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Formulation, nutritional evaluation and storage stability of gluten free quinoa biscuits for celiac disease patients

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Abstract

Improvisation towards a healthy lifestyle is a requirement of today. The aim of this research was to develop gluten-free biscuits with high nutritional value for celiac disease patients. Biscuits are a prominent food medium for nutritional fortification. They are convenient to prepare and transport ready-to-eat foods that are enjoyed by people of all ages. Quinoa grains have attained recent scientific attention amongst consumers due to their possible health promoting effects. In this study, quinoa seed flour was used to substitute maize flour in biscuit dough at 40, 50 and 60 per cent levels. Significant differences in sensory and nutritional properties of biscuits were observed. The developed biscuits were rich in protein, fibre, lysine and methionine content. Based on the sensory evaluation, 50% quinoa substituted biscuits were selected for nutritional evaluation. Biscuits could be stored in sealed polypropylene pouches at room temperature for 90 days without any significant alteration in texture.. It was concluded that quinoa substituted biscuits can be used as a protein, fibre, mineral and amino acid enriched gluten free product having acceptable sensory properties.

1. Introduction

An increasing number of people are being identified with food allergies and intolerances. Celiac illness, gluten sensitivity, and gluten ataxia all need the avoidance of gluten. Gluten free cereals such as amaranth, buckwheat, corn, millet and rice are examples of pseudo-cereals. Quinoa grains are high in amino acids, minerals, and fibre as well as other essential nutrients. In celiac disease, gluten causes inflammation in the small intestine, which leads to loss of various nutrients such as iron, folic acid, calcium and fat-soluble vitamins. This renders them susceptible to a variety of malabsorption-related conditions. The treatment of celiac disease is strict, life-long adherence to a gluten-free diet, ensuring that the foods and beverages are free of gluten. This remains a challenge for patients on such a diet. There are no drugs to cure celiac disease and no permanent treatment has been found yet (Ronda and Roos, 2011). A gluten-free diet is the only medically accepted treatment for celiac disease. Such a diet enables celiac patients to control their symptoms and avoid various complications related to this disease. Gluten-free food is usually seen as a diet for celiac patients, but people with a gluten allergy (an unrelated illness) should avoid all such grains (Chandralekha *et al.*, 2017). Celiac disease patients can consume a well balanced diet that includes a wide variety of foods. Instead of wheat flour, they can use pseudo-cereals like amaranth, buckwheat, quinoa, sorghum, maize, rice, pulses and soybean (Singh, 2018).

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Thus, an increasing trend in research is focusing on utilizing alternative grains potentially healthy to elaborate gluten-free products. Quinoa is a super smart pseudo-cereal food and is gaining popularity among health conscious clientele. It exhibits wonderful nutritional food quality; it is high in protein content with an abundance of essential amino acids, vitamins and minerals (Chandra *et al.*, 2018; Nisar *et al.*, 2018). It has higher protein (16-18 %), and more than 37 per cent of the quinoa protein comprises essential amino acids (Drzewiecki *et al.*, 2003). Quinoa contained total dietary fibre content of 13.4 per cent consisting of 11.0 per cent insoluble and 2.4 per cent soluble fibre. Quinoa contains 4.4-8.8 per cent crude fat, with the essential fatty acids linoleic and linolenic acid accounting for 55 to 63 per cent of the total fatty acids and has lipid lowering effect (Alvarez *et al.*, 2010). In the present study, efforts were made to develop and analyse the nutritional characteristics of maize and quinoa flour biscuits. The widespread use of maize and quinoa flour in the formulation of gluten-free biscuits is expected to enhance the nutritional and health status of celiac disease patients. The preparation of such items can help celiac patients with their impoverished palates, as well as be offered to children to meet their diet diversity and nutrient demands.

2. Materials and Methods

2.1 Procurement of raw materials

The study was undertaken in the Department of Foods and Nutrition, CCS HAU, Hisar from year 2020 to 2022. Quinoa and maize grains were procured from the local market of Hisar. Quinoa and maize grains were cleaned and finely milled to flour. All other ingredients were procured from local market of Hisar.

2.2 Formulating quinoa biscuits

The ingredients used for preparation of biscuits were quinoa seed flour, maize flour, ghee (40 g), milk (25 ml) and sugar (60 g). Biscuits were prepared from different blends of maize and quinoa flour in the respective ratios of Control (100% maize flour), Type- I (60:40), Type-II (50:50) and Type-III (40:60). Maize biscuits (100% maize flour) were taken as control. Biscuit dough was prepared manually and rolled into a thin sheet and cut into desired shape using mould. The cut pieces were baked at 160°C in a pre-heated oven. After baking biscuits were cooled and stored in air tight containers.

