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Incorporation of ghee residue protein hydrolysate powder in healthy biscuit

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ABSTRACT

Ghee residue (GR) protein hydrolysate was utilized in study to enrich biscuit with healthy content. In this first maida and sugar were first replaced with healthier alternatives like jowar and jaggery. Then to increase protein content of biscuit ghee residue hydrolysate were added to it. 5%, 10%, 15% incorporation level was checked. At 10% addition of hydrolysate powder sensory panel reported good acceptability which get decreased on 15% addition of powder. So finally 10% incorporation was selected which increased protein content of significantly. Further aerobically packaged biscuit were taken for shelf life evaluation till 60 days period in which biscuit performed well. Thus GR protein hydrolysate incorporated biscuit can provide healthier alternative with sustainable use for byproduct.

Keywords: Bitter peptides, Enzymatic hydrolysis, Freeze dried, Dairy byproduct

Introduction

Rapidly growing dairy industry of India needs sustainable utilization of animal products, which can be achieved through circular economy. But it is incomplete without addressing issue of byproduct utilization. Thus using complete potential of byproducts is possible only after properly processing it to harness its full nutritional content. One of such byproduct in dairy industry is ghee residue which is being underutilized in current situation. To, utilized it properly in various studies it has been hydrolysed using various methods. However this hydrolysis had resulted in generation of bitter peptides in final product. Thus incorporating it in regular diet got challenging thus hampering its ultimate usability. Thus in order to harness its full potential and put this byproduct origin product again into food chain the current study had incorporated it in biscuit. Biscuit is important food product of day in Indian homes loved by millions of Indians. However refined wheat flour which is chief ingredient in it, causing many health challenges and though the morning biscuits it disposing people to many health hazards. Also the no protein content in morning diet is very concerning situation in already protein deprived Indian population. Thus to address this protein need in morning diet, a healthy biscuit with enriched protein can easily address issue. Similarly for vegetarian population of India it can provide good start of day with balance nutrition of carbohydrates, proteins and fat. Similarly it will effectively utilize protein content of this bitter powder and will ensure sustainable use of this important product of dairy industry.

2. Materials and Methods

2.1 Biscuit preparation

Concentrations of ingredients (Table-1) were selected for preparation of biscuits. Baking temperature and time had been standardized based on the sensory evaluation on eight point hedonic scale. Replacement of *Maida* with different level of Jowar (sorghum) and sugar with Jaggery at selected baking time temperature combination had been standardized based on sensory evaluation.

Table 1. Different base ingredients in biscuit preparation

Ingredient	Weight(g)
<i>Maida</i>	100
Sugar	30
Salt	0.5
Oil	10
Butter	10
Baking powder	1.5

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Table 2. Different cooking time temperature combinations for biscuit preparation

Treatment	Temperature	Time
T_1	180°C	10 min
T_2	180°C	12 min
T_3	180°C	15 min
T_4	160°C	10 min
T_5	160°C	12 min
T_6	160°C	15 min

Where T - treatment

Table 3. Different level of incorporation of Jowar (sorghum) in biscuit

Ingredient	Control	C_1 (weight in grams)	C_2 (weight in grams)	C_3 (weight in grams)
Maida	100	75	50	25
Sugar	30	30	30	30
Salt	0.5	0.5	0.5	0.5
Oil	10	10	10	10
Butter	10	10	10	10
Baking powder	1.5	1.5	1.5	1.5
Jowar	-	25	50	75

Where C_1 : 25g Jowar, C_2 : 50g Jowar, C_3 : 75g Jowar

Table 4. Different level of incorporation of Jaggery in biscuit

Ingredient	Control	G_1 (weight in grams)	G_2 (weight in grams)	G_3 (weight in grams)
Maida	50	50	50	50
Jowar	50	50	50	50
Sugar	30	20	15	10
Jaggery	-	10	15	20
Salt	0.5	0.5	0.5	0.5
Oil	10	10	10	10
Butter	10	10	10	10
Baking powder	1.5	1.5	1.5	1.5

Where G_1 : 10g jaggery, G_2 : 15g jaggery, G_3 : 20g jaggery

All the ingredients were mixed with optimum mechanical action to incorporate more and more air into dough of biscuit. The dough was cast into biscuit using mould. Baking was done in air fryer (AGARO Regal plus, Model/year: 34003/2024). Standardisation for cooking time and temperature was done and at selected time temperature combination concentration of biscuit ingredients were standardised. First maida replacement was check followed by replacement of sugar.

