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CONTENTS

Suitability of Quinoa Grains (*Chenopodium Quinoa Willd.*) for development of Low Glycemic Index Biscuits

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ABSTRACT: The super grain quinoa has high protein content with a balanced amino acid profile, as well as high fiber content, essential fatty acids, vitamins, and minerals. Studies suggest that it can help diabetic people in managing their blood glucose levels and may prevent other diseases. The goal of the current study was to examine the physical, functional, and nutritional quality of quinoa and to elucidate how quinoa-incorporated biscuits affected blood sugar levels. The physical properties of quinoa grains showed that thousand kernel weight, thousand kernel volume and hydration capacity were 2.433g, 2.46 ml and 0.012 g, respectively. Pericarp color of quinoa grain was pale yellow and bulk density of quinoa grains was 0.750 g/cc. The functional properties of quinoa flour revealed that water absorption capacity, fat absorption capacity, emulsifying capacity and emulsion stability were 136 %, 43 %, 76 % and 40%, respectively. According to the proximate composition of quinoa flour, its moisture, total ash, crude protein, crude fat, crude fiber, carbohydrate and physiological energy values were 10.28%, 2.19%, 13.52%, 5.25%, 3.44%, and was 362 kcal/100g. Biscuits incorporating quinoa were found to contain 4.75% of moisture, 2.33% of total ash, 8.51% of crude protein, 3.12% of crude fat, and 21.3% of crude fiber, 59.9% of carbohydrate and 465 kcal/100g of physiological energy. The corresponding values for control biscuits made with refined wheat flour were 4.32%, 1.24%, 5.86%, 22.1%, 1.2%, 65.28%, and 482 kcal/100g respectively. Using refined wheat flour, the glycemic index of the biscuits was 61.7, whereas using quinoa, it was 46.1. Low glycemic index of formulated biscuits indicates its suitability in prevention and control of diabetes.

Key words: Diabetes, glycemic index, low GI biscuits, quinoa

Presently, world is facing two types of nutritional problems *viz.* nutrient deficiency diseases and degenerative diseases. Protein energy malnutrition and degenerative illnesses such as diabetes, cardiovascular diseases, cancer, arthritis, metabolic syndrome, and high blood pressure are a dual burden in India. Chronic degenerative diseases account for more than 71% of all fatalities in India (WHO, 2021). Cardiovascular disorders account for 34.3% of all fatalities as the leading causes of death in India followed by chronic respiratory disease (22%), cancer (12%), and diabetes (3%)(Arokiasamy, 2018). Diabetes is a serious health issue that is linked to a number of other degenerative conditions. In India, it is a significant cause of illness and mortality. Type 2 diabetes is becoming more common. Currently, there are 422 million diabetic patients in the world and 74 million people are living with diabetes in India (WHO, 2021). The health risk has doubled as a result of increased consumption of refined grains. This is related to an increased risk of insulin resistance as well as metabolic syndrome symptoms including low HDL cholesterol levels (Hazard *et al.,* 2020).Consumption of whole grains, high fibre foods and low GI diets has protective action against all these illnesses(Slavin, 2004).

The concept of measuring the blood glucose response to the consumption of foods high in carbohydrates is known as the glycemic index (GI). This idea was initially presented as a ranking scheme for carbohydrates based on how they would immediately affect blood glucose levels (Jenkins *et al.,* 1981)and further GI was developed for diabetics as a dietary selection guide (Jenkins *et al.,* 1983). Due to the lower glycemic response after consumption compared to high GI foods, lower GI foods were thought to be beneficial. Later on, various studies on low glycemic index diets showed that these foods have multiple favourable effects other than lowering of blood glucose. Studies have demonstrated that low glycemic index diets have favourable effect on weight management(Van Dam and Seidell, 2007), reduction in circulatory triglyceride levels specially raising HDL cholesteroland improvement of blood pressure (Ford and Liu, 2001). Therefore,it has been suggested that a low-glycemic index diet will likewise have a positive impact on fibrinolytic activity (Jarvi *et al.,* 1999).

