluate the effects of either completely or partially replacing germinated quinoa flour for soybean flour on the physicochemical and sensory characteristics of vegan burgers, as well as their impact on the biological activities of diabetic rats.

Materials and Methods

Materials

Ingredients

Quinoa seeds (Chenopodium *Quinoa*) were supplied by the Agriculture Research Center (Desert Research Center, Cairo, Egypt). The 48% protein soybean (SB) flour supplied by the Food Technology Research Institute (Agriculture Research Center, Giza, Egypt). The additional ingredients for the soybean or quinoa burger—spices, white and black pepper, onion powder, garlic powder, and salt—were purchased from the local market in Cairo, Egypt.Vitamins, minerals, cellulose, choline, and casein were purchased from El-Gomhoria Company (Cairo, Egypt), for use in rat diets.

Rats

Thirty-five adult male Sprague-Dawley rats, weighing around 200±10 g, were obtained by the Helwan Experimental Animals Farm, Egypt.

Chemicals

Streptozotocin was obtained from Sigma Company (St. Louis, MO, USA). Kits for biochemical analysis were purchased from Gama Trade Company for Pharmaceutical and chemicals, Egypt. The 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazinyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich, St. Louis, MO, USA.All of the reagents and chemicals were analytical grade and were obtained from various sources.

Methods

Preparation of germinated quinoa

Quinoa seeds were cleaned with cold water and germinated according to Padmashree et al. (2019). The germinated quinoa seeds were dried at 50 °C before being processed into powder in a high-speed mixer mill (WK-1000A; Qing Zhou Machinery Co., Ltd.) at a speed of 25000/min. After that, it was stored at 4±2 °C in polyethylene bags until used.

Vegan burger preparation

A soy burger made with soybean (SB) flour served as the vegan burger control. The SB flour in the control vegan burger was substituted with 20, 50, 80, and 100% germinated quinoa seeds powder (GQ) to create four vegan burger treatments (Table 1). All ingredients were weighed, mixed, and manufactured according to the method described with some modification by Mohamed et al. (2024). After mixing all the ingredients together, each selection was formed into vegan burger pancakes using an 8-cm-diameter burger making machine (Export Company, Shanghai, China). In an air oven preheated to 180±2°C, the vegan burger patties were cooked for 20 min. After 10 min, they were turned over.

TABLE 1. The formulations of the vegan burger made with soybean flour and/or germinated quinoa seed flour.

Ingredient (g/100 g)	Vegan burger treatments				
	SBB_{100}	GQB_{20}	GQB_{50}	GQB_{80}	GQB_{100}
Fat	10.0	10.0	10.0	10.0	10.0
Water	16.0	16.0	16.0	16.0	16.0
Salt	1.0	1.0	1.0	1.0	1.0
White and black pepper	1.0	1.0	1.0	1.0	1.0
Garlic and onion powder	2.0	2.0	2.0	2.0	2.0
Soybean flour	70.0	56.0	35.0	14.0	0.0
Germinated quinoa seeds	0.0	14.0	35.0	56.0	70.0

SBB_{100}, a vegan burger made with 100% soybean flour; GQB_{20}, $GQB_{50,}$$GQB_{80}$, and $GQB_{100,}$ vegan burgers made by replacing 20, 50, 80, and 100% of soybean flour by germinated quinoa seedflour, respectively.

Chemical analysis

The contents of moisture, total nitrogen, fat, ash, and crude fiber in the cooked burger samples were determined using the AOAC (2012) methods. The percentage of total nitrogen was multiplied by 6.25 to obtain the protein content.

Antioxidant activities

The antiradical activities of cooked burger samples were determined using the stable DPPH radicals (DPPH) and stable ABTS radicals (ABTS) assays according to Brand-Williams et al. (1995) and Re et al. (1999), respectively. In summary, 3 g of burger samples were weighed into 25 mL centrifuge tubes. 6 mL of methanol were added for the DPPH assay, and 12 mL of methanol were added for the ATBS assay. The tubes were then vortex vigorously for 2 min. The tubes were stored at 5±1 °C for an overnight, after which they were vortex vigorously for 2 min again and centrifuged at 6000 g for 10 min. 100 µL of clear methanol extract were added to 3.9 mL of DPPH working solution (25 mg DPPH/L methanol) or ABTS working solution (7 mM ABTS solution with 2.45 mM $K_2S_2O_8$). The mixture was incubated for 20 min in the dark at room temperature. The degree of decolorization was measured in a spectrophotometer at 517 nm for the DPPH and at 734 nm for the ATBS assay. In the same way as the assay mixture, a control solution (DPPH or ABTS solution with methanol) was made. The following formula was used to calculate the DPPH radical-scavenging activity:

$$\text{Radicalsscavenge activity (\%)} = [(A0 - A1)/A0] \times 100$$

A0 is the absorbance of the control and A1 is the absorbance of the sample.