2.3 Organoleptic evaluation

Among different types of maize and quinoa biscuits, Type-II (50% maize flour + 50% quinoa flour) biscuits scored maximum mean scores for all sensory attributes, *i.e.*, 8.0 ('liked very much'). Type-II biscuits were selected for nutritional evaluation on the basis of organoleptic scores (Figure 1).

2.4 Devised biscuits were chemically analyzed for following parameters

2.4.1 Proximate composition

Moisture, crude protein, fat, ash and crude fiber were estimated by employing the standard method of analysis (AOAC, 2000). Conversion factor of N × 6.25 was used for estimation of crude protein (Khan and Das, 2019).

2.4.2 Dietary fiber and total mineral content

Total, soluble and insoluble dietary fiber constituents were determined by the enzymatic method discussed by Khare *et al.* (2021). Total mineral content was determined following the methods mentioned by Lindsey and Norwell (1969). Two gram sample was taken in a 150 ml conical flask. To this, added 20 ml diacid mixture (HNO_3:$HClO_4$: 5:1,v/v) and kept overnight and digested next day to white precipitates. The precipitates were dissolved in double distilled water and filtered through Whatman No. 42. The filtrate so obtained was diluted to 100 ml with double distilled water and determined calcium, phosphorus, iron and zinc.

2.4.3 Mineral availability

Available calcium and zinc (*in vitro*) were determined by Atomic Absorption Spectrophotometer. Samples (2 g) were rehydrated, added 20 ml of pepsin solution (0.1 % pepsin in 0.1 N HCl) and incubated (37°C in a shaker cum water bath, 1 h). pH raised (6.8) with sodium bicarbonate solution, added 2.5 ml of suspension containing 0.5 % pancreatin and 5 % bile and again incubated (37°C, 1 h). Total volume was made to 50 ml with distilled water, centrifuged and filtrate was oven dried, digested in the diacid mixture, and preceded for the estimation of available minerals. Free iron was extracted using 0.5% pepsin in 0.1 N HCl, reacted with α^1, α^1dipyridyl and determined (AOAC, 2010).

2.4.4 Amino acid analysis

Extraction of amino acids was done by hydrolyzing the samples in autoclave for 6 h at 15 lb pressure. After filteration, hydrolyzed samples were used for the determination of methionine (Horn *et al.*, 1946). Lysine was assessed by method of Booth (1971).

2.4.5 Shelf life

2.4.5.1 Subjective evaluation of fresh and stored biscuits

All types of biscuits were organoleptically evaluated, by a panel of ten semi trained judges. The scores were assigned using 9-point Hedonic Rating Scale (Saharan and Jood, 2020; Gautam *et al.*, 2020; Verma *et al.*, 2021; Thakur *et al.*, 2021).

2.4.5.2 Fat acidity

The fat acidity was determined by method elaborated by AOAC (2010). Sample (10 g) was extracted with petroleum ether on Soxhlet apparatus and mixed with 50 ml benzene-alcohol-phenolphthalein solution. Titrated against potassium hydroxide (1 g/lt) to orange pink colour. Blank was also titrated and the value was subtracted from titration value of the sample. Fat acidity was calculated as:

Fat acidity = 10 × (T – B), where, T = ml of KOH required to titrate sample extract and B = ml KOH required to titrate blank.

2.4.6 Statistical analysis

The values were taken in triplicate and data were subjected to statistical analysis using statistical Package for Social Science (SPSS) version 2.0. The significant difference was checked at 5 and 1 per cent level of significance.

3. Results

3.1 Proximate composition

On the basis of organoleptic scores, Type-II (50% maize + 50% quinoa flour) biscuits were selected for nutritional evaluation. Quinoa flour contained 10.10, 13.01, 5.25, 8.09, 2.87 and 60.68 g/ 100 g of moisture, crude protein, crude fat, crude fibre and ash content. The moisture content of control biscuits (100% maize flour) was 2.06 and that of quinoa maize biscuits (Type-II) was 3.14 per cent (Table 1). The moisture content of quinoa-maize biscuits (Type-II) was significantly higher than that of control. This may be attributed to the high water absorption capacity of quinoa flour due to its high soluble fiber (gums) and high protein which retained higher moisture content in the final product. The crude protein and fat contents of control biscuits (100% maize flour) was 9.01 and 23.66 per cent, respectively and that of quinoa biscuits was 12.04 and 26.26 per cent, respectively. The crude protein and fat contents of quinoa maize biscuits was significantly higher than that of maize biscuits. The increased protein and fat contents of supplemented biscuits were attributed to their higher contents in quinoa flour. The crude fibre and ash content of control biscuits was 2.05 and 1.47 per cent, respectively while the values for supplemented biscuits were 6.75 and 2.74 per cent, respectively.