Quinoa (*Chenopodium quinoa Willd.*) is an ancient grain crop which comes under *Chenopodiaceae* family. It is an incredibly nourishing food crop. Quinoa grains contain 77.6 % carbohydrate, 6.5 % fat and 13.8%- 16.5 % protein (USDA, 2015). Because it contains all the essential amino acids for humans, quinoa proteins are regarded as high-quality proteins (Maradini *et al.,* 2015).Quinoa also has a balanced high lysine and methionine content. Both dietary fiber and omega-3 fatty acids are abundant in it (Ando *et al.,* 2002).Compared to other cereals, quinoa has much greater levels of potassium, calcium, magnesium, phosphorus, and iron (Konishi *et al.,* 2004). Higher intake of fibre and protein are two important dietary factors required for blood sugar regulation. Quinoa contains diverse range of anti-inflammatory nutrients which makes it suitable for reducing the risk of diabetes. Quinoa is capable of lowering cholesterol level and also helps to maintain HDL cholesterol levels(Farinazzi-Machado *et al.,* 2012).The anti-inflammatory nutrients of quinoa have the ability to protect human blood vessels from inflammation(Yao *et al.,* 2014). Such kind of defence provides reduced risk of many CVDs, including atherosclerosis. The anti-inflammatory and antioxidant phytochemicals of quinoa are also helpful in lowering the risk of cancer in humans(Pasko *et al.,* 2010). Other important benefits of quinoa involve decreased risk of allergy especially for individuals allergic to gluten(Zevallos *et al.,* 2014).Several compounds with nutraceutical benefits are also found in quinoa such as polyphenols, flavonoids, phytosterols and high quality lipids(Navruz-Varli and Sanlier, 2016).

In developing countries like Asia and Africa, quinoa possesses potential ability to provide high nutrition under drought conditions. In India rapid urbanization and industrialization has lead to increased consumption of ready to eat foods. Biscuits are one of such items. The major drawback with variety of biscuits is that they are made up of refined wheat flour thus, contain very low amount of dietary fibre, minerals and increase the load of fat and sugar in the body. Quinoa can very well be utilized in formulation of biscuits.Therefore, this study was conducted to formulate low GI biscuits with quinoa flour and to see its effect on blood glucose response on young healthy subjects.

MATERIALS AND METHODS

Raw materials collection and preparation

Quinoa grains (*Chenopodium quinoa Willd.*)were purchased from Organic India Pvt. Ltd, Lucknow, Uttar Pradesh, India. Gluten was purchased from the Rich Cream Pvt. Ltd. Kashipur, Uttarakhand, India. Guar gum powder was purchased from the Ganpati Global Pvt. Ltd. Jaipur, Rajasthan, India. Soy lecithin was purchased from the Urban Platter Gourmet Grocery Store Mumbai, Maharashtra, India while other ingredients were obtained from Pantnagar's (Uttarakhand) local market.

Physical properties of quinoa grains

Physical characteristics of raw quinoa grains like thousand kernel weight, thousand kernel volume and hydration capacity were analyzed as per the method of Williams *et al.* (1983). Bulk density was measured as per the method given by Narain *et al.* (1978) and pericarp color was determined using Munsell Soil Colour Chart (Munsell, 1954).

Preparation of quinoa flour

In order to get rid of dirt, stones, and other contaminants, quinoa grains were cleaned. Vivek Mandua Thresher cum Pearler with 1hp motor (VPKAS) was used to dehusk the quinoa grains. To eliminate saponins, the grains were washed by rubbing them under running water until no froth formed. Washed grains were dried in a tray dryer oven for six hours at 50 ± 2°C. After drying, the grains were ground (Philips, HL7505) and put through a screen with a mesh size of 60 to achieve a homogeneous particle size. Thereafter, the flour was packaged and kept refrigerated until further usage (Malik *et al.,* 2021).

Functional properties of quinoa flour

Quinoa flour was used to determine functional properties like water absorption and fat absorption capacity (Lin *et al.,* 1974). Emulsifying capacity and emulsion stability were also analyzed as per the method given by Yasumatsu *et al.* (1972).

Preparation of low glycemic index biscuits

Biscuits were formulated as per the method mentioned by Malik *et al.* (2021)(Figure 1). The incorporation of quinoa flour was 50% in the formulated biscuits. Refined wheat flour (100%) biscuits were also prepared for the comparison.