Physical properties

Cooking loss: The cooking loss of the vegan burger patties was estimated after cooking according to the method of Crowe and Johnson (2001), which required determining the weight difference between the samples before and after cooking. The following formula was used to get the percentage weight loss:

Weight loss (%) = (Raw patty weight - Cooked patty weight)/Raw patty weight x 100

Shrinkage in size: The change in diameter of vegan burger patties was measured as shrinkage in size, according to Carvalho et al. (2017) also before and after cooking. Every patty represented the average of three replications, and ten measurements were made of each one. The following equation was used for calculating the percentage of the shrinkage in size:

Shrinkage (%) = (Raw patty diameter -cooked patty diameter)/Raw patty diameter x 100

Texture profile analysis: The cooked or fried vegan burger patties were cut into small pieces (~1.5 x 1.0 x 1.5 cm/piece) according to Forghani et al. (2018). The texture profile analysis (TPA) of a cooked burger was performed using a texture analyzer, TA-XT Plus (Stable Micro Systems, UK). An plot of force (N) versus time (sec) was made during the compression test used to take measurements. Samples were twice compressed at a rate of 2 cm/min. Records were kept for textural measures like cohesiveness, chewiness (N*mm), hardness (N), and springiness (mm).

Sensory evaluation

Sensory attributes of a cooked vegan burger was evaluated by nine panel members at the Agricultural Research Center in Cairo, Egypt.

The characteristics of a vegan burger include appearance, taste, juiciness, flavor, and overall acceptability using the hedonic score system of 9 points, knowing that 1 means very hated and 9 means very loved according to the classification of sample quality according to Mihafu et al. (2020). Scores were collected and analyzed statistically.

Biological experiment

A week of adaptation was given to 35 male adult Sprague Dawely Strain albino rats, weighing 210±10 g, which were housed in clean, well-ventilated cages (Reeves et al., 1993). The rats were divided up into two main groups. The first group (A, 5 rats) was maintained as the negative control group (Control -ve) and fed the basal diet (Table 1). To induce hyperglycemia, the second group (B, 30 rats) fasted overnight and was injected (IP) with a low dose of streptozotocin (STZ, 35 mg/kg b.w.), which was dissolved in a pH 4.4 citrate bufferat a concentration of 15 mg/mL. Rats with a blood glucose level >200 mg/dL were considered diabetic, while the animals in the negative control group were injected with buffer only, according to Zhang et al. (2008). Diabetic rats were also divided into six subgroups, each with 5 rats: subgroup 1 fed on basal diets, which served as the positive control group (Control +ve), and subgroups 2, 3, 4, 5, and 6 fed on basal diets supplemented with SBB_{100}, QBB_{20}, QBB_{50}, QBB_{80}, and QBB_{100}, respectively. The added amount of vegan burger was calculated by deducting an equal amount of casein that was used to prepare the basal diet. After 8 weeks, rats were fasted overnight. Blood samples were collected from the medial canthus of the eyes of rats by means of fine capillary glass tubes without any anticoagulants and centrifuged for 20 min at 3000 rpm to get serum, which was stored at -80 °C until later biochemical examination.

Biological evaluations: Every day, the amount of food consumed and/or wasted was recorded and total feed intake (FI) was calculated. Every week, the rats in each group had their individual body weight (BW) recorded. The formulas used to calculate the feed efficiency ratio (FER) and body weight gain percentage (BWG %) were designed by Champman et al. (1959).

Biochemical analysis: Blood serum hormones like insulin, leptin, and cortisol were determined according to Naito and Kaplan (1984), Rudovich et al. (2004), and Chernow et al. (1987), respectively. Uric acid, creatinine, and urea as kidney functions were determined according to methods described by Young (2001). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as alkaline phosphatase (ALP), as liver functions, were determined according to Bergmeyer et al. (1978) and Belfield and Goldberg (1971), respectively. Lipid profiles such as serum total cholesterol (TC) (Richmond, 1973), triglycerides (TG) (Wahlefeld, 1974), and high-density lipoprotein (HDL-c) (Albers et al., 1983) were measured according to the reported methods. However, low-density lipoprotein (LDL-c) and very low-density lipoprotein (VLDL-c) were calculated according to the equation of Fridewald et al. (1972).