Selection was done by sensory evaluation

Sensory evaluation

A semi-trained panel, comprising scientists and postgraduate students from the LPT Division, assisted in carrying out the sensory evaluation of the product. Sensory testing sessions were consistently held be-

tween 3:30 to 4:00 pm. biscuits were assessed based on their appearance, Texture, flavor, crunchiness, aftertaste, and overall acceptability. The evaluation employed an 8-point hedonic scale, where a score of 8 indicated "like extremely" and 1 represented "dislike extremely" (Annexure-2) (Keeton, 1983) [1]

2.2 Optimization of incorporation level of GR hydrolysate in the basic product

In selected base product enzymatically hydrolysed freeze dried powder (Khawale, 2025) [2] had been added at level of 5%, 10% and 15% (Table-5) level and sensory evaluation was done for selection of optimum incorporation of powder due to its slightly bitter aftertaste. For incorporation of powder, replacement of Maida with powder had been checked.

Table 5. Different level of incorporation of GR in biscuit

Ingredient	Control Weight (g)	GR_1 Weight (g)	GR_2 Weight (g)	GR_3 Weight (g)
Maida	50	45	40	35
Jowar	50	50	50	50
Sugar	15	15	15	15
Jaggery	15	15	15	15
Salt	0.5	0.5	0.5	0.5

(continued on next page)

Ingredient	Control Weight (g)	GR₁ Weight (g)	GR₂ Weight (g)	GR₃ Weight (g)
Oil	10	10	10	10
Butter	10	10	10	10
Baking powder	1.5	1.5	1.5	1.5
Ghee Residue Hydrolysate	0	5	10	15

Where, Control: no ghee residue, GR₁: 5% ghee residue, GR₂: 10% ghee residue, GR₃: 15% ghee residue

2.3 Estimation of shelf life of GRH incorporated biscuits

Aerobic packaging of Biscuits was done in laminate pouches and stored for 60 days, periodically after every 10 days samples were drawn and evaluated.

2.3.1 Water activity

Measured using a digital water activity meter, where the samples were filled in plastic cups provided with the instrument and placed in the analyzer at 25 °C

2.3.2 TBARS value estimation

10 grams of the sample were weighed and transferred into a clean beaker. To this, 25 ml of 20% pre-cooled trichloroacetic acid (TCA) solution was added. The mixture was then homogenized at 15,000 rpm for two minutes using a homogenizer. The homogenate was rinsed with 25 ml of pre-cooled distilled water (DW). The entire content was then filtered through Whatman No. 1 filter paper, and the filtrate was collected as the TCA extract.

Subsequently, 5 ml of the TCA extract was combined with 5 ml of a 5 mM thiobarbituric acid (TBA) solution in a test tube. A blank solution

was prepared by mixing 5 ml of the 20% TCA solution with 5 ml of 5 mM TBA solution. The test tubes were incubated for 30 minutes at 70 °C in a water bath. After the incubation, the tubes were cooled under running tap water for 2–3 minutes. The absorbance was measured at 523 nm using a Thermo Scientific GENESYS 10S UV-Vis spectrophotometer. The absorbance value was then multiplied by 5.2 to convert it to milligrams of malondialdehyde per kilogram of the sample. (Witte *et al.*, 1970) [3]

2.3.3 Peroxide value

A 5 g sample was combined with 30 ml of chloroform and 1 g of anhydrous sodium sulfate, blended for 2 minutes using a homogenizer. The mixture was passed through a Whatman No. 1 filter paper. From the resulting filtrate, 25 ml of chloroform extract was taken and placed in a 250 ml conical flask. To this, 30 ml of glacial acetic acid and 2 ml of saturated potassium iodide were added, with the mixture being swirled intermittently for 2 minutes. The reaction was stopped by adding 100 ml of distilled water, followed by the addition of 2 ml of 1% fresh starch solution. Finally, the contents were titrated with 0.1 N sodium thiocyanate or sodium thiosulfate until the non-aqueous layer became colorless (Koniecko, 1979) [4].

