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FOOD SCIENCE AND TECHNOLOGY

Micronutrient Analysis by Ion Chromatography and AAS, Pasting Properties, and Shelf-life Study of Sugar Free Biscuit Inriched with Fenugreek Seed Powder and Natural Sweetener Stevia

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ABSTRACT

The colour of sugar free biscuit was recorded by hunter colour flex and the L*, a*, b*, value was 46.40, 12, 20.29, respectively. Developed sugar free biscuit was rich in calcium and magnesium and their concentration were observed 294.8mg/100g and 451.5mg/100g respectively by ion chromatography. Atomic absorption spectroscopy of developed sugar free biscuits was also performed for mineral analysis and reported that it contains iron (1.93mg/100g), copper (0.065mg/100g), and zinc (0.325 mg/100g). Fortified sugar free biscuits was also analysed by rapid visco analyser for pasting properties of starch, gelatin ionization of starch. The shelf-life of sugar free biscuit was lower at 37°C than at 10°C and 25°C. The HMF (Hydroxy Methyl Furfural) concentration which indicates the mallard reaction at a higher temperature and with increasing days of storage, the HMF concentration was also increased. The HMF increased to 6.1, 6.3, 6.5 µmol/100g at 10, 25, 37°C respectively after 30 days from 5.1 mol/100g which was the HMF value initially. The TBA (Thiobarbituric acid) also increased the most at 30 days storage at 10 °C as compared to 25 °C and 37°C, TBA value was 0.254, 0.265 and 0.286 respectively. The same result was seen in moisture content 5.1, 5.25., 5. 5% at 10 °C, 25 °C, 37 °C respectively from 4% of moisture initially content. The Microbiological studies concluded that the product was safe with no yeast and mould growth and coliform was also not detected. Only total plate count after 30 days was found 3×10 CFU/ml. The product was found safe for consumption even after 30 days of storage.

High Lights

- In the present research analysed micronutrients (calcium, magnesium, copper, zinc, etc.) by AAS and Ion chromatography of developed sugar free biscuit through fenugreek seed powder and natural sweetener stevia was found rich in micronutrient.
- Analysis of pasting properties by Rapid Visco Analyser (AVR), shelf-life study at different time and temperature combination, and microbiological analysis of sugar free biscuit.
- Chemical properties analysis by Fourier-transform infrared spectroscopy (FT-IR) and colour intensity was measure from hunter colour flex.

Keywords: AAS, sugar free biscuit, ion chromatography, hunter colour flex

Diabetes is the very serious problem all over the world in general, particularly in India. Now a days in India lack production of sugar free product. In the current research work developed sugar free biscuit with fenugreek seed powder and natural sweetener stevia and its also rich in calcium and magnesium which in analyzed by Atomic Absorption Spectrometry, Ion-Chromatography and l,a,b value is analyzed by hunter colour flex, digital bomb calorimeter for energy value calculation, functional component by FT-IR (Fourier-transform infrared spectroscopy) and pasting property of

starch by Rapid Visco Analyzer. And also shelf-life study of the final product at different time and temperature, with microbial analysis of the final sugar free biscuit (yeast and mould grouth, TPC, Coliform test). The consumers demand has increased for the quality food products with taste, safety, convenience and nutrition. Thus nutrition has emerged as an added dimension in the chain of food product development (Shaghir and Mashir *et al*. 2014). The *trigonella foenum-graecum* cold water extract, known as fenugreek tea, has been traditionally used against respiratory infections and since it nourishes the body during illness. Fenugreek seed has a central hard and yellow embryo which is surrounded by a corneous and comparatively large layer of white and semi-transparent endosperm (Betty, 2008). The fenugreek herb has also been used to reduce fever, when taken with lemon and honey. *Trigonella foenum-graecum* has been found to have potential health benefits effects such as hypoglycemic, antihypertensive, and hypolipidemic activities (Micallef and Garg 2009). Stevia (*Stevia rebaudiana*) is a small shrub native to subtropical and tropical South America and Central America (North to Mexico, Paraguay and Brazil). Native Indians of the Guarani Tribe appear to have used the leaves of this herb as a sweetener since pre-Columbian times.

MATERIALS AND METHODS

Measurement of Energy value of sugar free biscuits by bomb calorimeter

Principle: The gross energy is the amount of heat produced from unit feed when it is completely burnt down to its ultimate oxidation products (CO_2 and H_2O). The feed is burnt is a closed container (Bomb calorimeter) and heat produced from it is measured. A bomb calorimeter is used to measure the heat created by a sample burned under an oxygen atmosphere in a closed vessel (bomb), which is surrounded by water, under controlled conditions. The measurement result is called the Combustion-Calorific- or BTU-value.

Determination of mineral content by Atomic Absorption Spectroscopy (AAA) and Ion Chromatography

Digestion Procedure for mineral content in sugar free biscuit: 1 gram dried and powdered biscuit sample (20 mesh) was taken in a 50 mL digestion tube and 10 mL di-acid mixture (9:4 v/v HNO_3: $HClO_4$) was added to it and was kept overnight. It was then digested on a block digester till a colourless solution was obtained. The volume of acid was reduced till the flask contained only moist residue. The flask was cooled and 25 mL of distilled water was added. The solution was filtered into a 50 mL volumetric flask and diluted up to mark.

