Proximate Analysis, Phytochemical Analysis, Colour Estimation, Antioxidant, Antibacterial Analysis, Shelflife Analysis of Sugarfree Burfi Optimization from Quinoa Seed Powder and Stevia

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Authors’ contributions
This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT
Quinoa seed powder and stevia has several health benefits, stevia has been naturally incorporated with sugar making it beneficial for diabetic patients. Burfi was an Indian popular sweet with harmful content and qualities. So, burfi was prepared using quinoa seed powder and stevia to incorporate benefits of plant compounds quercetin and kaempferide with the help of physicochemical analysis, proximate analysis, phytochemical analysis, antioxidant analysis shelf-life analysis and colour estimation analysis. The moisture content of optimized burfi and control burfi was obtained to be 15-20% percent respectively. The protein content of optimized burfi and control burfi have value 18.97-20.286% percent respectively. The fat content of optimized burfi and control burfi were 18.630-21.972% percent respectively. The ash content of optimized burfi and control burfi 3.4-4% percent respectively. The Titrable acidity of optimized burfi and control burfi were 1.035-1.16% percent respectively. The carbohydrate of optimized burfi and control burfi were 31.66-25.708% percent respectively. The crude fiber of optimized burfi and control burfi were 0-1.25% percent respectively. The calcium of optimized burfi and control burfi were 571-520 mg respectively. The pH of optimized burfi and control burfi were 7.99-8.03 pH respectively etc. Antioxidant activity was analysed using
1. INTRODUCTION

Quinoa (Chenopodium Quinoa Willd) is a grain-like harvest which is customarily utilized for nourishment and food to Andean native societies for quite a long time. As of late, lights were tossed on quinoa and it was portrayed as "one of the 21st century's grains" for what it's worth of a high healthy benefit; it is a gluten free and having restorative properties permitting it to be utilized as a nutraceutical and a utilitarian food [1-3]. The high dietary benefit of quinoa might be credited to its exceptional substance organization as it contains a protein of a high amount and quality with a reasonable fundamental amino corrosive example. Additionally, it contains vitamins such as vitamins E, C, B2, B6 and folic acid with relatively high amounts [4-7]. Additionally, its mineral substance is of an incredible worth as it contains calcium, magnesium, copper, iron, zinc and potassium with generally high sum contrasted with different grains and a considerable lot of these minerals are of a decent bioavailability enough to from a reasonable eating routine. In addition to high healthy benefit and being liberated from gluten, quinoa was likewise answered to have numerous medical advantages [8,9]. It tends to be utilized for the two youngsters and old, for lactose bigotry, the individuals who are experiencing either pallor or heftiness or diabetes or celiac sickness or dyslipidaemia. It has a high cancer prevention agent and mitigating strength and can be utilized as anticancer, neuroprotective and immunomodulatory [10]. These medical advantages are because of its substance of protein, mineral, nutrients and fiber notwithstanding its substance of phytochemical and bioactive parts. Among the phytochemicals of quinoa, it has been accounted for that quinoa was among the most extravagant wellsprings of phytoecdystroids containing from 138 to 570 µg/g. Phytoecdystroids are polyhydroxylated steroids associated with plant protection and they have a wide scope of medical advantages including hostile to osteoporotic, anabolic execution upgrading, and against diabetic.

Quinoa is rich in its nutritive worth, with remarkable protein content and its protein supplement is equivalent to that of milk protein [11,12]. It likewise has a magnificent amino corrosive creation and amino acids like lysine, methionine and cystine, additionally presents in higher sums than normal oat and vegetables and can go about as a potential protein substitute in food [13-15].

Stevia rebaudiana Bertoni is a little enduring bush of the Asteraceae (Composite) family that is local to Paraguay, Brazil, and Argentine. The leaves of this plant have been utilized by native individuals for quite a long time in drugs and to improve beverages like mate, a green natural tea [16-19]. The plant was first brought to the consideration of the remainder of the world by the botanist Moises Santiago Bertoni in 1887, who gained of its properties from the Paraguayan Indians. The compound portrayal of the normal constituents of the plant known as steviol glycosides, which are answerable for its particular sweet taste, was not recognized until 1931 when 2 French scientific experts, Bridel and Lavielle, secluded stevioside, an essential steviol glycoside from stevia leaves. Stevia is a rich wellspring of numerous bioactive compounds (Vincent Kevin Tajo.,2014).