$$\text{Peroxide Value (meq/Kg)} = 0.1 \times 0.1N \text{ of Sodium thiosulphate} \times \frac{\text{weight of sample (gm)} \times 100}{\text{weight of sample (gm)}} \times 100$$

2.3.4 Microbiological parameters

Preparation of sample and serial dilution

The packaged samples were unsealed, and 1 gram of the sample was aseptically prepared in 9 ml of sterile 0.1% peptone water. The sample was mixed thoroughly, representing a 10⁻¹ dilution. To prepare the 10⁻² dilution, 1 ml of the initial solution was transferred into a sterile test tube containing 9 ml of sterile 0.1% peptone water, followed by thorough mixing. Further dilutions were made by transferring 1 ml from the previous dilution into 9 ml of 0.1% peptone water. Serial dilutions were performed as needed. The entire process of sample preparation and serial dilution was conducted near an open flame within a horizontal laminar air flow cabinet (Airstream®, ESCO class II BSC).

Total plate count

Plate count agar (23.5 grams) dissolved in 1000 ml of double-distilled water. The media was sterilized by autoclaving at 121 °C, 15 lbs of pressure for 15 minutes. After autoclaving, the medium was cooled to 45 ± 2 °C. The final pH was adjusted to 7.0 ± 0.2. One ml of the diluted sample from the required dilution was poured into sterile, disposable Petri dishes near an open flame within the horizontal laminar air flow cabinet. Each dilution was plated in triplicate. Approximately 10-12 ml of the cooled medium was added, and the Petri dishes were gently rotated clockwise and counter-clockwise to ensure even distribution of the medium and sample. The plates were left undisturbed until the agar solidified. Once solidified, the plates were inverted and incubated at 35 °C for 48 hours in an incubator (NSW®). After incubation, plates with 30-300 colonies were counted, and the colony count was multiplied by the inverse of the dilution factor, with results expressed as log₁₀ cfu/g of the sample.

Yeast and Mould count

Potato dextrose agar (39 grams) dissolved in 1000 ml of double-distilled water. The medium was sterilized by autoclaving at 121 °C, 15 lbs of pressure for 15 minutes. After autoclaving, the medium was cooled to 45 ± 2 °C. The sterile, cooled medium was acidified to a pH

of 3.5 ± 0.1 by adding 10 ml of 10% sterile tartaric acid, taking care to prevent heating of the acidified medium. One milliliter of the desired dilution was poured into sterile, disposable Petri dishes near an open flame in the horizontal laminar air flow cabinet. Each dilution was plated in triplicate. Approximately 10-12 ml of the medium was added, and the Petri dishes were gently rotated clockwise and counter-clockwise to ensure even distribution of the medium and sample. The plates were left undisturbed until the agar solidified. Once solidified, the plates were inverted and incubated at 25 °C for 5-7 days. After incubation, the colonies on the plates were counted, multiplied by the inverse of the dilution factor, and expressed as log₁₀ cfu/g of the sample

3. Result and Discussion

3.1 Standardization of formulation and processing conditions for biscuit preparation

To determine the best baking conditions, six different treatments were evaluated based on sensory scores from a semi-trained panel comprising scientists and postgraduate students from the LPT Division (Table-6). In terms of appearance, the panelists gave significantly ($p < 0.01$) higher acceptability to treatment T₅, which scored within the 'liked very much' range. However, significant ($p < 0.01$) differences were observed among the other treatments as well. For texture and flavor, all treatments showed insignificant ($p > 0.01$) differences, but T₅ still received a 'liked very much' score. For crunchiness and after-taste, T₅ again showed significantly ($p < 0.01$) higher acceptability compared to other treatments, with the highest overall acceptability score of 'liked very much' from the panelists.

