Effect of Storage on Moisture, Free Fatty Acid and Peroxide Value of Products Developed by Incorporating Modified Rice Starch

Gopika C. Muttagi\(^1\*) and Usha Ravindra\(^1\)

\(^1\)Department of Food Science and Nutrition, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Author GCM conducted research, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author UR designed the study and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Products were prepared by incorporating modified starch extracted from selected traditional rice varieties. The products were standardized by incorporating modified rice starch served as test product group and without incorporating modified rice starch served as control product group. The developed products such as sweet cookies, masala cookies and khakhra were stored up to 90 days at room temperature (25-30°C) and 40 to 60 per cent of relative humidity. The packaging material used was 300 gauge low density polyethylene (LDPE). Samples from sweet cookies and masala cookies were drawn in triplicates for evaluation when fresh and after 15, 30, 45, and 60 days of storage, whereas samples from khakhra were drawn when fresh and after 30, 60, and 90 days of storage duration. The products were evaluated for their microbial load, moisture content, free fatty acid (FFA), and peroxide value (PV). Total bacterial colony count increased as the storage duration increased. No colony counts for E.coli were detected. The fungi colony counts were detected at 60 and 90 days of storage duration in sweet cookies and masala cookies respectively, whereas no fungi counts were detected in product Khakhra. Moisture, free fatty acid

*Corresponding author: Email: gopika.cm@gmail.com;
(FFA) and peroxide value (PV) of stored products significantly increased as the storage duration increased. The products such as sweet cookies, masala cookies and khakhra, developed by incorporating modified rice starch were acceptable up to 45, 60 and 90 days respectively.

Keywords: Modified rice starch; storage; microbial load; lipid oxidation.

1. INTRODUCTION

Rice starch is one of the key ingredients of various food products. Traditionally, there have been basic attributes associated with rice starch that have given it merit over other cereal and non-cereal starches. These properties include hypoallergenicity [1], digestibility, bland taste, bland flavour, small granule (2-10 μm), white colour, greater freeze thaw stability of pastes and a wide range of amylose/amyllopectin ratios; it is smooth, creamy and spreadable. In contrast, corn starch is yellowish white and forms a firm gel. Rice starch granules are of the same size as homogenized fat globules; therefore they provide a texture perception similar to fat. The interest in developing functional foods has increased due to demanding foods that can improve the health and well-being of consumers. Obtaining rice starch with a high content of resistant starch (RS) is of particular interest because it shows a slow rate of starch hydrolysis [3] and would therefore potentially provide rice with lower glycemic index. Consumer demand towards traditional foods provides wider scope for research on health and nutritional benefits. Therefore traditional rice varieties with high amylose content are in great demand. Storage of foods is also a contributing factor to the changes in the content of the product. Processed foods containing fats and oils, oxidize slowly during storage and various oxidation products cause rancidity and deterioration of the sensory properties of the food products [4]. Limited literature is available on the incorporation of modified rice starch extracted from traditional rice varieties into food products which are popularly consumed in India. Thus keeping in view of above facts present study was conducted to evaluate the effect of storage on moisture, free fatty acid and peroxide value of products developed by incorporating modified rice starch extracted from traditional rice variety of Karnataka, India.

2. MATERIALS AND METHODS

2.1 Standardization of Products by Incorporating Modified Rice Starch

Three traditional food products viz. sweet cookies, masala cookies, and khakhra, were developed by incorporating modified rice starch. The products contained either wheat flour (Control) or the wheat flour and modified rice starch (1:1, Test). The following paragraphs depict the preparation of above products.

2.2 Sweet Cookies

Margarine was creamed with sugar, whole egg. Then added wheat flour plus modified rice starch and mixed thoroughly. Dough was rolled out to a 2 cm thickness on flat surface. Cookies were cut with a circular mould and placed on greased aluminium cookie tray. The cookies were baked at 160°C for 20 min in a baking oven. Baked cookies were allowed to cool down for 30 min and stored in a polythene covers.