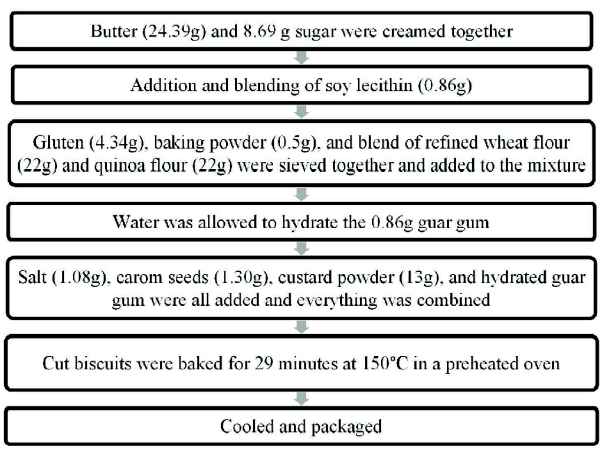


Fig. 1: Flow chart for preparation of quinoa flour incorporated biscuits

Nutritional quality evaluation

The proximate composition of quinoa flour as well as quinoa flour incorporated biscuits and refined wheat flour biscuits (control biscuits) were analyzed. Moisture, ash, protein, crude fiber, and fat content were assessed using the procedures recommended by the AOAC (1995). Carbohydrate content was determined by difference method. By multiplying the percentages of protein, fat, and carbohydrates in the sample by 4, 9, and 4 correspondingly, the physiological energy value was determined.

Evaluation of glycemic index

From the university's girls hostel, ten healthy subjects between the ages of 22 and 25 who had a body mass index between 19.43 and 22.16 kg/m^2 and were free of any illnesses were randomly chosen. Each subject was given a written explanation of the study's objective and written consent was taken from each participant. Throughout the study time, the subjects were instructed to refrain from any physical activity, take any medications, fasting and feasting. As all the subjects were sharing same hostel kitchen for food, their dietary pattern was almost same. They were instructed to take their daily diets. Food intake of the subjects was assessed by three day estimated food record method (Gibson, 1990). Besides, the subjects were asked for keeping record, if any additional food item was taken by them. Nutrients from food intake were calculated (Longvah *et al.*, 2017).

The subjects who had fasted overnight underwent the glucose tolerance test (GTT) with a 50 g glucose load. Blood sugar levels were checked at 0, 30, 60, 90, 120, and 150 minutes. Every alternate morning, 83 g of quinoa flour incorporrated biscuits and 76.5 g of biscuits made with refined wheat flour containing 50 g of accessible carbohydrate each were supplied. The participants were instructed to finish their meals in 10 to 15 minutes. The contour TS glucometer was used to perform finger prick blood glucose measurements at predetermined intervals. The glucose-oxidase method was used to measure blood glucose. The respondents were questioned about any side effects they may have had from eating the biscuits. Using the following formula (Wolever, 1990), the incremental area under the blood glucose response curve (iAUBGR) was calculated:

$$\frac{\Delta_0 30t}{2} + \Delta_0 30t + \frac{(\Delta_0 60 - \Delta_0 30)t}{2} + \Delta_0 60t + \frac{(\Delta_0 90 - \Delta_0 60)t}{2} + \Delta_0 90t + \frac{(\Delta_0 120 - \Delta_0 90)t}{2} + \Delta_0 120t + \frac{(\Delta_0 150 - \Delta_0 120)t}{2} + \Delta_0 150t \ldots etc$$

where, Δ_0 is change in blood sugar in mmol/l from the starting point to 30, 60, 90, 120, 150, and so forth.

The time interval between blood samples is denoted by t.

The formula provided by Wolever (1990) was used to compute the glycemic index of food items.

$$GI = \frac{Incrementalareaunderbloodglucoseresponsecurveforfoodproducts}{Correspondingareaafterequicarbohydrateportionofglucose} \times 100$$

The GI value of each type of biscuit was determined using the average GI of 10 replicates.

Statistical analysis

For nutritional quality, physical properties, and functional properties, all results are shown as mean and SD (standard deviation) of three replicates. To determine the significant difference between the glycemic index of the quinoa incorporated biscuits and the control refined wheat flour biscuits, a one-way ANOVA was utilized.

RESULTS AND DISCUSSION

Physical properties of quinoa grains

Determination of physical properties of grains helps in identifying varietal differences of the same species and differences with other food grains as well. Genetic and environmental conditions are the two major factors which largely affect the physical properties of grains (Hadjichristodoulou, 1990).Results pertaining to physical properties of quinoa grains have been presented in Table 1.