VLDL-c = TG/5, LDL-c = TC - (HDL-c + VLDL-c)

Oxidative stress biomarkers: Oxidative stress biomarkers like malondialdehyde (MDA) and glutathione peroxidase (GPX) levels in samples of serum were measured according to Satoh (1978) and Paglia & Valentine (1967).

Statistical analysis

Using the SPSS program (2015) software, the ANOVA procedure was used to do statistical analysis. The means were compared using Duncan's multiple comparison procedure. A probability of ($P \leq 0.05$) was used to establish statistical significance (Snedecor and Cochran, 1989).

Results and Discussion

Chemical properties

Chemical properties of post-cooked quinoa burgers made with different levels of germinated quinoa seed flour (GQ) as a replacement for soybean (SB) flour are presented in Table 2. When GQ was added in place of SB flour, in general, protein, fat, fiber, and ash content decreased; this decrease was proportionate to the addition level. The decrease was statistically significant ($p < 0.05$) until 80% replacement ($P \leq 0.05$), while there was no significant difference in protein, fat, fiber, or ash content between GQB_{80} and GQB_{100} ($P > 0.05$). A similar trend was observed when SB concentrated replaced with bulgur flour in beef burgers (Abd-El-Aziz et al., 2018). This decrease is explained by the fact that SB flour has more protein, fat, fiber, and ash content than GQ flour ($P \leq 0.05$). However, the moisture content was not affected by the type of flour or substitution level. The moisture content was ranged between 2.04 and 2.21%.

The antioxidant properties of natural materials are often evaluated using the DPPH and ABTS radical-scavenging activity assays (Shazly et al., 2022). The antioxidant activities of quinoa post-cooked quinoa burgers (GQB), which are made with different levels of GQ flour as a replacement of SB flour in soybean burgers (SBB$_{100}$), are shown in Fig 1. Antioxidant activity against the DPPH and ABTS radicals was generally stronger ($P < 0.05$) in in all GQB than SBB$_{100}$. Consequently, as the proportion of GQflour increased,the antioxidant activity also increased; however, this increase was only significant in GQB$_{80}$ that contained 80% GQ flour. The antioxidant activity against DPPH and ABTS radicals increased from 24.72 and 50.51% in SBB$_{100}$ to 35.50 and 62.97% in GQB$_{80}$, respectively. In addition, the GQB$_{80}$ exhibited more antioxidantactivity than the vegan burger sample made with 100% GQ (GQB$_{100}$) or 100% SB(SBB$_{100}$). This suggests that the combination of the compounds may exhibit more antioxidant activity than either of them alone. In similar lines, Ujiroghene et al. (2019) found that germinated quinoa yoghurt showed high levels of antioxidant capacity when tested using DPPH, FRAP, ABTS, and ORAC assays. The concentration of total phenols increased as the germination period increased (Ramos-Pacheco et al., 2024). During germination, secondary metabolites—which include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, and lignin—may undergo changes that raise their content and antioxidant activity (Enciso-Roca et al., 2021).

TABLE 2. Chemical properties of post-cooked vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour.

Vegan burger treatments	Chemical Properties				
	Moisture	Protein	Fat	Fiber	Ash
	-- (%) --				
SBB$_{100}$	2.05±0.21^{a}	16.71±0.71^{a}	27.23±0.85^{a}	10.89±0.61^{a}	4.21±0.11^{a}
GQB$_{20}$	2.21±0.18^{a}	14.43±0.62^{b}	25.65±0.93^{ab}	9.76±0.42a^{b}	3.41±0.08^{b}
GQB$_{50}$	2.18±0.13^{a}	12.43±0.23^{bc}	22.57±1.31^{bc}	9.40±0.48^{ab}	3.08±0.10^{bc}
GQB$_{80}$	2.12±0.11^{a}	11.33±0.69^{cd}	19.91±0.56^{c}	9.36±0.31^{ab}	2.70±0.11^{c}
GQB$_{100}$	2.04±0.09^{a}	9.62±0.43^{d}	19.07±0.65^{c}	9.18±0.29^{b}	2.12±0.21^{d}

The same-letter means (n = 3, ±SD) do not differ significantly at $P \leq 0.05$.
See footnotes at the bottom of Table 1

TABLE 3. Antioxidant activities of post-cooked vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour.

