Review Form 1.6

Journal Name:	**European Journal of Nutrition & Food Safety**
Manuscript Number:	**Ms_EJNFS_88234**
Title of the Manuscript:	**Proximate Analysis, Phytochemical Analysis, Colour Estimation, Antioxidant, Antibacterial Analysis, Shelflife Analysis of Sugarfree Burfi optimization from quinoa seed powder and stevia.**
Type of the Article	**Original Research Article**

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty'**, provided the manuscript is scientifically robust and technically sound.
To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(https://www.journalejnfs.com/index.php/EJNFS/editorial-policy)

Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	**Check grammatical errors in following sections of research article and correct the statements:** ❖ **Abstract:** (1) Optimized quinoa burfi was investigated for their in-scavenging activities using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). (2) Coliform will be 0. (3) The quinoa seed powder expands DPPH searching action up to 43.13µg µg of focus Other khoa burfi making it good for diabetic and ordinary individuals. ❖ **Introduction:** (1) Additionally, it contains nutrients like nutrients E, C, B2, B6 and folic corrosive with somewhat high sum. (2) Among the phytochemicals of quinoa are, it has been accounted for that quinoa is among the most extravagant wellsprings of phytoecdystroids containing from 138 to 570 µg/g. (3) During the information mining system, it was tracked down that few vegetative parts, yet primarily from the leaves of stevia. (4) Dairy and dairy food products are highly nutritious and important role in income generation and food security. ❖ **Materials and Methods: [Write Experimental Methods in Past Tense]** ● **PREPARATION OF SAMPLE** Burfi is ready by following the traditional technique of prepare. To obtain standard sample. In sample 1 we will replace sugar with stevia. Then we will do the burfi preparation in which we will put 4% stevia in gm khoa. To obtain standard sample. In sample 2 we will add quinoa to the burfi till the nutritive value of the burfi increase. Then we will make burfi mixed 20% quinoa seed powder in 74 gm khoa and add 6 gm stevia. ● **Characterization of burfi** The Functional and dietary portrayal of burfi arranged utilizing quinoa seed powder and stevia the accompanying portrayal quantitatively: ● **Determination of Moisture content** Take 5gm a sample in a Petri plate which is made constant previously and keep it in a hot air oven 110^{o}C for 1 hour. Take the weight after an hour and again keep it for 30 min if the weight content. Calculate the moisture conduct by the following : ● **Determination of Ash content** In this strategy ,2 grams of sample weighing by using weighing balance. Ignite the dish and charring for 15-20 minute. Then put the crucible in the muffle furnace (525^{o}C) for 2:30 hours. Put crucible in desiccator for 10 min. then weight the crucible. ● **Determination Fat content** In this method, weight the flask makes it constant. Take 5 gm dried sample make a thimble and keep the product unit. Prepare the setup, use benzene as a solvent run the solvent for 3 hours. Vaporize the solvent from the flask, weight the flask. ● **Determination of Protein**	

In this method, (Reagents preparation) mixed indicator 0.1 % bromocresol green and 0.1 % methyl red indicator in 95 % alcohol 10 ml bromocresol green $_+$ 2ml of methyl red -solutes. 10 gm boric + 500 ml of boiling distilled water. 0.01N Hcl. 30 % NaOH (150 gm of NaOH + 350 ml of distilled water. Catalyst for digestion, mixed 0.5 g of K_2So_4 and 20 gm of $CuSo_4$, $5H_2O$. 2gm dried sample. 2 gm catalyst mixture + 25 ml H_2So_4 for digestion 60^o C till solution become transparent blue. Keep for distillation make up to ml of digested sample. Take 20 ml of sample. Receiving flask 20 ml Boric acid + 4 drops of indicator. 10 ml NaOH. 40^oC is distillation. The sample was titrated with NaOH with end point indicated by a change greenish to pink colour. The volume of the acid for each sample distilled was noted as well as that of clear.

- **Determination of Crude fiber**

  2 gm of burfi sample. Wash with distilled water on hot plate for 10 min at 60^o C. Treat with 1.25% dilute H_2So_4 on plate for 15 min. wash with distilled water for 10 mi. Wash with 1.25 % NaOH on hot plate. Wash with water 2 times on hot plate for 10 min. Dry in hot air oven at 100^oC for constant weight. Take out from the hot air oven and cool it in the desiccator. Keep in muffle at 550^oC for 2 hr.

  Crude fiber formula -

  % Crude fiber = T_1 -$T_{2\times}$ 100 ÷ T_O **[Mention meaning of T_0, T_1, T_2]**

- **Determination of calcium**

  Pipette an aliquot (20ml) of the ash solution obtained by dry ashing to a 250ml beaker. Add 25 to 50 ml of H_2O +10 ml of saturated ammonia oxalate solution and 2 drops of methyl red indicator. Make the solution slightly alkaline by add of dil ammonium and then slightly acidic with a few drops of acetic acid until the colour is faint pin or pH. That the solution to the boiling point and leave overnight. filter through Whatman paper No. 42 paper and wash with distill H_2O till the filtrate become oxalate free. (Chloride test using $AgNO_3$) beaker the point of the filter with glass rod washes the pipette first using distilled water using wash bottle into the beaker in which the calcium was precipitated. Wash with hot distilled water. titrate while hot with 0.01N potassium permanganate to the first permanent pink colour. Add filter paper to the solution and complete the titration.

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

  In a test tube, place one milliliter of burfi extract and add 1 ml of 5 % $FeCl_3$ into it. The resulting dark blue and green- black indicator that tannin is present in a extract.