In this study the color of quinoa grains was pale yellow. According to James (2009), quinoa grains are often a light yellow tint, yet they can also be pink or black. The color of quinoa grains can vary from white to beige to gold to even red depending on variety, environmental factors, and soil type (Vega-Galvez *et al.,* 2010).

Table 1: Physical properties of quinoa grains

Physical properties	Mean±S.D.
Thousand kernel weight (g)	2.43 ± 0.015
Thousand kernel volume (ml)	2.46 ± 0.057
Hydration capacity (ml)	0.012± 0.004
Pericarp color	Pale yellow
Bulk density (g/cc)	0.75± 0.009

*All results are mean± standard deviation of three values

Table 2: Proximate composition of quinoa flour

	Quinoa flour
Moisture (%)	10.28± 0.01
Crude protein (%)	13.52± 0.07
Crude fat (%)	5.25± 0.04 5
Crude fibre (%)	3.44± 0.02
Total ash (%)	2.19± 0.01
Carbohydrate (%)	65.33±0.11
Physiological energy (Kcal/100g)	362±0.03

*All results are mean± standard deviation of three values

Thousand kernel weight (W_{1000}) measured for quinoa grains was 2.43g. According to Vilche *et al.* (2003), W_{1000} values ranged from 2.5 to 3.11 g. Beniwal *et al.*(2019) also reported the similar value of 2.57g. However, slightly higher value of 4.13g was reported by Contreras-Jimenez *et al.* (2019) in their study. W_{1000} is associated with the endosperm content of the grain. Other factors affecting the W_{1000} are variety, size and moisture content of grain (Beniwal *et al.,* 2019).

Thousand kernel volume of quinoa grains was evaluated as 2.46 ml. Similar values ranging from 1.63 to 2.87 ml were reported by Altuntas *et al.* (2018). Slightly higher 1000 kernel volume of 2.86 ml was reported by Sindhu *et al.* (2019)which is also higher than the another pseudo cereal amaranth.
Bulk density of quinoa grains in the present study was 0.75 g/cc which was equivalent to the value (0.76g/cc) reported by Sindhu *et al.* (2019).Vilche *et al.*(2003) also observed bulk density of quinoa seeds ranging from 0.66 to 0.74g/cc, depending on the varying moisture content (4.6–25.8%). Bulk density of food grains affects various aspects like storage, packaging, transportation thus ultimately affecting cost involved in the whole post harvest process.

Functional properties of quinoa flour

The functional properties of flour are crucial in the production of food products. The functional properties of flour play important role in the manufacturing of products. Functional properties greatly affect textural quality, sensory properties and storage life of final food product. Quinoa flour's water absorption capacity (WAC) in the current investigation was 136%; this value is higher than that reported by Beniwal *et al.* (2019) but lower than that for amaranth flour (153%). However, higher WAC value of 147% was reported by Ogungbenle (2003). WAC depends on the availability of hydrophilic groups which bind water molecules (Kulkarni *et al.*, 2002).There are various factors that

Table 3: Proximate composition of refined wheat flour biscuits and quinoa flour incorporated biscuits

Proximate composition	Control refined wheat flour biscuits	Quinoa flour incorporated biscuits	S.Em.	CD at 5%
Moisture (%)	4.32±0.27	4.75±0.18	0.29	0.10
Total ash (%)	1.24±0.18	2.33±0.25	0.25	0.85
Crude protein (%)	5.86±0.26	8.51±0.17	0.26	0.89
Crude fat (%)	22.1±0.17	21.3±0.32	0.81	0.28
Crude fibre (%)	1.2±0.43	3.12±0.19	0.35	0.12
Carbohydrates(by difference) (%)	65.28±0.18	59.9±0.16	0.32	1.12
Energy (kcal/100g)	482±4.33	465±3.76	0.38	1.31

*All results are mean± standard deviation of three values

Table 4: Blood glucose levels at different time intervals for refined wheat flour biscuits and quinoa flour incorporated biscuits against glucose