Estimation of mineral

The mineral content in biscuit was determined by using atomic absorption spectrophotometer (ELICOS SL 194) as per procedure outlined by Lindsay and Norwell 1978

Calculation

Dilution factor = (50/1) = 100 times

Elemental concentration (mg kg^{-1}) = AAS reading × 100

Pasting Properties of sugar free biscuits by Rapid Visco Analyser

The Rapid Visco-Analyser (RVA) was used to assess the quality sugar free biscuit. The pasting properties of starch and starch-containing products are readily assessed in the RVA. During the test, the starch is gelatinised with consequent rise in viscosity, subject to high temperature and controlled shear during which its stability is revealed and then cooled to provide an indication of setback during gelation. Samples can be assessed for pasting temperature, peak paste viscosity, time to peak, temperature at peak, hot and cold paste viscosity, breakdown, setback, final viscosity and other parameters.

Method

- Switched on the RVA and allowed 30 min warm up. Switch on associated computer, run the RVA control software, and select the desired profiles.
- Measured 25.0 ± 0.1 ml of distilled water (corrected to compensate for 14% moisture basis correction of sample) into a new canister.
- Finely, grounded sample was taken (3.5 ± 0.01 g) (14% moisture basis) into a weighing vessel and transfer sample onto the water surface.
- A paddle was placed into the canister and vigorously stirred through the sample up and

Table 1: Instruments used for manufacturing and analysis of sugar free biscuit

Sl. No.	Name	Company, Model and Country
1	Electronic weighing Balance	Metller Toledo, JBI 603-CIF act, Switzerland
2	Texture profile analyse	TA.XT plus texture profile analyser, stable Micro systems, UK
3	Vortex shaker	Macro scientific works Pvt. Ltd., Delhi
4	Hot air oven	Perfit, 992110, India
5	Laminar air flow	Labtech LCB 1201v, Daihan Pvt. Ltd., India
6	Centrifuge machine	Sigma, 3-30k, Germany
7	High pressure steam sterilizer (Vertical Autoclave)	Tomy, SX-500, japan Pelican
8	Incubator	Remi, India
9	Spectrophotometer	Model: 3375 Input voltage : AC220V/50Hz, SN: YH211705012
10	Hot plate	TARSONS, SPINOT MODEL MC-09, CAT.6030, S.NO. 1601120, 220V 50Hz 4.9Amps, 1170 Watts
11	FT-IR	Company; Thermo Scientific; Model Nicolet i S5
12	AAS	
13	OIN Chromatography	Metrohm (930 compact IC, Switzerland) Column used: Metrosep C4 (for cation) Metrosep A Supp.5 (for anion)
14	Rapid Visco Analyzer (RVA)	Tec master

down 10 times. If any lumps remain on the water surface or adhere to the paddle then repeat the jogging action.

- Place the paddle into the canister and insert the canister into the instrument. Initiate the measurement cycle by depressing the motor tower of the instrument. Remove canister on completion of test and discard.
- From the pasting curve, the pasting temperature, peak viscosity, time to peak, breakdown, minimum viscosity, setback and final viscosity may be measured.

Physico-chemical changes during storage

Shelf life study for sugar free biscuit was conducted at different temperature for 1 month at the interval of ten days. Samples were packed, sealed and kept at 10, 25 and 37 °C for storage study. The freshly prepared biscuit after being cooled were packed and analyzed for different parameters to be shelf stable. Thiobarbituric acid, Hydroxy methyle furfural content and moisture of the product were observed at the interval of ten days.

Hydroxy Methyle Furfural (HMF)

Total HMF in the sugar free biscuit was determined by taking 0.5 g of sample which was then thoroughly mixed with 9.5 ml distilled water, 5 ml 3 N oxalic acid was added and the tube were cooled and 5 ml of 40% Trichloroacetic acid solution was added. The precipitated mixture was filtered through Whatman filter paper No.42. 0.5 ml of the filtrate was pipetted out into a 5 ml test tube and added with 3.5 ml of distilled water and 1 ml of 0.05 M Thiopbarbituric acid solution and mixed well.

The tubes were then kept in a water bath at 40 °C for 50 minutes. After cooling to room temperature absorbance was measured at 443 nm (Shimadzu Corporation, Japan). A blank test was carried out in the same manner as above substituting distilled water for sugar free biscuit.

A standard curve of HMF concentration and optical density at 443 nm was made using a standard stock solution (10 µmole/ml HMF concentration). The dilutions were treated same as the sample for HMF estimation. From the standard curve, the HMF content in the samples was determined using the following regression equation:

Total HMF (µ mole/100gram) = (Absorbance – 0.55) × 87.5 × 0.4

Fig. 1: Ion Chromatography

Fig. 2: Atomic Absorption spectroscopy

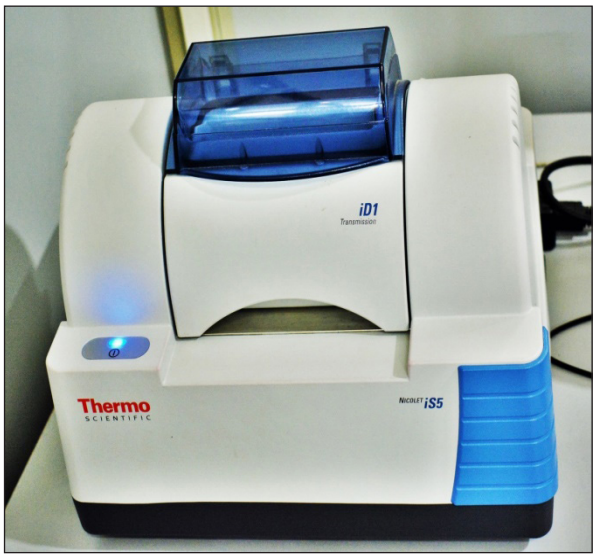

Fig. 3: FT-IR

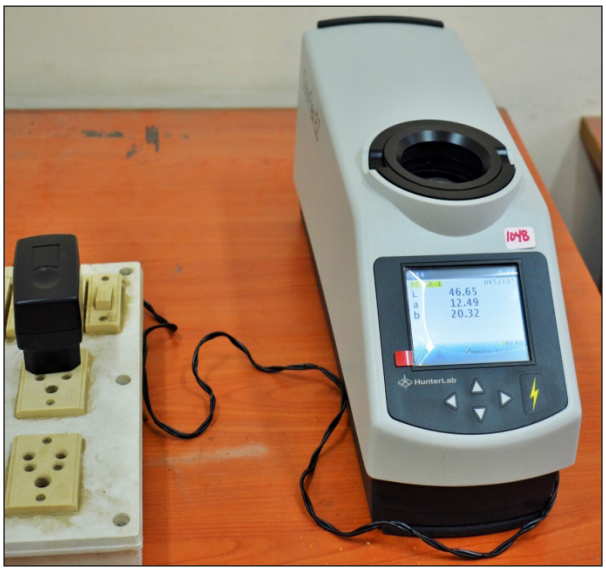

Fig. 4: Hunter Lab (Colorflex)

Fig. 5: Digital Bomb Calorimeter

Fig. 6: Rapid Visco Analyzer (RVA)

Thiobarbituric Acid (TBA)

The extent of oxidation of fat in the sugar free biscuit was measured in terms of increase in TBA value. For TBA value determination, about 2 gram sample of biscuit was taken and blend with 50 ml of 20 % TCA (Trichloroacetic acid) and 50 ml of distilled water and left undisturbed for 10 minute then the contents were filtered through whatman No. 1 filter paper. The filtrate (5ml) was then pipeted out in a test tube and added with 5ml of 0.01M 2-Thiobarbituric acid. Colour was developed by incubating the tube in boiling bath for 30 minutes at 100 °C. The content were then cooled to room temperature and absorbance was determined at 532 nm (Shimadzu corporation Japan). Blank determination were made sing distilled water in place of sample TBA value was expressed at absorbance at 532 nm.

Moisture content (AOAC 1995)

Procedure: In washed, preheated, cooled and weighed empty silica crucible, 2 gram of samples were weighted in duplicate. The crucibles were then placed in preheated, hot air oven at 100±5 °C for 24 hours. After drying, the crucible were cooled in the desiccator and weighed.

% moisture content =

$$\frac{\text{weight after drying} - \text{Initial weight}}{\text{Weight of sample}} \times 100$$

Determination of Microbial Population

Preparation of the sample (Serial dilution)

1 g of sample was taken and transferred to the test tube with 9 ml of normal saline solution

(0.9% NaCl). The sample was serially diluted up to 10^{-10} dilution. The test tube containing samples were homogenized for proper mixing.

Total plate count

Total plate count (TPC) was used for determination of bacterial count.

Method

Sterilization: The prepared media was autoclaved for 15 minute at 15 psi and temperature at 121 °C. All glassware's and necessary item were properly autoclave to avoid contamination.

Pouring: Pouring was done in the laminar- air flow chamber. A flame was lighted and petri-dishes were slightly opened near the flame and the media was poured in the petri-dishes and kept for solidification in incubator.

Inoculation of sample: Inoculation was done aseptically in laminar air flow chamber by taking 0.1 gm of the sample suspended in saline solution from 10^{-2} dilution and transferred to a petri dish with label 10-2 of nutrient agar media. Similarly, all the samples were taken and transferred into their respective petri-dishes of nutrient agar media. Duplicate sample was taken for each dilution, a control of nutrient agar media was also kept without inoculation. The inoculated petri dishes were incubated in incubator for 24 hour at 37 ±1 °C temperature .Total plate count was noted after 24 hours.

TPC (CFU/ml) = No. of colonies / dilution factor × 0.1

Where,

CFU = Colony Forming Unit

Amount Plated = 0.1 g

Coliform count: Violet red bile agar was used for coliform count.

Method: In laminar air flow, the media was poured in sterile petri-dishes in hot condition and kept till it solidified. The plates were marked according to the sample in duplicate. 100 mg of sample was weighed in a sterilized beaker added to the first dilution tube and mixed thoroughly, which was then serial diluted till 10^{-10} was achieved. The 10^{-10} sample was then plated on solidification agar plates using spread plate technique.

The plates were then incubated at 37 °C for 48 hours in inverted position. The colonies were then counted.

TPC (CFU/ml) = No. of colonies /dilution factor × 0.1

Where;

CFU= colony forming unit

Amount plated =0.1 g

Yeast and mold: PDA (Potato dextrose agar) was used to determine the yeast and mold in the biscuit.

Method

Sterilization: The prepared media was heated for 15 min an autoclave maintained at 15 psi for sterilization at 121 °C. All glassware's and necessary item were properly autoclave to avoid contamination. Pouring was done in the laminar–air flow chamber. The flame was lighted and petri-dishes were slightly opened near the flame and the media was poured in the petri-dishes and kept for solidification.

Inoculation of sample: Inoculation was done aseptically in laminar air flow chamber by taking 0.1 g of the sample suspended in saline solution from 10^{-2} dilution and transferred to petri-dishes with label 10^{-2} of nutrient agar media. Similarly, all the samples were transferred to the respective petri-diishes of nutrient agar media . Duplicate sample were taken for each dilution and a control of nutrient agar media was also kept without inoculation. The inoculated petri-dishes were incubated in incubator for 72 hours at 25 °C temperature. Colony was counted after 72 hours.