Dairy and dairy food products are highly nutritious . In developing countries dairy industry is a direct source of income and provide employment to the poor and has a sustainable contribution in poverty reduction (Burchi et al.,2011).

Burfi is one of the most famous milk-based desserts in India. Burfi is ready by warming a combination of concentrated milk solids and sugar to a close to homogenous consistency followed by cooling and cutting into little cuboids. Beating and whipping tasks before cooling are at times rehearsed to acquire an item with smooth surface and intently weave body. A few assortments of burfi are accessible in the market like plain or mava/khoa burfi, foods grown from
the ground, cashew burfi, chocolate, saffron and rava burfi. Burfi sold industrially differs broadly in variety, body, surface, pleasantness and flavour attributes (Sarkar et al. 2002).

2. MATERIALS AND METHODS

- Preparation of Sample

Burfi was ready by following the traditional technique of prepare. Control sample were prepared according to the standard burfi preparation method given by adding 10% sugar in 90 gm khoa. To obtain standard sample. In sample 1 was replace sugar with stevia. Then we did the burfi preparation in which we added 4% stevia in gm khoa. To obtain standard sample. In sample 2 we added quinoa to the burfi till the nutritive value of the burfi increase. Then we made burfi mixed 20% quinoa seed powder in 74 gm khoa and add 6 gm stevia.

- Burfi Characterization

Moisture content b) Ash content c) Fat content d) Tithable acidity e) crude fiber f) protein g) calcium h) carbohydrate i) pH.

- Proximate Analysis

  - Determination of Moisture content

5 gram of burfi sample was weighed into preweighed sterilized petri dishes. Then it kept into the hot air oven to a temperature of 120 °C for 3 hours. This was then allowed to cool in a desiccator and weighed. This dish was returned into the oven for another half hour and again cooled and reweighed. The process was repeated until a constant weight was reached. Moisture content was determined by the following:

Formula of moisture content -

\[ \text{Moisture content} = \frac{w_1 - w_2 \times 100}{w_1} \times \frac{100}{\text{weight of sample}} \]

\[ W_1 = \text{Initial weight} \]
\[ W_2 = \text{Final weight} \]

  - Determination of Ash content

2 gram of burfi sample weighed by using weighing balance. Ignite the dish and charring for 15-20 minute. Then put the crucible in the muffle furnace (525°C) for 3 hours. Put crucible in desiccator for 10 min. then weight the crucible.

Formula – Ash % = (\( w_3 - w_1 \) \times 100 ÷ \( w_2 - w_1 \)) (gm)

\[ W_1 = \text{Weight of empty crucible.} \]
\[ W_2 = \text{Weight of the sample + (before drying)} \]
\[ W_3 = \text{weight of crucible + sample (after ashing)} \]

  - Determination Fat content

5 gram of burfi sample was weighed and put in thimbles using a dry paper and plugged with cotton wool. The thimbles were dried and inserted into a Soxhlet system. The extraction round bottom flask were dried and weighed and then 50 ml solvent (petroleum ether) was added in each round bottom flask. The samples were extracted for 15 minutes in boiling position. The extraction was carried out continuously for three hours. This was cooled and reweighed. Following formula for fat content was:

Formula of Fat –

\[ \text{Fat} = \frac{\text{weight of flask after} - \text{weight of flask before} \times 100}{\text{weight of sample}} \]

  - Determination of Protein

1.0 gram of the burfi sample was weighed into the digestion flask. Kjedahl catalyst (10 gm potassium sulphate +2 gm copper sulphate +0.25 gm selenium oxide) was added to the sample. 20 ml of concentrated sulfuric acid was added to the sample and then fixed for 8 hours in the digestion unit (450° C) of the Kjedahl apparatus in fume cupboard. The digest, pure yellow after cooling changed into a colourless liquid that was transferred into 100 ml volumetric flask and made upto mark with distilled water. 20 ml of 4% boric acid solution was pipette into conical flask as indicator. The sample was thereafter diluted with 75 ml of distilled water. About 10 ml of the digested was made alkaline with 20 ml of sodium hydroxide (20%) and distilled. The steam exit of the distillatory was closed and the change of colour of boric acid solution to green was timed. The mixture was distilled for 15 minutes. The filtrate was then titrated against 0.1 N Hydrochloric acid. The protein content was calculated Protein formula –

\[ \text{Nitrogen} = (\text{sample titer} - \text{blank titer}) \times \text{N of Hcl} \times 14 \times 100 \times 100 \times \frac{100}{\text{weight of sample} \times \text{Aliquot take} \times \text{for distillation} \times 1000} \]