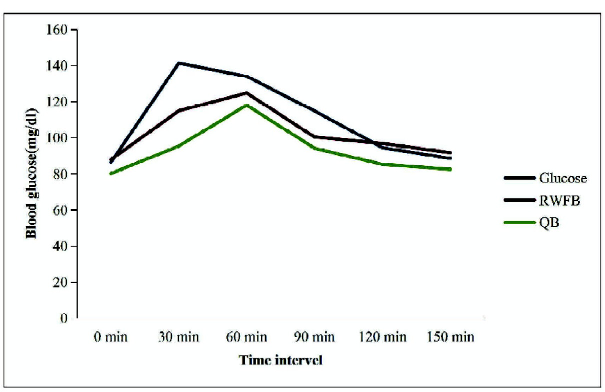
Time intervals (Minutes)	Glucose (mg/dl)	Refined wheat flour biscuits (mg/dl)	Quinoa flour incorporated biscuits (mg/dl)
0	86.3±1.24	87.87±1.43	80.1±2.62
30	141.3±3.42	114.86.1±3.24	95.4±3.77
60	134±6.31	124.8±4.63	118±6.34
90	114.8±5.73	100.37±5.68	94.2±9.54
120	94.5±3.57	96.87±3.45	85.2±3.68
150	88.54±2.52	91.7±3.61	82.5±1.84

*All results are mean± standard deviation of ten values

Table 5: Glycemic Index of refined wheat flour biscuits and quinoa flour incorporated biscuits

Food Product	Glycemic index (mean±SD)
Refined wheat flour biscuits	61.7±3.89
Quinoa flour incorporated biscuits	46.13±5.66
S.Em.	0.825
CD at 5 %	2.453

*All results are mean± standard deviation of ten values
*S.Em-standard error of mean, C.D- critical difference



RWFB- refined wheat flour biscuits, QB- quinoa flour incorporated biscuits

Fig. 2:Blood glucose response curve for refined wheat flour biscuits and quinoa flour incorporated biscuits against glucose

affect WAC of flour like; particle size, fibre content, protein content and amount of damaged starch in the flour. Fat absorption capacity of quinoa flour was slightly lower (43%) then the value of 46% reported by Ogungbenle (2003) and Oshodi *et al.* (1999). Oil absorption capacity is one of the most imperative aspects in food preparation since it imparts taste, flavor, mouth feel and enhances the palatability of the food products. Emulsifying capacity of quinoa flour was found as 76 % with an emulsion stability of 40%. However, emulsion capacity and stability reported by Ogungbenle *et al.*(2009) were 104% and 45%, respectively.

Proximate composition of quinoa flour

Proximate composition of quinoa flour has been presented in Table 2. The moisture content was determined to be 10.28% in quinoa flour, which is lower than the value of 11.20 % in quinoa flour reported by Ogungbenle(2003). Repo-Carrasco and Serna (2011) in theirstudy reported moisture content in the range of 10.78- 12.62% in quinoa flour. Stikic *et al.*(2012) also observed similar values of 10.87 % and 10.08 % in whole and dehulled quinoa seeds, respectively. Crude protein content analyzed in the current study was similar to the protein content (13.83%) observed by Rosell *et al.*(2009)but higher than the reported value of 11.32% (Collar and Angioloni, 2014)in quinoa flour. However, higher protein values of 16.7% and 17.41% were reported by Wright *et al.* (2002) and Stikic *et al.* (2012) in whole quinoa seeds, respectively which may be suggestive of protein loss during milling and sieving process.

Crude fat content of 5.25 % was observed in quinoa flour. Average oil content of quinoa is around 6 % as reported by USDA (2005). Rizzello *et al.*(2016) also reported values of 6.0% and 6.1% in quinoa flour and seeds, respectively. Ruales and Nair(1993)observed the fat content of 9·7 % in raw quinoa seeds. Ogungbenle(2003)reported the fat content of 6.3 % in quinoa flour. However, a lower value of 3.44% was reported by Collar and Angioloni(2014) in quinoa flour.

Quinoa flour showed the crude fibre content of 3.44 % which is in the range of 2.5-3.9 % reported by Valencia-Chamorro (2003).According to Stikic *et al.* (2012), whole grains of quinoa contain 10.32% crude fiber, while dehulled grains have 6.80% crude fiber. According to Villa *et al.* (2014), quinoa has 2.2% crude fiber. As compared to other reports a very high crude fibre content of 9.5 % was observed by Ogungbenle(2003) in quinoa flour.

Regarding ash content various reports were in accordance with the ash content observed in the current study ranging from 2.20% to 2.82% (Rizzello *et al.*, 2016;Wang *et al.*, 2015; Iglesias-Puig *et al.*, 2015; Axel *et al.*, 2015).