Considering the sensory scores across all attributes, the baking condition of T₅ (160⁰ for 12 minutes) was selected for further study ($n = 6$); Mean±SE with different superscripts column wise (A,B,C) differ significantly

Where T- treatment

Table 6. Sensory evaluation of different cooking time temperature combination

Attribute	T1	T2	T3	T4	T5	T6
Appearance	6.833 ± 0.211 ^B	7.000 ± 0.316 ^B	6.417 ± 0.154 ^B	6.500 ± 0.183 ^B	7.333 ± 0.167 ^A	6.500 ± 0.183 ^B
Texture	6.917 ± 0.239 ^A	6.750 ± 0.250 ^A	6.333 ± 0.167 ^A	6.417 ± 0.154 ^A	7.083 ± 0.154 ^A	6.833 ± 0.211 ^A
Flavour	7.083 ± 0.239 ^A	7.417 ± 0.239 ^A	6.750 ± 0.112 ^A	6.833 ± 0.211 ^A	7.417 ± 0.239 ^A	7.417 ± 0.239 ^A
Crunchiness	6.250 ± 0.214 ^C	7.083 ± 0.239 ^B	7.083 ± 0.154 ^B	6.500 ± 0.183 ^C	7.667 ± 0.167 ^A	6.917 ± 0.154 ^C
After Taste	6.583 ± 0.239 ^B	6.583 ± 0.239 ^B	6.533 ± 0.203 ^B	6.917 ± 0.154 ^B	7.333 ± 0.167 ^A	6.417 ± 0.153 ^B
Overall Acceptability	6.242 ± 0.108 ^C	6.833 ± 0.167 ^B	6.167 ± 0.105 ^C	6.417 ± 0.154 ^C	7.500 ± 0.134 ^A	6.500 ± 0.183 ^C

Treatment	Temperature	Time
T ₁	180°C	10 min
T ₂	180°C	12 min
T ₃	180°C	15 min
T ₄	160°C	10 min
T ₅	160°C	12 min
T ₆	160°C	15 min

After standardizing the time-temperature combination for biscuit preparation, the replacement of *Maida* with Jowar flour at different levels (25%, 50%, and 75%) was evaluated (Table-7) based on sensory evaluation. The evaluation focused on key attributes including appearance, texture, flavor, crunchiness, aftertaste, and overall acceptability of the biscuits. The sensory scores for each of these attributes were assessed to determine the optimal level of Jowar substitution for a desirable biscuit product. In this 50% replacement of *Maida* with Jowar had shown significantly ($p < 0.01$) higher score for all the parameters over other preparations. With notably higher score recorded for aftertaste and overall acceptability at 50% replacement of *Maida* with Jowar. Based on this results biscuits with 50% replacement of *Maida* with Jowar were taken for further experiment where replace-

ment of sugar with jaggery were tested in similar way on same parameters. In this 30 gram sugar in base preparation was replaced by different level (10g, 15g, 20g) of Jaggery (Table-8). In sensory evaluation 15g replacement of sugar with Jaggery performed best over other two replacements so finally it was selected. At the end biscuit preparation of 50% replacement of *Maida* with Jowar and 15g replacement of sugar with Jaggery was selected to incorporate enzymatically hydrolysed freeze dried Ghee Residue powder (EHFGR) in biscuit to check inclusion level.

($n = 6$); Mean±SE with different superscripts column wise (A,B,) differ significantly ($p < 0.01$)

Where C₁: 25g Jowar, C₂: 50g Jowar, C₃: 75g Jowar

Table 7. Sensory evaluation of Different level of incorporation of Jowar (Sorghum)

Attribute	Control	C1	C2	C3
Appearance	7.436 ± 0.156 ^A	6.833 ± 0.247 ^B	7.333 ± 0.167 ^A	6.933 ± 0.138 ^{AB}
Texture	7.333 ± 0.166 ^A	6.583 ± 0.201 ^C	7.167 ± 0.105 ^{AB}	6.750 ± 0.250 ^B
Flavour	6.333 ± 0.167 ^B	6.500 ± 0.183 ^B	7.500 ± 0.183 ^A	7.500 ± 0.183 ^A
Crunchiness	7.083 ± 0.239 ^{AB}	6.667 ± 0.211 ^B	7.417 ± 0.239 ^A	6.750 ± 0.214 ^B
After Taste	6.167 ± 0.105 ^B	6.583 ± 0.239 ^B	7.333 ± 0.167 ^A	6.750 ± 0.250 ^B
Overall Acceptability	6.241 ± 0.108 ^C	6.333 ± 0.167 ^C	7.667 ± 0.167 ^A	6.750 ± 0.112 ^B