  - Determination of Crude fiber

2 gram of fat free sample was taken in a thimble. Washing with distilled water was done for 10
minutes at 60 degrees Celsius in a hot plate. Treat with 1.25% dilute sulfuric acid on hot plate for 15 minutes, again wash with distilled water for 10 minutes. Thereafter the sample was treated with 1.25 % of sodium hydroxide for 10 minutes. Place the treated sample into a muffle furnace for ashing at 550 degrees Celsius for 2 hours. Weigh the remaining residue and calculate fibre content by the following:

Crude fiber formula -

\[
\% \text{ Crude fiber } = \frac{W_1 - W_2}{W} \times 100
\]

- **Determination of Total Titratable acidity**

2 gm sample was dissolved in 30 ml of water. Mixed then filtered and make up to 100 ml. 10 ml of the filtrate was pipetted into a beaker. Add 2-3 drops of phenolphthalein indicator. Titrated against the standard 0.01 N NaOH solution until a light pink colour was attained. noted burette reading.

- **Determination of pH**

1 g burfi sample dissolved in 10 ml distilled water pour in the beaker and mixed the sample. The pH checked using the pH meter. Check the reading from the pH meter. The pH meter was standardized using standard buffer of pH 4.0 and 7.0.

- **Determination of Carbohydrate**

Carbohydrate formula –

\[
\% \text{ carbohydrate } = 100 - (\% \text{ protein } + \% \text{ ash } + \% \text{ fat} + \% \text{ moisture} + \% \text{ crude fiber})
\]

- **Determination of calcium**

Pipette an aliquot (20 to 100 ml) of the ash solution of burfi solution obtained by dry ashing to a 250 ml of beaker. Add 25 to 50 ml of water, if necessary. Add 10 ml of saturated ammonium oxalate solution and 2 drop of methyl red indicator. Make the solution slightly alkaline by the addition of addition of alkaline by addition of dilute ammonia and then slightly acid with a few drops of acetic acid until the colour is faint pink (pH 5.0). Heat the solution to the boiling point. Allow to stand for at least 4 hours overnight. Filter through Whatman no 42 paper and wash with water, till the filtrate is oxalate free. Break the point of the filter paper with platinum wire or pointed glass rod. Wash the precipitate first using hot dilute sulfuric acid from wash bottle into the beaker in which the calcium was precipitated. Titrate with 0.01 N Potassium per magnate to the permanent pink colour.

The formula for calcium –

\[
\text{Calcium (mg/100)} = \text{titer} \times 0.2 \times \text{total made up of ash solution} \times 100 \times \text{aliquot take for titration} \times \text{weight of sample taken for ashing.}
\]

- **Phytochemical analysis**

- **Phytochemical screening for tannin content of burfi**

In a test tube, place one milliliter of burfi extract and add 1 ml of 5 % FeCl₃ into it. The resulting dark blue and green-black indicator that tannin was present in a extract (Trease and Evans 1996).

- **Phytochemical screening for flavonoid content of burfi**

To 1 ml of extract add 3-4 ml of sodium hydroxide drop by drop. The existence of yellow color indicator the presence of flavonoid content in burfi (Odebiyi and sofowara,1978).

- **Phytochemical screening for quinones content of burfi**

Briefly add 1 ml of extract into test tube and concentrated sulfuric acid up to 1 ml. The red color indicates the presence of quinone (G Jayapriya, 2014).

- **Phytochemical screening for phenols content of burfi**

2 ml distilled water is added to 1 ml burfi extract in a test tube, along with a few drops of 10 % chlorine. Concentrations of phenols are indicated by the blue-green appearance of the extract (G Jayapriya, 2014).