2.3 Masala Cookies

Wheat flour, modified rice starch, baking powder and salt were sieved together. The mixture was rubbed in fat, added green masala and sugar. The mixture was kneaded to soft dough with curd. The dough was rolled in to a thin sheet and cookies were cut with biscuit cutter. Masala cookies were baked at 160°C for 30 min.

2.4 Khakhra

Wheat flour, resistant starch, salt and masala are mixed. Water was added and kneaded to make soft dough. The dough was rolled into small balls and were flattened. Khakhra were dry roasted on slow heat till it becomes crisp and light brown in colour. Khakhras were cooled and stored in polythene covers.
2.5 Storage Stability of Food Products

The developed products such as sweet cookies, masala cookies and khakhra were stored up to 90 days at room temperature (25-30°C) and 40 to 60 per cent of relative humidity. The packaging material used was 300 gauge low density polyethylene (LDPE). Samples from sweet cookies and masala cookies were drawn in triplicates for evaluation when fresh and after 15, 30, 45, and 60 days of storage, whereas samples from khakhra were drawn when fresh and after 30, 60, and 90 days of storage duration. The products were evaluated for their microbial load, moisture content, free fatty acid (FFA), and peroxide value (PV).

2.6 Microbiological Study of the Food Products

The microbiological analysis of the developed value added products was carried out as per the standard method [5] for Coliforms using the Eiosine Methalene Blue Agar (EMBA), for general bacteria Nutrient Agar (NA) and for fungi using Rose Bengal Agar (RBA). Ten gram of sample was diluted in 90 ml of buffer blanks and subsequent dilutions were prepared up to $10^6$ dilution. Three dilution factors were used for plating of Coliforms, general bacteria and fungi viz., $10^1$, $10^2$, $10^4$, $10^6$ and $10^8$. The numbers of microbial counts were calculated using the following formula.

$$\text{No. of Microorganisms} \left( \frac{\text{per g}}{\text{ml}} \right) = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Weight/volume of aliquots taken} \frac{\text{g}}{\text{ml}}}$$

2.7 Extraction of Total Lipids from Food Products

Total lipids were extracted using the Bligh and Dyer [6] method. The lipids were extracted and analysed for free fatty acid and peroxide value in a mixture of chloroform and methanol (2:1 v/v).

2.7.1 Acid value

Acid value of products was determined according to Raguramulu et al. [7]. Percentage free fatty acids (FFAs) were calculated using oleic acid as a factor.

$$\text{Acid value} = \frac{\text{Titre value} \times 0.00561 \times 1000}{\text{Weight of sample (g)}}$$

2.7.2 Peroxide value

Peroxide value was detected by the liberation of iodine from an acid solution of potassium iodide according to the method by Raguramulu et al. [7].

$$\text{Peroxide value of oil} \left( \frac{\text{meq}}{\text{kg of sample}} \right) = \frac{(\text{Titre} - \text{blank})x N \times 1000}{\text{Weight of oil (g)}}$$

2.8 Statistical Analyses

All the analyses were performed in triplicates. All data were analysed by two way ANOVA procedure and independent ‘t’ test using SPSS software version 16.0. Differences were declared statistically significant when $P = .05$. Where significant differences were detected, the means were separated by Duncan’s multiple range test (DMRT) at 5% probability level using the MSTAT-C statistical package.

3. RESULTS AND DISCUSSION

3.1 Shelf Life of Selected Food Products

Effect of storage and product groups on microbial load, moisture, free fatty acids (FFA), and peroxide value (PV) of sweet cookies, masala cookies and khakhra were studied. The results are presented under following sub headings from Tables 1 to 5.

3.2 Effect of Storage and Product Groups on Microbial Load

Microbes such as bacteria and fungi play major role in the spoilage of the baked products. The fresh bakery products are sterile but soon become contaminated when it comes in contact with the air and other material. Improper handling also introduces contamination. Coliforms and generic E. coli species are commonly used as hygiene and safety indicators in many food industries to assess the microbiological safety of processing environments and foods. Thus, the present study analysed microbial load of food products during storage. The data presented in Table 1 reveals the effect of storage duration and product groups on the microbial load of sweet cookies, masala cookies and khakhra.