Table 8. Sensory evaluation of Different level of incorporation of jaggery

Attribute	Control	G1	G2	G3
Appearance	6.241 ± 0.108 ^B	6.583 ± 0.239 ^B	7.437 ± 0.157 ^A	6.767 ± 0.214 ^B
Texture	6.333 ± 0.167 ^B	7.167 ± 0.279 ^A	7.167 ± 0.169 ^A	6.517 ± 0.212 ^B
Flavour	6.17 ± 0.105 ^B	6.583 ± 0.201 ^B	7.450 ± 0.243 ^A	6.500 ± 0.183 ^B
Crunchiness	6.933 ± 0.138 ^B	7.000 ± 0.183 ^B	7.650 ± 0.161 ^A	6.667 ± 0.174 ^B
After Taste	6.416 ± 0.137 ^B	6.583 ± 0.239 ^B	7.350 ± 0.161 ^A	6.583 ± 0.239 ^B
Overall Acceptability	6.666 ± 0.210 ^B	6.667 ± 0.247 ^B	7.333 ± 0.167 ^A	6.417 ± 0.138 ^B

($n = 6$); Mean±SE with different superscripts column wise (A,B,) differ significantly ($p < 0.01$)

Where G₁: 10g jaggery, G₂: 15g jaggery, G₃: 20g jaggery

3.2 Optimization of incorporation level of GR hydrolysate in the basic product

In the preparation of the biscuit base, the recipe initially consisted of 50g maida and 50g jowar. To incorporate the enzymatically hydrolyzed freeze-dried ghee residue (EHFGR) powder, maida was replaced with 5g, 10g, and 15g of EHFGR powder, representing 5%,

10%, and 15% replacement of the base with EHFGR powder, while keeping all other ingredients constant as standardized in section 4.5.1. This modification aimed to assess the impact of varying EHFGR powder inclusion on the sensory attributes and overall quality of the biscuits.

In the sensory evaluation (Table-9) of these biscuits, there were no significant differences observed in appearance and texture. However, flavor and aftertaste exhibited significant differences ($p < 0.01$) with the 15% addition of EHFGR powder compared to the other two formulations and control. Sensory panelists noted a significant ($p < 0.01$)

bitterness in the aftertaste of biscuits with a 15% addition of EHFGR powder. Although there was insignificant ($p > 0.01$) difference in control, 5% and 10% incorporation level, as powder is protein rich commodity and to utilize more and more powder in biscuit 10% level was selected for further experiment.

Findings of this experiment are in accordance to previous studies by *Abraha et al. (2018)* [5] *Sinthusamran et al. (2019)* [6] in which they have reported significantly higher protein in biscuits fortified with protein hydrolysate. *Singh et al. (2020)* [7] reported 15% addition of hydrolysate in biscuit which is more incorporation level as compared to our finding which is due to debittering treatment done by them

could have beneficial effect on incorporation level into biscuit as bitterness in aftertaste is major factor limiting incorporation of hydrolysate into biscuit in our study. Similarly higher incorporation level (15%) was reported by *Zhang et al. (2023)* for whey protein hydrolysate in which phenylalanine content was lowered thus reduced bitterness in product.

($n = 6$); Mean $\pm$ SE with different superscripts column wise (A,B,) differ significantly ($p < 0.01$)

Where Control: no ghee residue, GR₁: 5% ghee residue, GR₂: 10% ghee residue, GR₃: 15% ghee residue

Table 9. sensory evaluation for Level of incorporation of ghee residue in biscuit