- **Phytochemical screening for alkaloids content of burfi**

A test tube was filled with two milliliters of burfi extract followed by two milliliters of 1 %
concentrated hydrochloric acid, and 2-3 drops of Mayer's reagent. Green or white color gives positive result of alkaloids in burfi extract (Ogukwe et al.2004).

- **Phytochemical screening for anthocyanin and Betacyanin**

In a test tube, 1ml burfi extract and add 1ml 2N Sodium hydroxide heat for 5 min at 100°C. Bluish green color indicator the ± ce anthocyanin and formation of yellow colour indicates the ± ce of betacyanin.

- **Antioxidant analysis**

- **Procedure of DPPH inhibition method**

The antioxidant activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) was calculated via spectrophotometer with small modifications. In methanol, the color of DPPH is dark blue. In its reduced form, the antioxidant compound changes color from purple to yellow, allowing DPPH to gain electrons. DPPH shows strong absorption at 517 nm, determined by 1,1-diphenyl 1 - 2 pyridyl hydroxylase (DPPH). Briefly, 0.1 ml DPPH solution was mixed with 1ml of optimized burfi prepared in various concentrations (20,40,60,80,100 µg/ml). A control sample of 1 ml of methanol was prepared and incubated in the darkroom for 30 minutes at ambient temperature. After incubation, the absorbance of the sample was read at 517 nm using a UV Visible spectrophotometer methanol used as a blank. Reduction in the absorbance value, shows high activity in scavenging free radicals.

**Note:** The test tube was covered with brown paper as DPPH was very sensitive to light.

The formula for DPPH:

\[ \text{%DPPH scavenging activity} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100 \]

- **Determination of shelf-life analysis**

- **Yeast and Mold**

Chloramphenicol yeast agar (YGCA) was used to determination the yeast and mold maintained at 15 psi for sterilization at 121°C. prepare media and distilled water and all glassware autoclaved temperature 20°C and time 30 min. After 30 min the media and petri plate and test tube will be autoclaved after the pressure released. Then the media and Petri plate placed in the laminar. Then pour 25 ml media in the Petri plate. Then put the petri plate on the U.V light and keep it in the laminar for 10 min for the media to be solidified. Media plate, test tube, distilled water placed in laminar and U. V light was turned on for 15 min. Then 1 ml 10^{-2} dilution sample spread in media plate. The inoculation petri dishes were inoculation in incubator for 72 hours at 25°C temperature. Colony counted after 72 hours.

Yeast and Mold count (CFU in log_{10}) = \log_{10}(A×B)

- **Coliform**

MacConkey agar was used to determination coliform in the quinoa seed burfi sample. The preparation media heated for 15 min in autoclave maintained at 15 psi for sterilization at 121°C. prepare media and distilled water and all glassware autoclaved temperature 20°C and time 30 min. After 30 min the media and petri plate and test tube will be autoclaved after the pressure released. Then the media and Petri plate placed in the laminar. Then pour 25 ml media in the Petri plate. Then put the petri plate on the U.V light and keep it in the laminar for 10 min for the media to be solidified. Media plate, test tube, distilled water placed in laminar and U. V light is turned on for 15 min. Then 1 ml 10^{-2} dilution sample spread in media plate. The inoculation petri dishes were inoculation in incubator for 72 hours at 25°C temperature. Colony counted after 72 hours.

Coliform formula:

\[ \text{Coliform} = \frac{\text{(sum of colony count from plates } \times 100)}{\text{sum of the filtered volume}} \]

- **Antimicrobial analysis**

- **Violet red bile agar**

Violet red bile agar was used microbial analysis in the quinoa seed powder sample. The preparation media 15-10 min heated media in hot plate not used autoclave. then media pour 25 ml per Petri plate. 100µm/l sample spread in petri plate. Then put in the petri plate in the incubator 32°C for 24 hours.

\[ \text{Violet red bile agar} = \frac{\text{No of colonies } \times \text{ dilution factor}}{\text{volume of culture plate}} \]
- **MRS Agar**

MRS agar was used to determine microbial activity in the quinoa seed burfi sample. (MRS agar 33.35g per 500 ml of distilled water) The preparation media heated for 15 min in autoclave maintained at 15 psi for sterilization at 121°C. Cool 50°C, properly mixed and pour into sterile Petri dishes. Take 0.1 gm sample. Take the MCT tube, then 1 ml distilled water put in the MCT tubes with the help of pipette, then add the sample and mix it. Then put it in a 100 micro liter sample and spread it by putting in the media plate, then put in the incubator.