TPC (CFU/ ml) = No. of colonies /dilution factor × 0.1

Where,

CFU= Colony Forming Unit

Amount plated = 0.1 g

RESULTS AND DISCUSSION

Mineral analysis of Sugar Free Biscuit by Ion Chromatography

For the analysis of minerals in sugar free biscuits were compared with blank. The blank sample was prepared from the di-acid mixture 9:4 (v/v HNO_3: $HClO_4$) and the mineral content found in blank such as anion, chloride was 1.87 ppm concentration (Table 2) and (Fig. 7) whereas cations Viz. Sodium, potassium, magnesium, calcium concentration in blank sample was 3.075, 0.699, 1.526, and 10.309 ppm respectively (Table 2, Fig. 8).

The mineral content was also analysed in sugar free biscuit and found more in concentration than blank sample. The anion content in sugar free biscuits like chloride, bromide, sulphate was 0.961, 3.236, and 4.897 ppm respectively (Table 3, Fig. 9) and whereas cations viz. magnesium, and calcium was 6.041, 13.257 ppm respectively (Table 3, Fig. 10)

The result from mineral analysis by Ion Chromatography, it is recommended that the developed sugar free biscuit, fortified with fenugreek seed powder and natural sweetener stevia, rich in calcium and magnesium and their concentration 294.8 mg/100 g and 451.5 mg/100 g respectively. Rao *et al.* (2017) also reported the similar result for biscuit from wheat flour have calcium concentration 98.66mg/100g.

FT-IR analysis of Sugar Free Biscuits

FTIR spectroscopy was used to analyse the chemical structure and functional group. (Fig. 11) showed the FTIR spectra at around 2923.80 cm^{-1} the intensity of peak absorbance which is C-H group, around 1745 cm^{-1} which is C=O, 1164 cm^{-1} C-O group is present in the sugar free biscuit similarly reported in (Abi. *et al.* (2017).

Pasting properties of Sugar Free Biscuits and Refined wheat flour by Rapid Visco Analyser (RVA)

Pasting properties of Sugar Free Biscuits

There is very little gelatinization was found in sugar free biscuit as shown in (Fig. 12). There is no clear peak viscosity or hot paste viscosity evident was

Table 2: Mineral content in blank sample

			Anion		
Sl. No	**Component name**	**Retention time (min)**	**Height (µs/cm)**	**Area[(µs/cm)×min]**	**Concentration (ppm)**
1	Chloride	9.92	1.011	0.198	1.87
			Cation		
2	Sodium	4.17	3.326	0.677	3.075
3	Potasium	5.75	0.326	0.090	0.699
4	Magnesium	13.17	1.147	0.545	1.526
5	Calcium	16.00	2.716	2.140	10.309

Table 3: Mineral content in sugar free biscuit

Sl. No	Component name	Retention time (min)	Height (µs/cm)	Area[(µs/cm)×min]	Concentration (ppm)
			Anion		
1	Chloride	9.92	0.772	0.150	0.961
2	Bromide	14.00	0.205	.262	3.236
3	Sulphate	25.75	1.214	.677	4.897
			Cation		
4	Magnesium	12.74	3.782	2.293	6.041
5	Calcium	15.82	3.249	2.759	13.257