The carbohydrate content in quinoa flour was 65.33%. Prego *et al.*(1998) reported that carbohydrate content varied from 67 to 74 % in quinoa. Ogungbenle(2003)reported 58.3 % carbohydrate in quinoa flour. Villa *et al.*(2014), Schoenlechner *et al.*(2008)reported carbohydrate values of 55.3%, 64.2% and 69%, respectively in quinoa seeds.

Quinoa flour was shown to have 362 kcal of physiological energy per 100g, compared to 368 kcal of energy per 100g given by the USDA (2015).

Proximate composition of quinoa flour incorporated biscuits and refined wheat flour biscuits

The proximate composition of quinoa flour incorporated biscuits and refined wheat flour biscuits are given in Table 3.In comparison to control biscuits, which had proximate composition of 4.32%, 5.86%, 22.1%, 1.24%, 1.2%, and 65.28% for moisture, crude protein, crude fat, total ash, crude fiber, and carbohydrates, quinoa flour incorporated biscuits had moisture (4.75%), crude protein (8.51%), crude fat (21.3%), total ash (2.33%), crude fiber (3.12), and carbohydrates (59.9%)(Malik *et al.*, 2021).

Glycemic Index

The subjects consumed an average of 296.43g of carbohydrates, 45.23g of protein, 39.45g of fat, and 1717 kcal per 100g, respectively. The blood glucose response to the ingestion of foods containing carbohydrates in proportion to glucose is described by the glycemic index. Table 4 displays the increase in blood sugar levels following consumption of glucose, control biscuits and quinoa flour incorporated biscuits. After consuming glucose, the blood glucose level peaked after 30 minutes, whereas it took 60 minutes for the blood glucose level to peak after consuming control and quinoa biscuits (Figure 2). Area under blood glucose response curve was found to be significantly higher for glucose (5820 mg min/100ml), followed by refined wheat flour biscuits (3590 mg min/100 ml) and quinoa flour incorporated biscuits (2685 mg min/100ml).The glycemic index of biscuits made with refined wheat flour (61.7) and quinoa flour incorporated (46.13) differed significantly (Table 5). Quinoa has a GI that varies from 35 to 53 depending on the cooking method as well as cooking duration (Atkinson *et al.,* 2008). The inclusion of guar gum to the biscuits is another factor in the low glycemic index of quinoa biscuits. Guar gum's ability to make gels from soluble fibers lowers blood glucose levels. As a result of the prolonged stomach emptying, it increases satiety. Guar gum-supplemented diets reduce hunger, appetite, and the desire to consume (Butt *et al.,* 2007). Guar gum greatly slows down starch digestion, according to an *in vitro* study. It serves as a barrier between starch and the enzymes that break down starch (Dartois *et al.,* 2010). Low GI foods include beans, nuts, dairy products, and pasta. Intermediate GI foods include muesli and some breads, and high GI foods include white wheat bread (>70)(Brand-Miller *et al.,* 2008). Low glycemic index foods are those with a glycemic index of less than 55 (Brand-Miller and Foster-Powell, 1999). Foods with a low glycemic index are beneficial for preventing obesity, cardiac disease, and hypertension (Radulian *et al.,* 2009). A low GI diet might also improve thrombolytic performance. Twenty people with type 2 diabetes were shown to have a 53% lower activity of plasminogen activator inhibitor-1, a thrombolytic factor that increases clot and plaque formation, after 24 days on a low GI diet compared to a high GI diet (Jarvi *et al.,* 1999).In contrast to refined wheat flour biscuits, which fall under the category of intermediate glycemic index foods, quinoa flour incorporated biscuits fall under the category of low glycemic index foods.

CONCLUSION

The present study provides evidence to support good nutritional quality, physical and functional properties of quinoa flour. Low glycemic index of formulated biscuits indicate its suitability in prevention and control of various degenerative diseases specially diabetes. Additional research is required to assess the long-term effects of quinoa biscuit consumption on patients with diabetes and cardiovascular disease's blood lipid profiles and glycosylated hemoglobin. Low GI diets have been demonstrated to reduce free fatty acids, enhance lipid profiles, improve glucose levels, and prevent weight gain by assisting in controlling appetite by delaying gastric emptying. These eating plans help lower insulin resistance and the risk of lifestyle diseases like cardiovascular disease, diabetes, and several types of cancer.

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