Vegan burger treatments	DPPH scavenging activity (%)	ABTS scavenging activity (%)
SBB$_{100}$	24.72±1.37^{c}	50.50±2.06^{c}
GQB$_{20}$	26.86±2.49^{bc}	55.89±3.76^{bc}
GQB$_{50}$	28.14±1.65^{bc}	57.78±3.49^{ab}
GQB$_{80}$	35.50±1.54^{a}	62.97±3.39^{a}
GQB$_{100}$	31.43±2.21^{ab}	55.61±2.41^{bc}

The same-letter means (n = 3, ±SD) do not differ significantly at $P \leq 0.05$.
See footnotes at the bottom of Table 1

Physical properties

The parameters, such as physical attributes (Cooking loss, Shrinkage in size and texture profile), can be used to evaluate a food product's quality.Table 4 showed that GQB_{100} had a higher cooking loss than other vegan burgers; however, the difference was only statistically significant when compared to GQB80 ($P \leq 0.05$). This implies that the internal structural network of SBB_{100} was more effective in retaining moisture during cooking when compared to GQB patties. Therefore, the presence of SB flour with GQ could reduce loss during cooking.The higher WHC in SBB_{100} patties is probably due to the higher concentration of soy-based water-soluble proteins (Samard and Ryu, 2019). Conversely, shrinkage in size was the lowest in GQB_{100} followed by that in GQB_{80}. This means that both GQB_{100} and GQB_{80} are more stable against shrinkage in size than other burger samples (SBB_{100}, GQB_{20}, and GQB_{50}). The shrinkage in size percentage ranged between 1.61 and 4.54% Whig was lower than that reported by Bakhsh et al. (2021) in plant-based meat analog. According to earlier research, adding fiber and non-meat proteins could help prevent shrinkage and weight loss after cooking (Gujral et al., 2002).

Table 5 given the texture profile attributes of vegan burgers, including hardness, cohesiveness, springiness, and chewiness. The hardness, cohesiveness, and springiness of SBB_{100} was the highest ($P \leq 0.05$), which significantly decreased as the level of replacement with GQ flour increased ($P \leq 0.05$). The values of hardness, cohesiveness, and springiness decreased from 380.34N, 1.97 and 0.82mm on SBB_{100} to 261.40 N, 0.85and 0.615mm in GQB_{100}, respectively. Similar, the hardness decrease from 292 to 252 N when SB concentrated replaced with bulgur flour in beef burgers (Abd-El-Aziz et al., 2018). These findings could be explained by SB flour higher protein and fiber content than GQ flour (Table 2). Because of the higher protein content, the network formation may be stronger inside, increasing its ability to resist compression and decreasing its hardness, cohesiveness, and springiness (Bakhsh et al., 2021). Conversely, the chewiness of the vegan burger increased as the concentration of GQ flour increased

TABLE 4. Cooking loss and shrinkage in size of post-cooked vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour.

Vegan burger treatments	Cooking Loss (%)	Shrinkagein Size (%)
SBB_{100}	24.44 ± 0.64^{ab}	3.53 ± 0.29^{a}
GQB_{20}	24.18 ± 0.29^{ab}	4.54 ± 0.31^{a}
GQB_{50}	23.97 ± 1.4^{ab}	4.45 ± 0.19^{a}
GQB_{80}	23.14 ± 0.90^{b}	3.07 ± 0.40^{ab}
GQB_{100}	25.24 ± 0.37^{a}	1.61 ± 0.35^{b}

The same-letter means (n = 3, ±SD) do not differ significantly at $P \leq 0.05$.

See footnotes at the bottom of Table 1

TABLE 5.Texture profile analysis of post-cooked vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour.

Vegan burger treatments	Texture Profile Analysis			
	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (N*mm)
SBB_{100}	380.34 ± 0.88^{a}	1.97 ± 0.06^{a}	0.82 ± 0.09^{a}	68.27 ± 1.11^{e}
GQB_{20}	342.07 ± 1.84^{b}	1.48 ± 0.05^{b}	0.78 ± 0.06^{b}	98.77 ± 2.20^{c}
GQB_{50}	292.25 ± 1.50^{c}	0.95 ± 0.03^{c}	0.71 ± 0.06^{c}	106.19 ± 2.29^{b}
GQB_{80}	281.55 ± 2.37^{d}	0.88 ± 0.05^{d}	0.66 ± 0.07^{d}	110.73 ± 4.53^{a}
GQB_{100}	261.40 ± 1.80^{e}	0.85 ± 0.03^{d}	0.62 ± 0.09^{e}	85.72 ± 1.55^{d}

The same-letter means (n = 3, ±SD) do not differ significantly at $P \leq 0.05$.