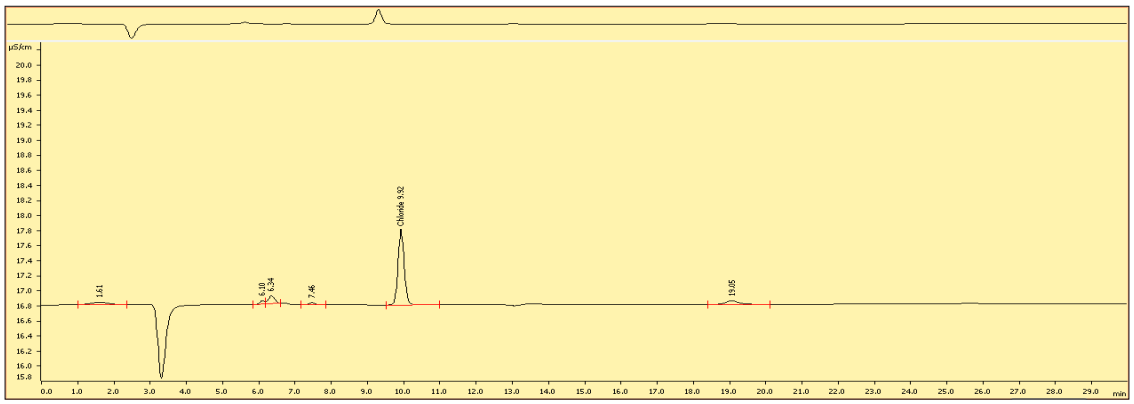

Fig. 7: Anion content in blank sample

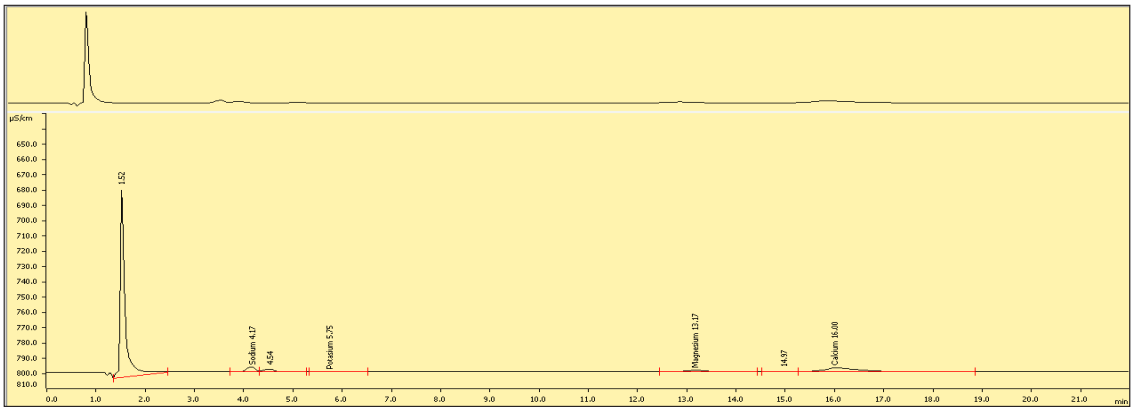

Fig. 8: Cation content in blank sample

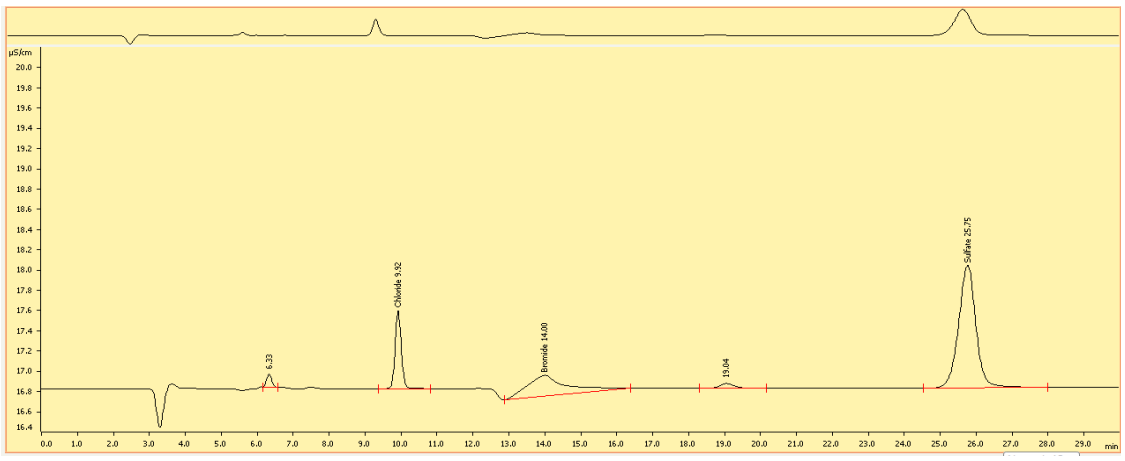

Fig. 9: Anion content in sugar free biscuit

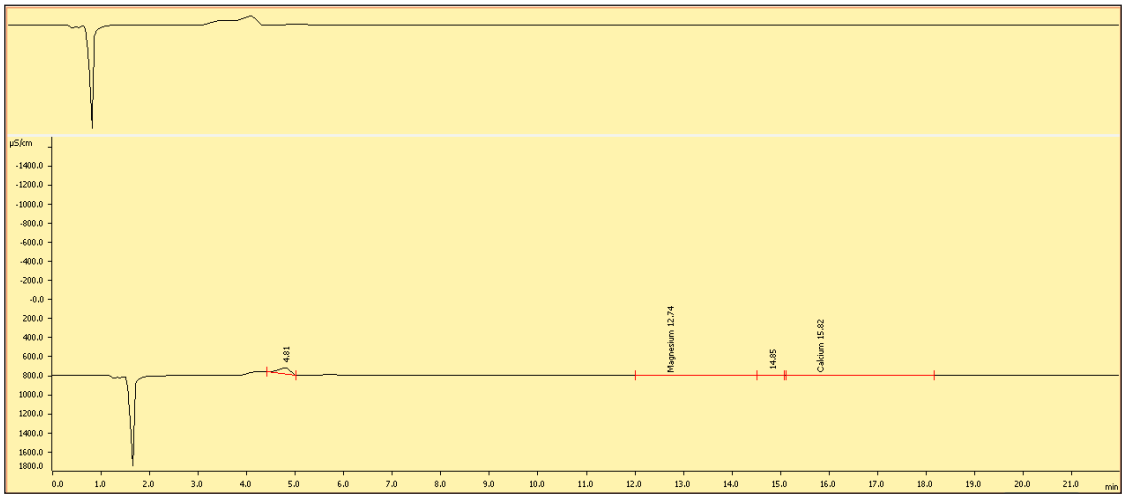

Fig. 10: Cation content in sugar free biscuit

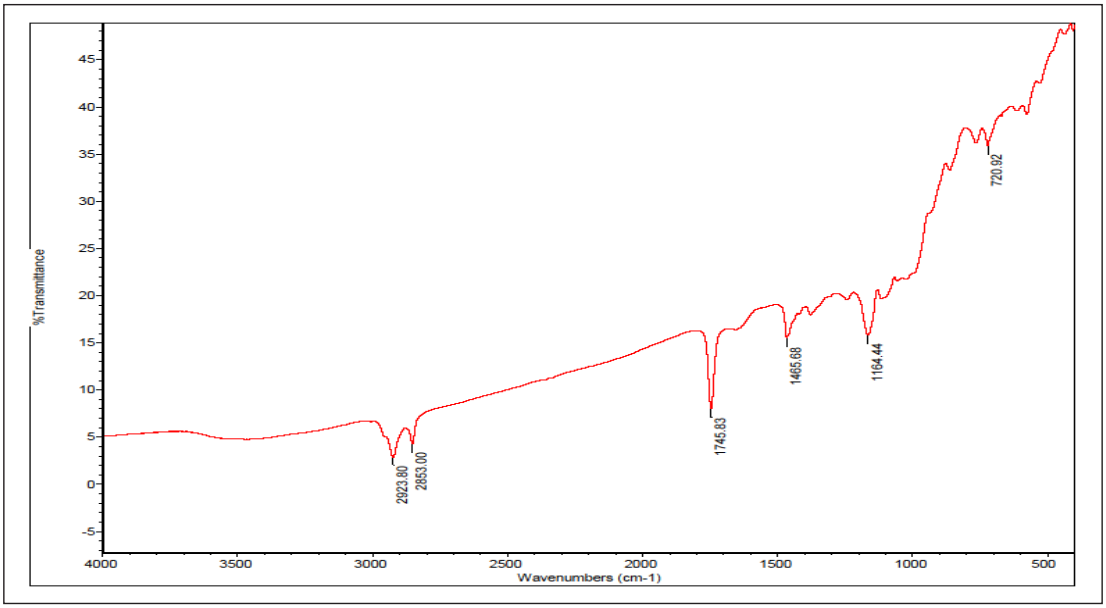

Fig. 11: FT-IR analysis of sugar free biscuit

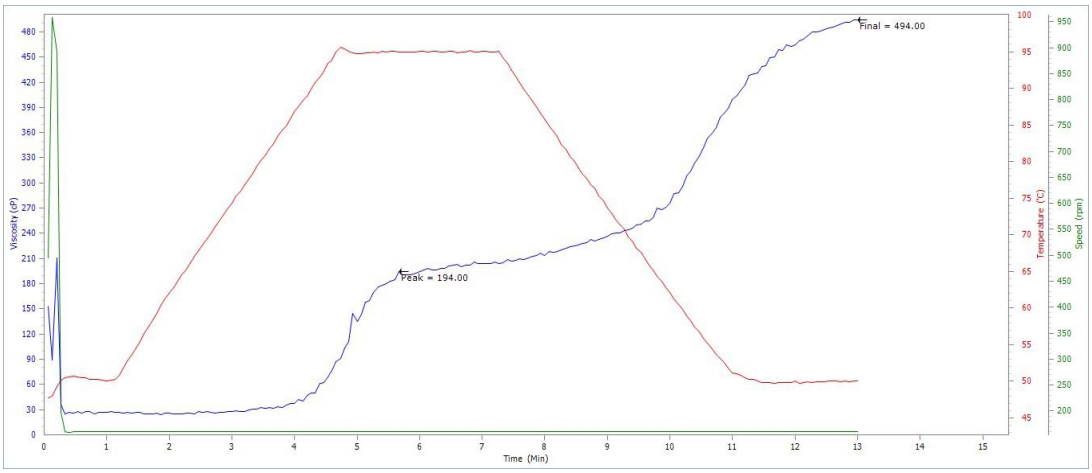

Fig. 12: Graph of pasting properties, peak viscosity and breakdown viscosity of sugar free biscuits

found. Peak viscosity is an indicator of amylose content and water absorption by the starch. A higher peak indicates a good proportion of amylose present which gives a greater cooking or hot paste viscosity. This pattern of Fig. was obtained because of the fact that the biscuit sample being tested here was a pre-cooked sample at a high temperature, where most of the starch and protein would have got gelatinized and denatured respectively.

The peak viscosity mentioned in the report was merely the value of viscosity obtained, at a point just before the shear sets in. There was no hold viscosity found and the peak viscosity being reported was the viscosity found at the end of test. The final viscosity at large depends upon the amylopectin component of starch being tested. This is because; amylopectin is resistant to water uptake and shear forces. Amylopectin, thus, is responsible for cool paste viscosity or retrograded viscosity. There was no breakdown also reported. Breakdown points indicates the sample's ability to resist shearing stress. It mainly is the result of amylose content of the