Attribute	Control	GR1	GR2	GR3
Appearance	7.437 $\pm$ 0.157 ^A	7.367 $\pm$ 0.156 ^A	7.317 $\pm$ 0.174 ^A	6.800 $\pm$ 0.208 ^A
Texture	7.167 $\pm$ 0.169 ^A	7.067 $\pm$ 0.167 ^A	7.050 $\pm$ 0.161 ^A	6.567 $\pm$ 0.203 ^A
Flavor	7.450 $\pm$ 0.243 ^A	7.650 $\pm$ 0.161 ^A	7.750 $\pm$ 0.092 ^A	6.633 $\pm$ 0.156 ^B
Crunchiness	7.650 $\pm$ 0.161 ^A	7.767 $\pm$ 0.105 ^A	7.550 $\pm$ 0.229 ^A	6.733 $\pm$ 0.174 ^B
After Taste	7.350 $\pm$ 0.161 ^A	7.267 $\pm$ 0.167 ^A	7.233 $\pm$ 0.193 ^A	6.283 $\pm$ 0.209 ^B
Overall Acceptability	7.333 $\pm$ 0.167 ^A	7.550 $\pm$ 0.050 ^A	7.667 $\pm$ 0.167 ^A	6.367 $\pm$ 0.148 ^B

3.3 Study of complete product profile of the developed biscuits

3.3.1 Proximate composition of biscuit (on dry matter basis)

The proximate composition of the EHFGR-incorporated biscuit (Table-10) provides valuable insight into its nutritional profile. The moisture content of the biscuit was found to be $3.04 \pm 0.39\%$, while

the protein content was $14.75 \pm 0.79\%$, and the fat content was $10.75 \pm 0.39\%$. The total ash content was observed to be $2.89 \pm 0.37\%$. The carbohydrate content, calculated by the difference method, was $68.58 \pm 0.90\%$, indicating that carbohydrates, primarily derived from the flour and sugar, constitute the highest proportion of the biscuit's composition. This suggests that the biscuit is energy-dense and primarily serves as a quick energy source.

Table 10. Proximate composition of biscuit

Parameter	Control	Sample
Moisture	3.31 $\pm$ 0.57	3.04 $\pm$ 0.39
Protein	5.78 $\pm$ 0.68	14.75 $\pm$ 0.79
Fat	11.02 $\pm$ 0.28	10.75 $\pm$ 0.39
Total Ash	2.24 $\pm$ 0.38	2.89.37
Carbohydrate	77.65 $\pm$ 0.47	68.58 $\pm$ 0.90

Notably, there was a significant increase in protein content compared to the control biscuit, with the added protein primarily coming from the EHFGR powder. For comparison, some popular biscuit brands currently available in the market claim a protein content of about 4%. However, some brands marketing "high-protein" biscuits may claim protein contents ranging from 10 to 50g per 100g of product.

Also similar study of incorporation of protein hydrolysate in biscuit by *Abraha et al. (2018)* [5] had reported $19.52 \pm 0.12\%$ crude protein in biscuit with 10% incorporation of hydrolysate and *Sinthusamran et al. (2019)* [6] had reported $11.82 \pm 0.21\text{g protein/100g biscuit}$ at 5% incorporation of hydrolysate.

Table 11. characteristics of biscuit

Parameter	Control	Sample
pH	7.182 $\pm$ 0.051	7.338 $\pm$ 0.012
Water activity	0.218 $\pm$ 0.005	0.276 $\pm$ 0.009

3.4 Estimation of shelf life of GRH incorporated biscuits

Table 12. Storage stability study of ghee residue incorporated biscuits

Parameter	0 Day	10 Day	20 Day	30 Day	40 Day	50 Day	60 Day
pH	7.338 $\pm$ 0.012 ^A	7.306 $\pm$ 0.004 ^B	7.283 $\pm$ 0.004 ^C	7.241 $\pm$ 0.006 ^D	7.20 $\pm$ 0.006 ^D	7.17 $\pm$ 0.005 ^E	7.1 $\pm$ 0.004 ^F
Moisture	2.558 $\pm$ 0.233 ^B	3.058 $\pm$ 0.335 ^B	3.192 $\pm$ 0.451 ^B	3.235 $\pm$ 0.377 ^B	3.737 $\pm$ 0.372 ^B	3.935 $\pm$ 0.054 ^A	4.350 $\pm$ 0.129 ^A
Water Activity	0.276 $\pm$ 0.009 ^E	0.351 $\pm$ 0.013 ^D	0.395 $\pm$ 0.010 ^{CD}	0.429 $\pm$ 0.020 ^C	0.496 $\pm$ 0.005 ^B	0.554 $\pm$ 0.010 ^A	0.580 $\pm$ 0.014 ^A