See footnotes at the bottom of Table 1

Sensory evaluation

Table 6 displays the sensory evaluation of GQB prepared with various proportions of GQ as flour a replacement for SB flour in SBB_{100} as a control. GQB samples were evaluated using sensory methods to quickly determine if there were noticeable differences in appearance, taste, juiciness, flavor, and overall acceptability compared with SBB_{100}. The addition of GQ flour as a SB flourr eplacement generally significantly improved all sensory attributes when compared to SBB_{100} samples ($P \leq 0.05$). The improvement in sensory properties was proportional to the replacement ratio. GQB samples have a more appetizing flavor, desirable color, palatable taste, and juicy texture. The juiciness and overall acceptability scores of the vegan burger made by completely replacing GQ flour (GQB_{100}) for SB flour were the highest, but there were no appreciable variations between the GQB made by replacing GQ for 80 (GQB_{80}) or 100% (GQB_{100}) of the SB flour.

Biological activities

Body weight gain, feed intake, and feed efficiency ratio

Table 7 shows the body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of diabetic rat groups fed post-cooked GQB and/ or SBB_{100} diets in comparison to normal (Control -ve) and diabetic (Control +ve) rats fed the basal diet for 45 days.The normal group had the highest BWG (78.4), FI (23.0), and FER (3.41) compared to all diabetic rat groups fed on vegan burgers ($P < 0.05$). Inversely, the diabetic group showed body weight loss (-18.7), negative FER (-1.10), and the lowest FI (17.0) ($P < 0.05$).When a person has diabetes, which prevents the body from absorbing glucose from the blood into the cells for energy consumption, the body starts burning fat and muscle for energy, which lowers overall body weight. The kidneys also attempt to expel high glucose levels through urine. Dehydration and the loss of calories from the sugar that wasn't used as energy result in weight loss (Gardner, 2016).

TABLE 6.Sensory evaluation of post-cooked vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour.

Vegan burger treatments	Sensory Attributes				
	Appearance	Taste	Juiciness	Flavor	Overall Acceptability
SBB_{100}	7.66 ± 0.18^{c}	7.83 ± 0.26^{b}	7.50 ± 0.28^{c}	7.50 ± 0.20^{c}	7.66 ± 0.22^{c}
GQB_{20}	8.33 ± 0.32^{b}	7.66 ± 0.36^{b}	7.83 ± 0.27^{bc}	7.75 ± 0.15^{bc}	7.66 ± 0.18^{c}
GQB_{50}	8.60 ± 0.30^{ab}	9.00 ± 0.19^{a}	8.33 ± 0.18^{ab}	8.16 ± 0.18^{b}	8.00 ± 0.23^{bc}
GQB_{80}	9.00 ± 0.40^{a}	9.00 ± 0.11^{a}	8.33 ± 0.22^{ab}	9.00 ± 0.22^{a}	8.33 ± 0.16^{ab}
GQB_{100}	8.83 ± 0.17^{ab}	9.00 ± 0.21^{a}	8.83 ± 0.18^{a}	9.00 ± 0.21^{a}	8.66 ± 0.16^{a}

The same-letter means (n = 3, ±SD) do not differ significantly at $P \leq 0.05$.
See footnotes at the bottom of Table 1

TABLE 7. Body weight gain, feed intake, and feed efficiency ratio of diabetic rats fed on vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flourfor 45 days.

Rat groups	Body weight gain (g)	Feed intake (g/day)	Feed efficiency ratio
Control -ve	78.4 ± 2.73^{a}	23.0 ± 1.00^{a}	3.41 ± 0.33^{a}
Control+ve	-18.7 ± 2.24^{e}	17.0 ± 0.44^{c}	-1.10 ± 0.07^{f}
SBB_{100}	4.6 ± 1.02^{d}	20.4 ± 0.74^{ab}	0.23 ± 0.10^{e}
GQB_{20}	17.4 ± 2.13^{c}	21.4 ± 0.50^{ab}	0.81 ± 0.09^{d}
GQB_{50}	23.9 ± 1.98^{c}	19.8 ± 0.66^{b}	1.21 ± 0.17^{c}
GQB_{80}	27.4 ± 1.93^{c}	19.6 ± 1.43^{b}	1.40 ± 0.14^{bc}
GQB_{100}	34.0 ± 2.08^{b}	21.4 ± 1.02^{ab}	1.59 ± 0.26^{b}