3.2 Dietary fibre

The total dietary fibre content of control biscuits was 6.43 g/100 g and that of quinoa biscuits was 9.81 g/100 g, respectively (Table 2). The soluble dietary fibre content of control biscuits was 1.31 g/ 100 g and at 50 per cent supplementation levels it was 2.43 g/100 g, respectively and the insoluble dietary fibre content of control biscuits was 5.12 g/100 g and at 50 per cent level of supplementation it was 7.38 g/100 g, respectively. Significant increase was observed in dietary fibre contents of quinoa supplemented biscuits.

3.3 Total minerals

The calcium content in maize biscuits was 21.98 mg while the quinoa biscuits contained significantly higher calcium content of 128.52 mg (Table 3). The iron content in quinoa biscuits was 4.77 mg per 100 g which was significantly higher than the control biscuits *i.e.*, 2.12 mg/100 g. The zinc content of quinoa biscuits 2.87 mg was also significantly higher than that of control (1.63 mg per 100 g). The phosphorus content of quinoa supplemented biscuits 287.90 mg/100 g was also significantly higher than the control (198.80 mg/100 g).

Table 1: Proximate composition of quinoa flour supplemented gluten free biscuits (%, dry weight basis)

Biscuits	Moisture*	Crude protein	Crude fat	Ash	Crude fibre
Control M.F (100 %)	2.06 ± 0.04	09.01 ± 0.11	23.66 ± 0.87	1.47 ± 0.05	2.05 ± 0.09
Type II (MF:QF:: 50:50)	3.14 ± 0.08	12.04 ± 0.39	26.26 ± 0.45	2.74 ± 0.07	6.75 ± 0.24
‘t’ value	19.86**	12.97**	4.56**	25.45**	24.82**

Values are mean ± SE of three independent determinations
MF- Maize flour, QF- Quinoa flour
*Moisture content fresh weight basis
**Significant at 1% level of significance

Table 2: Dietary fibre contents of quinoa flour supplemented gluten free biscuits (g/100 g, on dry matter basis)

Biscuits	Total dietary fibre	Soluble dietary fibre	Insoluble dietary fibre
Control (MF 100 %)	6.43 ± 0.40	1.31 ± 0.31	5.12 ± 0.19
Type II (MF:QF::50:50)	9.81 ± 0.12	2.43 ± 0.02	7.38 ± 0.34
‘t’ value	13.97**	6.31**	10.09**

Values are mean ± SE of three independent determinations
MF- Maize flour, QF- Quinoa flour
**Significant at 1% level of significance

Table 3: Total minerals (mg/100 g) of quinoa flour supplemented gluten free biscuits (on dry matter basis)

Biscuits	Iron	Calcium	Zinc	Phosphorus
Control (MF 100 %)	2.12 ± 0.02	21.98 ± 1.13	1.63 ± 0.06	198.80 ± 1.87
Type II (MF:QF::50:50)	4.77 ± 0.30	128.52 ± 2.38	2.87 ± 0.09	287.90 ± 2.05
‘t’ value	15.51**	70.69**	20.16**	55.54**

Values are mean ± SE of three independent determinations
MF- Maize flour, QF- Quinoa flour
**Significant at 1% level of significance

Table 4: Lysine and methionine content of quinoa flour supplemented gluten free biscuits (g/100 g protein on dry weight basis)

Biscuits	Lysine	Methionine
Control (MF 100 %)	1.11 ± 0.08	0.99 ± 0.02
Type II (MF:QF::50:50)	3.34 ± 0.01	1.96 ± 0.04
‘t’ value	47.50**	37.88**

Values are mean ± SD of three independent determinations

MF-Maize flour, QF-Quinoa flour

**Significant at 1% level of significance

Table 5: *In vitro* mineral availability (%) of quinoa flour supplemented gluten free biscuits (on dry matter basis)