- **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.

- **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^oC. Bluish green color indicator the ± ce anthocyanin and formation of yellow colour indicates the ± ce of betacyanin.

- **Procedure of DPPH inhibition method**

  DPPH shows strong absorption at 517 nm, determined by 2, 2-diphenyl-22-pyridyl hydroxylase (DPPH).

  The formula for DPPH-

  %DPPH inhibition = AB-AS×100÷AS **[Mention meaning of AB, AS]**

- **Yeast and Mold**

prepare media and distilled water and all glassware autoclaved temperature 20^{o}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^{o}C temperature. Colony counted after 72 hours.

Yeast and Mold count (CFU in $\log_{10}$) = Log_{10} (A×B) [Mention meaning of A and B in formula]

- **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^{o}C. prepare media and distilled water and all glassware autoclaved temperature 20^{o}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^{o}C temperature.

- **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^{o}C for 24 hours.

- **MRS Agar**

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.

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

DRBC agar was used to determination 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^{o}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.

- **Determination of Color attributes –**

Variety credits of the example were assessment by estimating the L*(100 = white; 0 = dark), a*(+, red; -, green) and b*(+, yellow; -, blue) values utilizing a Minolta variety peruse (Hesham A. Ismail 2021) values are the mean of three assurance.

❖ **Results and Discussion: [Correct contradiction between result tables data and their interpretation]**

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.

Ash

The ash content of optimized burfi and control burfi 3.4-4 % percent respectively. The ash content control sample is higher than optimized product.

Protein

The protein content of optimized burfi and control burfi have value 18.97-20.286 % percent respectively. The protein content of optimized product is high due to incorporation of protein rich quinoa seed powder.

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.

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.

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.

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 T_2 sample show that the positive result tannin, flavonoid, phenol, quinones, alkaloids, anthocyanin/betacyanin.

COLOUR ESTIMATION OF BURFI

Interpretation of result table is completely missing.

Shelf-life analysis of burfi – [Correct sentence formation]

The quinoa burfi obtained from khoa, stevia and quinoa seed powder. Check the shelf life of quinoa Barfi. will 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 we check the Shelflife at 10 days. T_0 - 3.27×10^2 , T_1 - 3.36×10^2 ,T_2 - 4.72×10^2. Coliform will be 0.

Antimicrobial activity

Interpretation of result tables is completely missing.

ANTIOXIDANT ANALYSIS OF BURFI

Show the graph between concentration (µg) and antioxidant activity (%) of extract. According to, if DPPH value is 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 is above 150µg/ml it has weak antioxidant property. The antioxidant activity of quinoa burfi at different concentrations (Control, T_1, T_2) 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.

%DPPH inhibition = AB-AS×100÷AS

❖ **Conclusion:**

It was concluded from different analysis and experiment burfi was fused with quinoa seed powder and stevia. Quinoa seed have higher measure of protein, fiber, mineral and each of the nine fundamental amino corrosives. It has low glycaemic list which is really great for diabetic individuals. This item contains normal sugar stevia which make item sugar free. Burfi is consumed by wide gathering of populace. This item is ready with the intend to give sustenance to each individual. 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. The overall conclusion of the study is that the proximate analysis of optimized quinoa burfi. Carbohydrate, calcium, crude fiber, calcium, pH, total Titrable acidity check in quinoa burfi. Phytochemical analysis of optimized quinoa burfi. optimized quinoa burfi has good antioxidant activity. Antimicrobial analysis of optimized quinoa burfi. Shelflife analysis of optimized burfi .	
Minor REVISION comments	❖ **Key Words:** ***'Incorporate'*** – It cannot be considered as keyword for search options. Replace the word with any other scientific term. ❖ **Methodology:** Correct symbol of **'pH'** throughout the document.	
Optional/General comments	❖ Current research article **requires serious major revision and only be accepted for the publication in "European Journal of Nutrition & Food Safety"** after incorporation of necessary suggestions and improved documentation strategy in the revision document. ❖ **Current research article can be published in the international journal provided that serious grammatical errors in sentence formation throughout the article have been corrected and result section has been completely modified and enriched with representation of necessary research citations** in the revision document which are completely missing in support of obtained results. ❖ Presented research article has explored Proximate Analysis, Preliminary Phytochemical Analysis, Colour Estimation, Antioxidant Activity Analysis, Antimicrobial Analysis, Shelf-life Analysis of Sugar-free Burfi optimization supplemented with quinoa seed powder and stevia. ❖ 'Abstract' section has been presented very precisely revealing essential nutrients composition and antioxidant as well as antibacterial activities of sugar-free burfi prepared using quinoa seed powder and stevia. ❖ 'Introduction' section has documented scientific information regarding quinoa, stevia and burfi in brief. ❖ **Poorly documented methodology section completely sought for scientific representation of standard methods used for preparation, standardization and optimization of burfi samples.** ❖ **'Methodology' must be re-written and documented in past tense without grammatical errors.** ❖ **Mismatch of study results represented in tabular form and their interpretation must be strictly corrected in revision document.** ❖ **All the result data interpretation paragraphs must be supplemented with relevant reference citations.** ❖ **'Conclusion' section must be modified and re-written in scientific manner without any grammatical error in sentences.** ❖ **'Reference' section must be corrected and formatted as per journal's guidelines for maintaining uniformity in representation.**	

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

Name:	**Flora Shah**
Department, University & Country	**Indian Institute of Teacher Education (IITE) University, India**