starch which readily takes up water and swells up. The graph that has been obtained was most likely due to the presence of resistant starch that escapes any high heat treatment or because of the relative effect of various components present in the biscuit.

Pasting properties of refined wheat flour

Refined wheat flour (Maida) sample shows relatively low pasting temperature, indicating some modifications has been done (Fig. 13). It high peak viscosity with high trough values indicates that the sample has good proportion of amylose and thus has high water absorption and swells too many times their original size. This gives a greater cooking property. The high breakdown value indicates the sample was unable to resist shear stress, because of the high amylose content in it. It's nearly same final viscosity as its peak viscosity; show the partial waxy nature of the sample, implying that the proportion of amylose and amylopectin in the sample was nearly similar.

Determination of mineral content in sugar free biscuit by Atomic Absorption Spectroscopy

Iron, copper, magnesium and zinc content were analysed in sugar free biscuits by Atomic absorption spectroscopy. It was found that sugar free biscuit contain iron, 1.93mg/100g, copper, 0.065mg/100g, and zinc, 0.325 mg/100g. Similar finding were also reported by Rao *et al*. (2017) where biscuits fortified with spinach have iron content 2.85mg/100g.

Table 4: Mineral content in sugar free biscuit by AAS

Sl. No	Sugar free biscuit	Mineral content in mg/100g
1	Iron	1.93
2	cu	0.065
3	Zn	0.325

Energy value

Energy value of sugar free biscuit was measured by the digital bomb calorimeter and reported 484 kcal /100 g.