(continued on next page)

Parameter	0 Day	10 Day	20 Day	30 Day	40 Day	50 Day	60 Day
TBARS	0.142 ± 0.014 ^G	0.260 ± 0.013 ^F	0.320 ± 0.008 ^E	0.387 ± 0.007 ^D	0.450 ± 0.007 ^C	0.493 ± 0.007 ^B	0.542 ± 0.006 ^A
Mg							
Peroxide	0.917 ± 0.011 ^G	1.212 ± 0.011 ^F	1.573 ± 0.008 ^E	1.828 ± 0.009 ^D	2.138 ± 0.016 ^C	2.315 ± 0.009 ^B	2.417 ± 0.007 ^A
meq/Kg							

($n = 6$); Mean±SE with different superscripts column wise (A,B,C,D,E,F,G) differ significantly ($p < 0.01$)

3.4.1 pH change

The pH of the ghee residue incorporated biscuits exhibits a gradual decrease over the 60-day storage period, similar to the findings for enzymatically hydrolyzed freeze-dried powders. At Day 0, the pH is 7.338 ± 0.012 , and it decreased to 7.1 ± 0.004 by Day 60. This decrease in pH is statistically significant ($p < 0.01$), as indicated by the different superscripts (A, B, C, D, E, F) in Table-12. The superscripts denote significant differences in mean pH values across the different time points, with Day 0 showing the highest pH and Day 60 showing the lowest. This change could be linked to several factors, such as the chemical composition of the biscuits, interaction with moisture, or enzymatic processes during storage discussed in subsequent sections.

3.4.2 Moisture

Table-12 shows the moisture content of ghee residue-incorporated biscuits packed aerobically at various time points during a 60-day storage period. The moisture content is reported in percentage, the data indicate a gradual increase in moisture content over the course of storage, which is due to the absorption of moisture from the environment. Increase moisture content of ghee residue-incorporated biscuits showed significant ($p < 0.01$) differences between the various storage days. The gradual increase in moisture content is likely a result of various factors, such as humidity, temperature conditions during storage hygroscopic nature of biscuit which cause the biscuits to absorb moisture (Romani *et al.*, 2016; Cappelli *et al.*, 2022) [9, 8].

These results are in agreement with, findings of Balestra *et al.* (2019) [10] they reported similar significant increase in moisture content of biscuit during storage days for this they pointed towards hygroscopic nature of dried food and permeability of packaging material. Romani *et al.* (2016) [9] also reported similar trend of increase moisture content of biscuit during storage where they reported fastest increase in moisture content during first 15 days of storage due to hygroscopic nature and porous structure of product.

3.4.3 Water activity

Table-12 presents the water activity (a_w) values for ghee residue hydrolysate incorporated biscuits packed aerobically over a 60-day storage period. Water activity is a critical parameter in food preservation as it reflects the availability of water for microbial growth and chemical reactions. The data show that water activity increases progressively from Day 0 through Day 60 and these increase during storage is statistically significant ($p < 0.01$). Water activity increased from 0.276 ± 0.009 at day 0 to 0.580 ± 0.014 at day 60th, which is within the requirement to check growth of microbes in product at the end of storage period. Similar findings were reported by Kumar *et al.* (2016) [11], Romani *et al.* (2016) [9], Balestra *et al.* (2019) [10] where these

researchers found increasing trend of water activity for biscuits during storage life in respective studies. Romani *et al.* (2016) [9] highlighted porous nature of biscuit for this increased water activity.