The same-letter means (n = 5, ±SD) do not differ significantly at $P \leq 0.05$.
See footnotes at the bottom of Table 1

Feeding SBB_{100} sample improved BWG, FI, and FER compared to the positive group ($P \leq 0.05$). However, the improvement was more noticeable when GQ flour was used as a replacement of SB in GQB ($P \leq 0.05$); the improvement increased with the proportion of substitution. The diabetic rats fed GQB_{100}exhibited the greatest recovery.These findings are similar to those found by Barakat (2011) and Vega-Gálvez et al. (2010), who showed that consumption of quinoa plays a role in regulating energy homeostasis and maintaining body weight balance.

Glucose and insulin concentration

Changes in the serum glucose and insulin levels in the diabetic rats groups fed on SBB_{100} and GQB their combination flours for 45 days are presented in Table 8. Compared to the negative control group, the diabetic rats in positive group had a significantly higher blood glucose level (188.67mg/dL). The levels of glucose and insulin in blood serum gradually decreased in diabetic rats as GQ flour increased in the GQB compared to the control-positive group. However, the level of serum glucose was still significantly higher ($P < 0.05$) than that in the control normal group. The percent of insulin reduction was 30.99, 33.63, and 34.68% for the groups fed on GQB_{100}, SBB_{100}, and QBB_{80}, respectively. Quinoa has a wide range of compounds that may contribute to its hypoglycemic effects. Due to its ability to promote satiety, fiber may modify the postprandial insulin response. Additionally, quinoa protein, which slows down digestion, has also been linked to a low glycemic index (Graf et al., 2015). Furthermore, a particular class of polyhydroxylated steroids is responsible for this bioactivity. The amount of TPCs and tocopherols contained in the cereal bar's daily consumption was the main factor contributing to the drop in blood sugar(Pas´ko et al., 2010). Boath et al. (2012) reported that tannins, anthocyanin, and various polyphenols inhibit α-glucosidase and α-amylase, which slows down the rise in blood glucose that occurs after a meal. Quercetin, kaempferol, and their derivatives are among the flavanol-type flavonoids that make up the majority of the polyphenols in quinoa (Balakrishnan and Schneider, 2020). Furthermore, the process of germination leads to a notable increase in the overall phenolic content and antioxidant activity of quinoa, as reported by Ibrahim and Mohamed (2021), Bhinder et al. (2021).

Lipid profile

As shown in Table 9, triglycerides, total cholesterol, LDL-cholesterol, and VLDL-cholesterol were considerably elevated ($P \leq 0.05$) in diabetic rats (positive group), while the levels of HDLC-cholesterol were decreased ($P \leq 0.05$) when compared to control rats (negative group). Elevated blood glucose and insulin resistance are associated with high triglyceride levels. High triglyceride indicates insulin resistance; this is when cells (such as muscle cells) that normally respond to insulin become resistant to it. This prevents insulin from allowing cells to absorb glucose, requiring higher and higher levels of insulin. This leads to blood sugar levels higher than normal (Hartz et al., 2018). The lipid profile of diabetic rats was improved when rats were fed on SBB_{100} or when SB flour was replacement with GQ at different levels in vegan burgers, as long as the replacement ratio was at least 50% ($P < 0.05$). Furthermore, results showed that feeding GQB_{100} was the most effective strategy to improve the lipid profile, providing the group's level closest to that of the negative control. Similar findings were found by Alamri et al. (2023) in diabetic rats treated at concentrations of 25% quinoa seeds. According to Aniess et al. (2020), the high fiber content of quinoa improves the breakdown of cholesterol by binding to bile acid. Additionally,

TABLE 8. Glucose and insulin levels of diabetic rats fed on vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour for 45 days.

Rat groups	Glucose (mg/dL)	Insulin (mIU/L)
Control -ve	105.79 ± 4.51^{e}	16.78 ± 0.28^{e}
Control+ve	188.67 ± 6.21^{a}	34.58 ± 0.41^{a}
SBB_{100}	138.26 ± 2.58^{c}	28.45 ± 0.54^{b}
GQB_{20}	148.55 ± 4.19^{b}	27.94 ± 0.22^{b}
GQB_{50}	133.15 ± 2.35^{c}	25.57 ± 0.57^{c}
GQB_{80}	118.52 ± 3.65^{d}	19.85 ± 0.31^{d}
GQB_{100}	114.94 ± 4.51^{d}	17.89 ± 0.34^{e}

The same-letter means (n = 5, ±SD) do not differ significantly at $P \leq 0.05$.