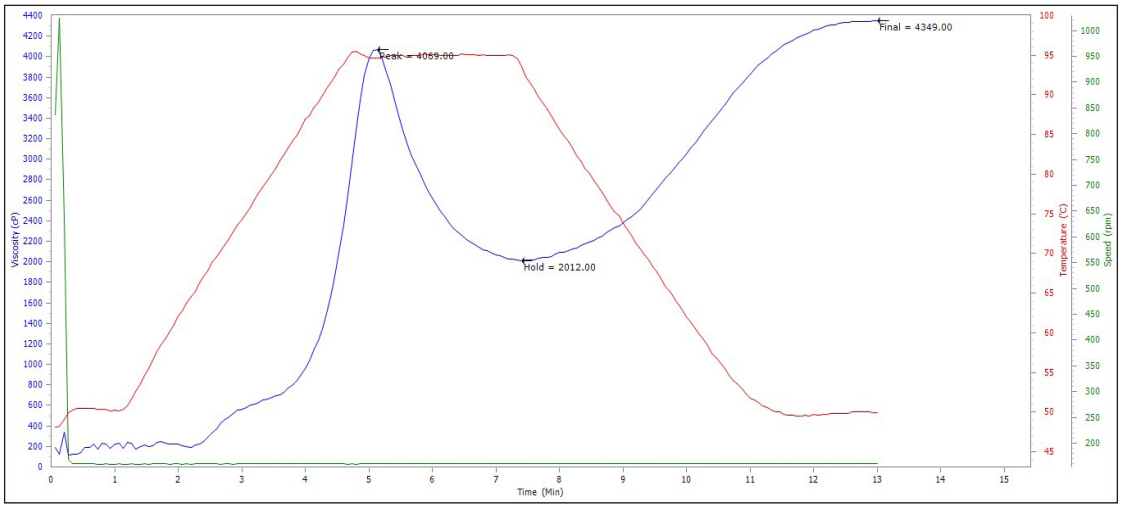

Fig. 13: Graph of pasting properties, peak viscosity and Breakdown viscosity of refined wheat flour

Reducing sugar

In the final optimized sugar free biscuit not detected reducing sugar.

Colour characteristics

The colour of sugar free biscuits was measured by Hunter lab (colour-flex). The final optimized sugar free biscuit is L- 46.40, a-12, b-20.29 and similar finding reported Rao *et al.* (2017) where L-66.15, a-11.86 and b-33.95. The developed sugar free biscuit have L and b value was low due to the fortification of fenugreek seed powder and as well as using of skimmed milk powder (SMP) and stevia.

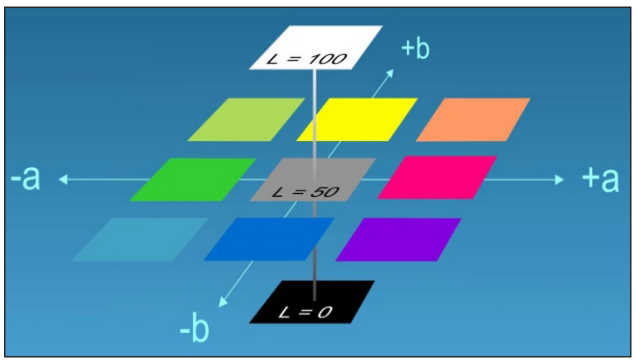

Fig. 14: Colour flex chart for L, a and b value

Shelf-life study of sugar free biscuit

Changes in Hydroxy Methyl Furfural (HMF) content during Storage

Hydroxymethyle furfural (HMF) in an organic compound derived from dehydration of certain sugars. HMF has been identified in wide variety of baked goods. It is practically absent in fresh food, but is naturally generated in sugar – containing food during heat – treatments like drying or cooking. Along with many other flavour and colour related substances, HMF is formed in the Maillard reaction as well as during caramelization. In these foods it is also slowly generated during storage and with increasing time.

The increase in HMF value is presented in figure. In fresh sugar free biscuit HMF value recorded was 5.1 mol/100g which increased to 5.34 mol/100g after storage of 10 days at 10 °C and 5.63 and 5.71 mol/100g at 25 and 37 °C respectively. After 20 days of storage the HMF increased to 5.81, 5.91, 5.96 mol/100g at 10 °C, 25 °C, 37 °C respectively. After 30 days of storage the HMF increased to 6.1, 6.3, 6.5 mol/100g at 10, 25, 37 °C respectively.

It was observed that the formation of HMF was lesser at 10 °C as compared to 25 and 37 °C. It can therefore be concluded that with increase in storage time and temperature the formation of HMF increases. At 10, 25, 37 °C, the total HMF increased to 6.1, 6.3, 6.5 mol/100g respectively after 30 day.

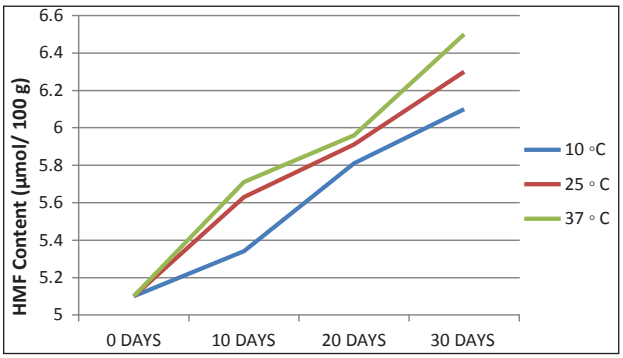

Fig. 15: Changes in HMF

Changes in Thiobarbituric acid (TBA) during storage

During storage fat or shortening used in bakery products such as biscuit, cookies, cakes etc undergo auto-oxidation which is often followed by storage odours. The TBA value is widely used for estimating peroxidation and rancidity in foods. The increase in (TBA) values is presented in Figure. In Fresh sugar free biscuit TBA value recorded was 0.178 which increased to 0.183 after storage 10 days at 10 °C and increased to 0.196 in 10 days at 25 °C and increased to 0.203 in 10 days at 37 °C after 20 days of storage the TBA increased to 0.212, 0.224 and 0.243 at 10, 25 and 37 °C respectively. After 30 days of storage the TBA increased to 0.254, 0.265 and 0.286 at 10, 25 and 37 °C respectively.