\[ \text{MRS} = \text{No of colonies} \times \text{dilution factor} \div \text{volume of culture plate}. \]

- **Yeast / mold (DRBC Agar (Dichloran Rose Bengal Chloramphenicol Agar)**

DRBC agar was used to determine microbial activity in the quinoa seed burfi sample. (DRBC agar 15.75 g per 500 ml of distilled water) The preparation media heated for 15 min in autoclave maintained at 15 psi for sterilization at 121°C. Cool 50°C, properly mixed and pour into sterile Petri dishes. Take the MCT tube, then 1 ml distilled water put in the MCT tubes with the help of pipette, then add the sample and mix it. Then put it in a 100 micro liter sample and spread it by putting in the media plate, then put in the incubator.

\[ \text{Yeast and mold} = \text{No of colonies} \times \text{dilution factor} \div \text{volume of culture plate} \]

- **Determination of Color attributes**

The color parameters of the burfi sample were measured using colorimeter of Color Tech PCM+ (Color Tec Associates Inc. Clinton NJ, USA). The color reading includes lightness (L*), redness (a*) and yellowness (b*).

### 3. RESULTS AND DISCUSSION

#### 3.1 Proximate Analysis of Burfi

**Table 1. Result of the proximate analysis of the quinoa burfi**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Control (T₀)</th>
<th>Stevia (T₁)</th>
<th>Optimized product (T₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>15.10%</td>
<td>17.89%</td>
<td>20.634%</td>
</tr>
<tr>
<td>Ash</td>
<td>3.4%</td>
<td>3.64%</td>
<td>4.1%</td>
</tr>
<tr>
<td>fat</td>
<td>18.630%</td>
<td>23.328%</td>
<td>21.972%</td>
</tr>
<tr>
<td>Protein</td>
<td>20.26g</td>
<td>21.911g</td>
<td>20.20g</td>
</tr>
</tbody>
</table>

#### 3.2 Moisture

The moisture content of optimized burfi and control burfi was obtained to be 15-20 % percent respectively. The moisture content of control sample is significantly higher than the optimized product. This might be due to the moisture content in quinoa seed powder. This may be because of the moisture content in quinoa seed powder.

#### 3.3 Ash

The ash content of optimized burfi and control burfi 3.4-4.1 % percent respectively. The ash content control sample is higher than optimized product.

#### 3.4 Fat

The fat content of optimized burfi and control burfi were 18.630-21.972 % percent respectively. The fat content of content sample is significantly higher than optimized product.

#### 3.5 Protein

The protein content of optimized burfi and control burfi have value 20.26–20.20 % percent respectively. The protein content of optimized product is high due to incorporation of protein rich quinoa seed powder.
3.6 Titrable Acidity

The Titrable acidity of optimized burfi and control burfi were 1.035-1.16 % percent respectively. The Titrable acidity content optimized product is higher than control sample.

3.7 pH

The pH of optimized burfi and control burfi were 7.99-8.03 pH respectively. The pH a significant optimized sample higher than control sample.

3.8 Crude Fiber

The crude fiber of optimized burfi and control burfi were 0-1.25% percent respectively. The crude fiber content is significant optimized burfi higher than control sample.

3.9 Calcium

The calcium of optimized burfi and control burfi were 571 and 520 mg, respectively. The calcium content a significant control sample higher than optimized sample.

3.10 Carbohydrate

The carbohydrate of optimized burfi and control burfi were 31.66-25.708 % percent respectively. The carbohydrate content of optimized product is significantly higher than control sample.

3.11 Phytochemical Analysis of Burfi

The phytochemical analysis showed burfi contain some secondary metabolism. The table shows the presence (+) and absence of (-) of phytochemical constituents in the tested sample of quinoa burfi. The burfi shows that the positive result of control sample quinone and negative result tannin, flavonoid, phenol, alkaloids, anthocyanin/betacyanin, and T2 sample show that the positive result tannin, flavonoid, phenol, quinones, alkaloids, anthocyanin/betacyanin.