See footnotes at the bottom of Table 1

the fermentation of fiber in the colon results in the production of short-chain fatty acids and decreases the synthesis of cholesterol in the liver.However, the addition of GQ flour at low concentrations (GQB_{20}) had adverse effects on lipid profile levels compared to diabetic rats fed on SBB_{100}.

Kidney functions

STZ injection into rats significantly (P≤0.05) increased the mean values of serum urea, creatine, and uric acid as compared to the control negative group. These parameters are considered important signs leading to renal dysfunction (Fekete et al., 2008). Moreover, it seems that the diabetic complications in the kidney are likely to be associated with alterations in enzyme levels (Zafar et al., 2009). In general, feeding diabetic rats with vegan burgers significantly (P ≤ 0.05) decreased the mean values of serum urea, creatine, and uric acid as compared to the control-positive group (Table 9). There were no significant changes (P > 0.05) in the mean values of serum urea, creatine, and uric acid among the rats fed on QB burgers with concentrations of 50, 80, and 100%. The addition of QB flour at levels of 80 and 100% improved kidney function, and there was no significant difference observed for serum uric acid, creatine, or urea levels among the rats in these groups and the control negative group (Table 9). Similar to lipid profile, but less marked; the addition of GQ flour at low concentrations (20%) had adverse effects on uric acid and urea levels.This suggests that it is best to add at least 50% GQ flour to the vegan burger.The improvement of kidney functions in diabetic rats may be due to some vitamins, minerals, and fibers in quinoa, such as selenium, magnesium, folic acid, and tocopherol, which act as antioxidants for renal cell membranes (Altunkaynak et al., 2008; Ibrahim and Mohamed, 2021).

Liver functions

The three enzymes that are most commonly used to diagnose liver damage are alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The marked increased release of AST, ALT, and ALP indicates severe damage to liver tissue membranes. As shown in Table 10, STZ injected into rats increased significantly (P < 0.05) serum AST, ALT, and ALP. These findings confirmed those of Arkkila et al. (2001), who demonstrated a correlation between certain liver enzyme changes and problems related to diabetes.Higher ALP levels in type 2 diabetic patients may be a risk factor for hepatic fibrosis (Kocabay et al., 2011). The high blood glucose level that causes oxidative stress and inflammation in the blood may be the reason for the raised serum transaminases (Alamri et al., 2023). When diabetic groups were fed a vegan burger, both serum AST and ALP levels significantly decreased. The addition of GQ at a concentration of 50% or higher resulted in a significant decrease in the serum ALT level as well (P < 0.05). When 100% SB flour (SBB_{100}) was replacement with GQ flour (GQB_{100}), the blood levels of AST, ALT, and ALP dropped to nearly the normal range of the negative group. These findings are consistent with those of Cao et al. (2020), who showed that feeding rats fed a high-fat diet quinoa for eight weeks enhanced liver tissue and the levels of transaminases like ALT and AST. It might be because quinoa seeds contain significant concentrations of dietary fiber, minerals, proteins, and bioactive substances which protect the liver from oxidative damage brought on by elevated blood sugar (Alamri et al., 2023).

TABLE 9. Kidney functions in diabetic rats fed on vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour for 45 days.

Rat Groups	Urea (mg/dL)	Creatine (mg/dL)	Uric acid (mg/dL)
Control - ve	21.33 ± 1.15^{c}	0.95 ± 0.05^{d}	3.66 ± 0.35^{c}
Control +ve	32.66 ± 1.52^{a}	1.24 ± 0.11^{a}	4.56 ± 0.41^{a}
SBB_{100}	23.46 ± 0.58^{bc}	1.19 ± 0.06^{ab}	4.15 ± 0.36^{abc}
GQB_{20}	25.43 ± 0.91^{b}	1.15 ± 0.03^{b}	4.36 ± 0.45^{ab}
GQB_{50}	22.08 ± 1.09^{c}	1.02 ± 0.08^{c}	4.23 ± 0.40^{ab}
GQB_{80}	22.91 ± 1.41^{c}	1.00 ± 0.09^{cd}	4.00 ± 0.12^{abc}
GQB_{100}	21.77 ± 1.52^{c}	0.96 ± 0.07^{cd}	3.85 ± 0.19^{bc}