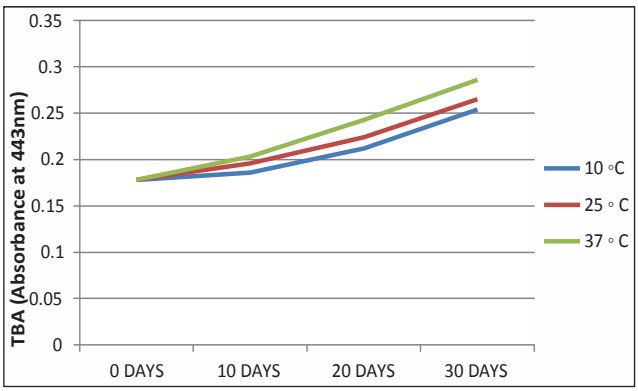

Fig. 16: Changes in TBA value

It was observed that the formation of TBA was lesser at 10◦c as compared to 25 and 37 °C. It can therefore be concluded that with increase in storage time and temperature the formation of TBA increases. At 10, 25 and 37 °C the total TBA increased to 0.254, 0.265, 0.286 respectively after 30 days of storage.

Changes in moisture during storage

Fig. 17 shows the moisture gain in sugar free biscuit during storage. The moisture content in the biscuit sample initially was 4% which increase to 4.21 and 4.32, 4.43% at 10, 25, 37 °C respectively in 10 days. In 20 days the moisture increased to 4.50, 4.58, 4.62, at 10, 25, and 37◦C respectively. The moisture increased after 30 days to 5.1, 5.25., 5. 5% at 10, 25, 37 °C respectively.

It is observed that the increase In moisture content was lesser at 10 °C as compared to 25 and 37 °C. It included that with increase in storage temperature and time the moisture content increased. This phenomenon of moisture absorption during storage is also supported by (Wade 1988), (Leelawathi and Rao 1993) and (Rao *et al*. 1995).

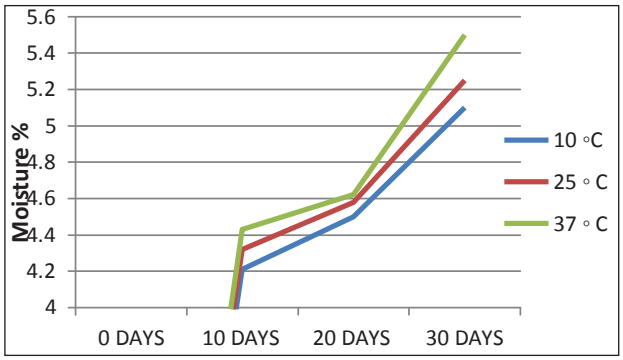

Fig. 17: Changes in moisture

Microbial Analysis

Microbiological studies were conducted for yeast and mould, total plate count and coliform. It was found that total plate count in fresh and after 10 days and 20 days storage period was not detected but after 30 days of storage the total plate count was found 3×10 cfu/ml which was well below the permissible limits. On the basis of these finding, it could be concluded that the product is safe to consume due to the high heat treatment and proper hygiene consideration during preparation of sugar free biscuit. The microbiological analysis is given below in (Table 5).

Table 5: Microbial Analysis

Sl. No.	Parameter	0 Days	10 Days	20 Days	30 Days
1	TPC (cfu/ml)	ND	ND	ND	3×10
2	Yeast and mould (cfu/ml)	ND	ND	ND	ND
3	Coliform count	ND	ND	ND	ND

ND- Not Detected.

CONCLUSION

The present research work "Micronutrient analysis by ion chromatography and AAS, pasting properties, and shelf life study of sugar free biscuit enriched with fenugreek seed powder and natural sweetener stevia. Developed sugar free biscuit was rich in calcium and magnesium and their concentration were observed 294.8mg/100g and 451.5mg/100 g respectively by ion chromatography. Atomic absorption spectroscopy of developed sugar free biscuits was also performed for mineral analysis and reported that it contains iron (1.93mg/100g), copper (0.065mg/100g), and zinc (0.325 mg/100g). The colour of sugar free biscuit was recorded by hunter colour flex and the L*, a*, b*, value was 46.40, 12, 20.29, respectively which is similar to other biscuits available in the market and acceptable by consumer. The pasting properties of sugar free biscuit was analysed by Rapid visco analyser there is very little gelatinization was found in sugar free biscuit. The shelf-life studies of the final product found that the shelf life of sugar free biscuit was lower at 37°C than at 10°C and 25°C. The HMF (Hydroxy Methyl Furfural) concentration which indicates the mallard reaction at a higher temperature and with increasing days of storage, the HMF concentration was also increased. The HMF increased to 6.1, 6.3, 6.5 µmol/100g at 10, 25, 37°C respectively after 30 days from 5.1 mol/100g which was the HMF value initially. The TBA (Thiobarbituric acid) also increased the most at 30 days storage at 10 °C as compared to 25 °C and 37°C, TBA value was 0.254, 0.265 and 0.286 respectively. The same result was seen in moisture content 5.1, 5.25, 5. 5% at 10 °C, 25 °C, 37 °C respectively from 4 % of moisture initially content. The Microbiological studies concluded that the product was safe with no yeast and mould growth and coliform was also not detected. Only total plate count after 30 days was found 3×10 CFU/

ml. The product was found safe for consumption even after 30 days of storage. So, from the above study, it can be concluded that fenugreek seed powder is the good source of mineral content and functional component trigonelline. The developed sugar free biscuit was also enriched with natural sweetener stevia. From the above data, it can be recommended that the developed sugar free biscuit have promising health benefits for diabetic patient.

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