3.12 Colour Estimation of Burfi

It was seen that in the Control burfi, there was no significant difference (p>0.05) in the color from day 1 to day 7. The burfi had an acceptable score of 8.4 throughout the 7 days which means the color of the burfi was liked very much till the day 7.

3.13 Shelf-life Analysis of Burfi

The quinoa burfi was obtained from khoa, stevia and quinoa seed powder. Check the shelf life of quinoa Burfi. Check shelf life on 0 days. No growth in any Sample at 0 days. Then after 5 days check the shelf life of burfi. Growth does not occur in any sample. Then check the burfi sample Shelf life at 10 days. T0 - 3.27×10², T1 - 3.36×10², T2 - 4.72×10². Coliform was 0.

Table 2. Result of Titrable acidity in quinoa burfi

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>T₁</th>
<th>T₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titrable acidity</td>
<td>1.035%</td>
<td>0.69%</td>
<td>1.61%</td>
</tr>
<tr>
<td>pH</td>
<td>7.99</td>
<td>7.88</td>
<td>8.03</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>0%</td>
<td>0%</td>
<td>1.25%</td>
</tr>
<tr>
<td>Calcium</td>
<td>571mg</td>
<td>581.482mg</td>
<td>502mg</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>31.66%</td>
<td>22.528%</td>
<td>25.708%</td>
</tr>
</tbody>
</table>

Table 3. Phytochemical analysis of quinoa burfi

<table>
<thead>
<tr>
<th>Testing</th>
<th>Control</th>
<th>Result T₁</th>
<th>Result T₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthocynin/betacyanin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 4. Colour estimation of sugarfree quinoa burfi

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Test Parameter (s)</th>
<th>Test method used</th>
<th>Result (unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L (T₀)</td>
<td>X-rite colour Lab</td>
<td>50.45</td>
</tr>
<tr>
<td>2</td>
<td>a (T₀)</td>
<td>X-rite colour Lab</td>
<td>+16.93</td>
</tr>
<tr>
<td>3</td>
<td>b (T₀)</td>
<td>X-rite colour Lab</td>
<td>+32.93</td>
</tr>
<tr>
<td>4</td>
<td>L (T₁)</td>
<td>X-rite colour Lab</td>
<td>58.63</td>
</tr>
<tr>
<td>5</td>
<td>a (T₁)</td>
<td>X-rite colour Lab</td>
<td>+12.58</td>
</tr>
<tr>
<td>6</td>
<td>b (T₁)</td>
<td>X-rite colour Lab</td>
<td>+36.92</td>
</tr>
<tr>
<td>7</td>
<td>L (T₂)</td>
<td>X-rite colour Lab</td>
<td>42.69</td>
</tr>
<tr>
<td>8</td>
<td>a (T₂)</td>
<td>X-rite colour Lab</td>
<td>+19.26</td>
</tr>
<tr>
<td>9</td>
<td>b (T₂)</td>
<td>X-rite colour Lab</td>
<td>+30.20</td>
</tr>
</tbody>
</table>

Table 5. Shelf-life analysis during storage

<table>
<thead>
<tr>
<th>Days</th>
<th>0 days</th>
<th>5 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>T₀</td>
<td>T₁</td>
<td>T₂</td>
</tr>
<tr>
<td>Yeast and mold</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coliform</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

3.14 Antimicrobial Analysis

3.14.1 De man, rogosa and sharpe agar (MRS)

The quinoa burfi was obtained from khoa, stevia and quinoa seed powder. Check the microbial activity of quinoa burfi. Check microbial activity 24 hr. No growth in all sample. Then after 48 hr check the microbial activity, control- 8×10³, T₁ - 2×10³, T₂ -1.5 ×10⁴.

3.14.2 Violet red bile agar

The quinoa burfi was obtained from khoa, stevia and quinoa seed powder. Check the microbial activity of quinoa burfi. Check microbial activity 24 hr. Control- 1×10³, T₁ - 1×10³ , T₂ - 4.0×10³. Then after 48 hr check the microbial activity, control-1.6 × 10⁴ , T₁-8.0 × 10⁴, T₂ -1×10³.