The same-letter means (n = 5, ±SD) do not differ significantly at P ≤ 0.05.
See footnotes at the bottom of Table 1

Serum lipid peroxidation

Malondialdehyde (MDA) is one of the main by-products of lipid peroxidation associated with the development of several chronic diseases. Glutathione peroxidase (GPx) possesses a single redox-sensitive selenocysteine amino acid, which is important for the enzymatic reduction of soluble lipid hydroperoxides (Handy and Loscalzo, 2022). According to Lekshmi et al. (2015), oxidative stress increases in diabetes lead to lipid peroxidation and increased MDA formation. The same trend was observed in the serum MDA and GPx concentrations when comparing the positive group with the negative group.In general, feeding a vegan burger caused a significant decrease in the serum MDA and Gpx concentrations in all diabetic groups ($P \leq 0.05$); the decrease was more pronounced when GQ flour was added at ≥ 50% from SB flour (Table 11). The decrease in serum MDA and GPx concentrations in the diabetic group fed on GQB_{100} (59.26nmol MDF/mL and 84.67 GPx u/mL) was very close to the negative control (22.62nmol MDF/mL and 49.96 GPx u/mL).The antioxidant compounds found in GQ flour, such as phenols, flavonoids, vitamin E, and others, may act as scavengers for free radicals by donating a hydrogen atom (H) to the hydroxyl groups in the radical chain reaction, preventing oxidative stress in hyperglycemia (Foti, 2007).

TABLE 10. Liver functions in diabetic rats fed on vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour for 45 days.

Rat groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control - ve	37.14 ± 1.46^{d}	40.19 ± 1.68^{c}	100.35 ± 1.64^{c}
Control +ve	49.26 ± 2.34^{a}	45.78 ± 1.36^{a}	118.11 ± 1.33^{a}
SBB_{100}	40.89 ± 1.40^{c}	43.32 ± 1.69^{ab}	104.52 ± 0.74^{b}
GQB_{20}	45.94 ± 2.04^{b}	44.50 ± 1.71^{ab}	104.60 ± 3.11^{b}
GQB_{50}	44.31 ± 1.09^{b}	42.53 ± 1.53^{bc}	102.22 ± 2.97^{bc}
GQB_{80}	41.38 ± 0.91^{c}	42.38 ± 1.78^{bc}	102.26 ± 2.00^{bc}
GQB_{100}	37.27 ± 1.84^{d}	40.43 ± 0.72^{c}	100.93 ± 3.18^{c}

The same-letter means (n = 5, ±SD) do not differ significantly at $P \leq 0.05$.
See footnotes at the bottom of Table 1

TABLE 11. Malondialdehyde and glutathione peroxidase concentrations in diabetic rats fed on vegan burgers made with different levels of germinated quinoa seed flour as a replacement for soybean flour for 45 days.

Rat groups	MDA (nmol/mL)	GPx (u/mL)
Control - ve	22.62 ± 2.81^{g}	49.96 ± 2.73^{g}
Control +ve	59.26 ± 0.98^{a}	84.67 ± 3.43^{a}
SBB_{100}	45.27 ± 0.88^{c}	77.58 ± 2.80^{c}
GQB_{20}	51.02 ± 2.70^{b}	80.29 ± 2.05^{b}
GQB_{50}	37.71 ± 1.43^{d}	61.01 ± 1.24^{d}
GQB_{80}	32.98 ± 2.04^{e}	55.33 ± 3.03^{e}
GQB_{100}	25.53 ± 2.12^{f}	52.98 ± 2.63^{f}

The same-letter means (n = 5, ±SD) do not differ significantly at $P \leq 0.05$.
See footnotes at the bottom of Table 1

Conclusion

The germination process of quinoa seeds produces a high content of phenolic compounds and antioxidant activity, which reducesanti-nutritional factors (Ibrahim and Mohamed, 2021). Using GQ flour as a partial substitute for SB flour in the production of vegan burgers with high nutritional value leads to an increase in its antioxidant content and leads to a significant improvement in the physical qualities of the product itself. Partial or complete replacement of GQ flour for SB flour improves the texture and organoleptic qualities of the vegan burger product. From here GQB can be adopted as a functional diet for diabetics, especially when consumed with a replacement rate of at least 80% to achieve the highest possible biological activity, which enhances body functions and reduces the risks related to diabetes.

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