3.4.4 TBARS value estimation

The Table-12 presents the values of TBARS (expressed as my; MDA/Kg) over a 60-day storage period for ghee residue hydrolysate incorporated biscuits packed aerobically. TBARS is a common indicator used to assess the extent of lipid oxidation in product particularly secondary products in lipid oxidation. In this study TBARS values showed increasing trend during storage study, observed TBARS value at each time point are significantly different from each other ($p < 0.01$). For instance, the TBARS value at day 60 (0.547 ± 0.006 MDA/Kg) is significantly higher than that at day 0 (0.142 ± 0.014 MDA/Kg), demonstrating a significant increase in lipid oxidation as the biscuits age. This suggests that the storage duration has a notable impact on the oxidative stability of the biscuits packed aerobically with a marked accumulation of oxidation products over time. Marcinkowska-Lesiak *et al.* (2018) reported similar increasing trend of TBARS value in storage study of biscuit. Also they reported increase in TBARS value even after addition of antioxidant powder however it performed well over control biscuit having no antioxidant powder. Similarly, Patrignani *et al.* (2014) [12] reported lipid oxidation in biscuits during storage study, noting that the highest levels of oxidation were found in samples with a higher content of unsaturated fatty acids. This could also explain the fat oxidation observed in the aerobic storage conditions of our study. Kumar *et al.* (2016) [11] observed an increasing trend in TBARS values of biscuits during storage and attributed this to the oxygen permeability of the packaging material, which facilitated the oxidation of lipid components of product.

3.4.5 Peroxide value (PV)

Peroxide values (meq/Kg) are a key indicator of lipid oxidation, particularly it indicate primary oxidation product formed due to oxidation of lipid component. In this experiment the oxidative stability of ghee residue hydrolysate incorporated biscuits packed aerobically were measured by peroxide value presented table Table-12 which shows increasing trend as time progresses, The significant ($p < 0.01$) increase in peroxide values over 60 days (2.417 ± 0.007 meq/Kg) demonstrates that lipid oxidation is progressively occurring in the ghee residue incorporated biscuits. Patrignani *et al.* (2014) [12] reported similar increase in PV during storage of biscuit in which unsaturated fatty acid content played significant role. El-Baset *et al.* (2023) [13] reported significant increase in peroxide value during storage study of biscuit in which they attributed towards various factors like temperature, light exposure, oxygen, permeability of packaging material

3.4.6 Microbiological parameters

Table 13. Storage stability study of ghee residue incorporated biscuits for microbial parameters

Parameter	0 Day	10 Day	20 Day	30 Day	40 Day	50 Day	60 Day
TPC	2.293 ± 0.002 ^G	2.343 ± 0.002 ^F	2.548 ± 0.002 ^E	2.683 ± 0.002 ^D	2.813 ± 0.002 ^C	2.915 ± 0.002 ^B	3.017 ± 0.002 ^A
log10 cfu/g							
Y&M	ND	ND	ND	ND	ND	ND	ND

($n = 6$); Mean±SE with different superscripts column wise (A,B,C,D,E,F,G) differ significantly ($p < 0.01$)

Total plate count

Table-13 outlines the results of a storage stability study on ghee residue hydrolysate incorporated biscuits, with a focus on Total Plate Count (TPC) as a measure of microbial growth, recorded in log10

colony-forming units per gram (log₁₀ cfu/g) over a 60-day period. TPC serves as a critical indicator of microbial load in food products, offering valuable information about the potential for microbial contamination or proliferation during storage. The data reveals a steady increase in TPC values throughout the 60-day storage, starting at 2.293 ± 0.002 log₁₀ cfu/g on Day 0 and rising to 3.017 ± 0.002 log₁₀ cfu/g by Day 60. Although an upward trend in TPC was observed, it is noteworthy that the water activity level measured on Day 60 was below the threshold needed for microbial growth. This suggests that the rise in TPC might be attributable to contamination occurring during post-production handling, rather than due to intrinsic microbial growth during storage, even though all hygiene protocols were adhered to.

Yeast and Mould count

During the storage of 60, no growth of yeast and mould were found in this experiment which could be due to low moisture and water activity of product during storage.

4. Conclusion

Jowar and jaggery has shown healthier and acceptable alternatives to maida and sugar respectively in biscuit preparation. Study had shown 10% incorporation is very much acceptable for bitter testing hydrolysate in prepared biscuits. Above 10% incorporation is mainly affected by bitter test thus de-bittering treatment can be further explored to increase this incorporation in biscuits and other suitable products.

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