3.14.3 Yeast / mold (DRBC Agar (Dichloran Rose Bengel Chloramphenicol Agar)

The quinoa burfi was obtained from khoa, stevia and quinoa seed powder. Check the microbial activity of quinoa burfi. Check microbial activity 24 hr. Control- 4×10⁴ T₁ - 0 T₂ - 0. Then after 48 hr check the microbial activity, control- 1.6 × 10⁴, T₁ - 0, T₂ - 4×10³.

Table 6. Result of MRS, violet red bile agar and yeast / mold (DRBC Agar (Dichloran Rose Bengel Chloramphenicol Agar)

<table>
<thead>
<tr>
<th>MRS Sample</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>8×10³</td>
</tr>
<tr>
<td>T₁</td>
<td>0</td>
<td>2×10³</td>
</tr>
<tr>
<td>T₂</td>
<td>0</td>
<td>1.5 ×10⁴</td>
</tr>
<tr>
<td>Violet Red Bile Agar Sample</td>
<td>24 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td>Control</td>
<td>1×10³</td>
<td>1.6 × 10⁴</td>
</tr>
<tr>
<td>T₁</td>
<td>1×10³</td>
<td>8.0 × 10⁴</td>
</tr>
<tr>
<td>T₂</td>
<td>4.0×10³</td>
<td>1×10³</td>
</tr>
<tr>
<td>DRBC Agar Sample</td>
<td>24 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td>Control</td>
<td>4×10⁴</td>
<td>1.6 ×10⁴</td>
</tr>
<tr>
<td>T₁</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T₂</td>
<td>0</td>
<td>4 × 10³</td>
</tr>
</tbody>
</table>
3.15 Antioxidant Analysis of Burfi

DPPH- DPPH is the most suitable way to determine the antioxidant property of a sample. Because DPPH free radicals are scavenged by antioxidant compound, the colour of the sample change from purple to yellow (Nirmala). Show the graph between concentration (µg) and antioxidant activity (%) of extract. By using a spectrophotometer, the optical density of a sample and the optical density of the control can be calculated to determine DPPH behavior in a sample. According to, if DPPH value was below 50 μg/ml it has a very strong antioxidant property, if it lies between 50-100 μg/ml has strong antioxidant property and if it was above 150μg/ml it has weak antioxidant property. The antioxidant activity of quinoa burfi at different concentrations (Control, T1, T2) was evaluation and the results obtained were illustrated.

According to these results, quinoa seed burfi concentration increases up to 43.13 µg. Afterward, the activity of antioxidant was constant.

\[
\% \text{DPPH scavenging activity} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100
\]

Table Showing the absorbance value obtained for quinoa seed burfi for DPPH radical scavenging activity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.23µg</td>
</tr>
<tr>
<td>T1</td>
<td>28.36µg</td>
</tr>
<tr>
<td>T2</td>
<td>43.13µg</td>
</tr>
</tbody>
</table>

Fig. 1. % DPPH activity of control and optimized burfi

**Shelflife Analysis**

Fig. 2. Yeast and mold plates
Antimicrobial activity

Fig. 3. MRS plates

Fig. 4. Yeast / mold (DRBC Agar (Dichloran Rose Bengel Chloramphenicol Agar)

Fig. 5. Violet red bile agar

Phytochemical analysis

Fig. 6. presence of phytochemical
5. CONCLUSION

(1) In the above study, burfi was mixed with stevia and quinoa seed powder. (2) Quinoa seed have higher amount of protein, fiber, mineral and each of the nine fundamental amino corrosives. (3) It has very low level of glycaemic index which is really great for diabetic individuals. (4) This item contains normal sugar stevia which make it sugar free. (5) Burfi was consumed by wide gathering of populace. (6) This item was ready with the intend to give sustenance to each individual. (7) Anyway, further examination work can be completed on this item to expand its timeframe of realistic usability with further developed surface properties by further developing assembling process or by utilizing novel bundling. (8) The overall conclusion of the study is that the proximate analysis of optimized quinoa burfi (9). Carbohydrate, calcium, crude fiber, calcium, pH, total Titrable acidity check in quinoa burfi. (10) Phytochemical analysis of optimized quinoa burfi. (11) Optimized quinoa burfi has good antioxidant activity. (12) Antimicrobial analysis of optimized quinoa burfi. Shelllife analysis of optimized burfi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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18. Ekanem Onok, Philippa Ojmelukwe. Potentials of coconuts milk as a substitute


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