Biscuits	Iron	Calcium
Control (MF 100 %)	25.99 ± 0.51	60.84 ± 0.34
Type II (MF:QF::50:50)	22.65 ± 0.19	56.96 ± 0.57
‘t’ value	10.67**	10.08**

Values are mean ± SD of three independent determinations

MF- Maize flour, QF- Quinoa flour

**Significant at 1% level of significance

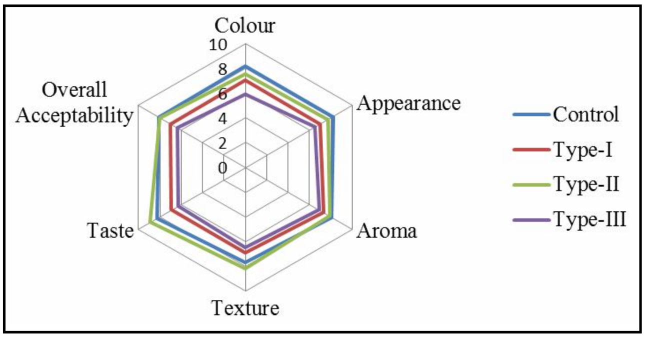

Figure 1: Sensory evaluation of biscuits.

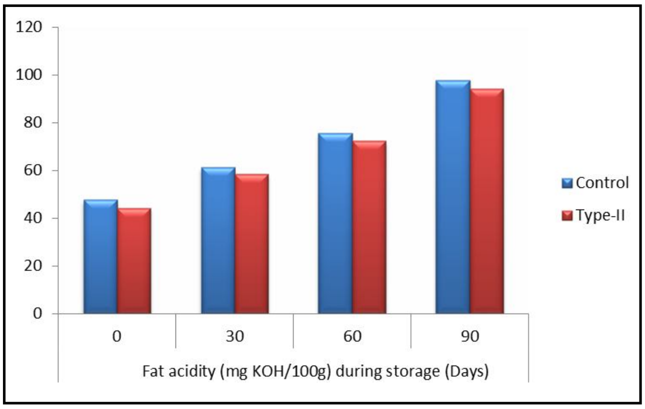

Figure 2: Fat acidity of stored biscuits.

Table 6: Storage stability of quinoa flour supplemented gluten free biscuits

Biscuits	Overall acceptability during storage (Days)				
	0	30	60	90	CD (P=0.05)
Control (MF 100 %)	8.1 ± 0.07	7.4 ± 0.03	7.2 ± 0.02	6.8 ± 0.05	0.13
Type II (MF:QF::50:50)	8.0 ± 0.06	7.2 ± 0.04	7.1 ± 0.03	6.7 ± 0.05	0.11
Values are mean ± SE of ten penalists					
Fat acidity (mg KOH/100g) during storage (Days)					
Biscuits	**0**	**30**	**60**	**90**	**CD (P=0.05)**
Control (MF 100 %)	47.73 ± 0.17	61.24 ± 0.82	75.55 ± 0.29	97.77 ± 0.61	1.03
Type II (MF:QF::50:50)	44.28 ± 0.05	58.53 ± 0.28	72.37 ± 0.26	94.29 ± 0.05	0.37
't' value	33.75**	5.43**	14.11**	9.78**	

Values are mean ± SE of three independent determinations

MF- Maize Flour QF- Quinoa flour

**Significant at 1% level of significance

3.4 Lysine and methionine content

Lysine content of control biscuits was 1.11 g/100 g and in 50 per cent quinoa supplemented biscuits, it was 3.34 g/100 g (Table 4). Significant increase was observed in lysine content of supplemented biscuits compared to control. Similarly, it was found that methionine content of quinoa supplemented biscuits was 1.96 g/100 g, respectively, which was significantly higher ($p \leq 0.05$) than control.

3.5 Mineral availability

Control biscuits possessed 25.99 and 60.84 per cent available iron and calcium, respectively, and in 50 per cent quinoa supplemented biscuits, the values were, *i.e.*, 22.65 and 56.96 per cent, respectively (Table 5). However, total amount of available minerals in quinoa biscuits was higher. The lower digestibility of maize-quinoa biscuits was explained to supplementation of quinoa in maize flour which might have led to increase in polyphenols and interference with digestibility (Schumacher *et al.*, 2010).

3.6 Storage stability

During storage (upto 90 days), the mean overall acceptability scores of maize flour biscuits ranged from 8.1 (liked very much) to 6.80 (liked moderately) (Table 6). Whereas, quinoa incorporated biscuits scores were 8.0 to 6.7 during storage. The mean overall acceptability scores of supplemented fresh biscuits ranged from "liked very much" to "liked moderately". Fat acidity content of maize (control) and quinoa flour biscuits significantly increased during storage (Figure 2). Maize flour biscuits fat acidity values varied between 47.73 to 97.77 mg KOH/100 g during storage. While, the values for 50 per cent quinoa supplemented biscuits were 44.28 mg KOH/100 g on zero day which amplified to 94.29 mg KOH/100 g on 90th day of storage (room temperature).

4. Discussion

Moisture, crude protein, crude fat, crude fibre and ash content of maize and quinoa biscuits were significantly higher than that of control (100% maize flour). Similar findings were reported by Puri *et al.* (2020) that 40 per cent quinoa flour supplemented biscuits contained higher ash content, crude fibre, protein content and lowest carbohydrate content compared to control. Results of present study also lend support to that of Kaur (2016) who reported that quinoa supplemented cookies contained more proximates than control except carbohydrate content. Demir and Kilinc (2017) also reported that ash, crude protein and crude fat content increased with addition of quinoa flour. According to Alvarez-Jubete *et al.* (2010), crude protein, ash and crude fat contents in quinoa are generally higher than in common cereals such as wheat. The value added biscuits were nutritionally superior to control due to higher nutritional quality of added quinoa. Total iron, calcium, zinc and phosphorus content in quinoa and maize biscuits were higher than control (100% maize flour). Similar findings were reported by Demir and Kilinc (2017) and Goyat *et al.* (2018) that control cookies contained less iron and zinc content compared to quiona flour supplemented cookies. Ascheri *et al.* (2002) found that quinoa flour is also rich in minerals particularly K (546 mg/100 g), Fe (11.77 mg/100 g), Mg (160 mg/ 100 g), Ca (38.26 mg/ 100 g) and P (357 mg/100 g). It is concluded that since quinoa flour has higher levels of many nutrients and is gluten free, so it can find the application in various processed foods aimed for celiac patients. Higher dietary fibre values were observed in quinoa added biscuits. These results are in close confirmation with the results obtained by Ghose *et al.* (2022) who also recorded more dietary fibre in quinoa flour supplemented cookies than 100 per cent cereal cookies. Lysine and methionine content were significantly higher in Type-II biscuits than control. Watanabe *et al.* (2014) also found higher lysine and methionine content in quinoa supplemented cookies compared to control. Available calcium and iron content was significantly higher in control (100% maize flour) biscuits compared to maize and quinoa biscuits. The lower values of per cent available calcium and iron in quinoa supplemented products were owing to high amounts of anti-nutritional factors as compared to control. These results are in confirmation with the results obtained by Valencia (2003) who reported that the available mineral content reduced in quinoa flour products compared to control. Sanchez *et al.* (2019) also reported that the available mineral content reduced in quinoa flour products compared to control. Overall acceptability scores of biscuits declined during storage period. Similar findings were reported by Godase *et al.* (2020) who found that overall acceptability scores of biscuits prepared from quinoa flour decreased during storage. Significant increase was observed in the fat acidity content of stored biscuits. The increase in fatty acids with the advancement in storage period might be due to increased hydrolysis of triglycerides resulting in formation of free fatty acids which increased the fat acidity

Goyat *et al.* (2018). Enhancement in the fat acidity in baked products with storage was possibly due to moisture gain and the commencement of fat break down with time passage and creation of free fatty acids. This also explains the descend in the acceptability scores of stored products Godase *et al.* (2020). Utilization of quinoa flour for formulation of different types of healthy food can be undertaken by food industries to develop products rich in essential amino acids like lysine and methionine, calcium, dietary fibre and protein which is the need of the hour. The quinoa added biscuits showed good sensory scores, revealing their potential for consumption by the general population and as an interesting option for individuals suffering with celiac disease and diabetes.

5. Conclusion

Quinoa seed flours were effectively incorporated in gluten free biscuits by partially substituting maize flour. Substitution of 50 per cent quinoa flour resulted in the biscuits with best sensory acceptability and high nutritional composition. Protein, dietary fibre, amino acids, minerals and bioactive substances all increased significantly after substitution of maize flour with quinoa flour. The standardized biscuits could be stored under packaged conditions for 90 days at ambient temperature. Quinoa seed flour can be suitably used to develop gluten free biscuits for celiac patients with enhanced nutritional property and storage stability.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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