3.2.1 Sweet cookies

Total bacterial counts were below detectable limits at initial and 15 days of storage duration in both control and test cookies. The bacterial count increased to 400 cfu/g and 440 cfu/g in control and test cookies at the end of storage period (60 days). There were no counts for E. coli for both product groups during storage duration. No fungi counts were detected at initial, 15 days, 45 days and 1.0 cfu/0.1 g at 60 days in both control and test cookies. The increase in bacteria and fungi counts as the storage duration lengthened might have been due to a corresponding increase in moisture content during storage duration. Increase in microbial counts as the increase in storage period was found by Nagi et al. [8] in cereal bran incorporated biscuits. Oat bran biscuits had least bacterial count (11.15 x 10² cfu/g) whereas rice bran biscuits had maximum bacterial count (21.68 x 10² cfu/g). According to Indian standards, total bacterial count should not be more than 10,000 cfu/g. E coli and fungi counts should be absent. In this regard, in the present study the control and test cookies were acceptable up to 45 days of storage duration.

3.2.2 Masala cookies

Total bacterial counts were below detectable limits at initial and 15 days of storage duration among control and test cookies. Storage duration showed increasing effect on microbial quality of cookies. The bacterial count increased from 150 (45 days) to 770 cfu/g (90 days) in control cookies and 163 to 750 cfu/g in test cookies. There was no E.coli counts were detected during storage duration among product groups. Both control and test cookies showed fungi counts at 90 days of storage duration (1.0 cfu/0.1 g). Garlic paste was added to masala cookies, which might have extended the shelf life of masala cookies (up to 60 days) compared to sweet cookies (up to 45 days). Increase in the garlic concentration showed a noticeable reduction in the microbial load of cookies produced from wheat flour fortified with mushroom flour [9]. Similar findings with the same formulation of cookies reported by Emmanuel et al. [10], in which garlic was replaced by curry leaves.

3.2.3 Khakhra

Total bacterial count was not detected during initial and 30 days of storage duration in the product groups of Khakhra. The bacterial counts were detected at 60 days (100 cfu/g) storage duration and increased to 300 cfu/g (90 days). E. coli and fungi counts were not detected during storage duration among both product groups.

Khakhra available in the market can be stored for 9 months, since they are usually vacuum packed and contain added preservatives to provide longer shelf life and also low in moisture content. In the present study, Khakhra was not vacuum packed and no added preservatives, however it was acceptable at the end of storage duration (90 days). The microbial analysis was done at the interval of three days after the preparation of Sorghum roti [11] and showed surface growth on 3 day. The total bacterial counts increased from 15 x 10¹ to 34 x 10² cfu/g whereas, yeast and mould counts increased from 5 x 10² to 34 x 10³ cfu/g. As per the WHO [12] guideline the total plate count, Yeast and mould count should be less than 2 x 10⁵ and 1 x 10⁴ per gram respectively.

3.3 Effect of Storage and Product Groups on Moisture, Free Fatty Acids (FFA) and Peroxide Value (PV) of Products

The physico-chemical and microbiological stability of food depends greatly on the water content and its interaction with food ingredients. Analysis of moisture content of stored foods is a reliable assessment of the microbial growth and chemical stability of foods following manufacture [13].

The structure of foods is changed during processing, and as a result, lipids may become more exposed to oxygen. In addition, naturally occurring antioxidant systems are impaired during processing, making processed food more susceptible to oxidation.

In the present study moisture, free fatty acids (FFA) and peroxide value (PV) were analysed and results are discussed under following paragraphs. Results are established in Tables 2 to 5. Analysis of variance test indicated that storage duration, product groups and interaction were found to have significant (P=.05) effect on all parameters studied except in the product sweet cookies, product group did not show significant effect on FFA and PV (Tables 2, 3 & 4).
Table 1. Effect of storage and product groups on microbial load

| Products          | Groups | Duration | Colony forming units (CFU) at 10⁻¹ dilution | Total bacterial count (per g) | E.Coli (per g) | Fungi (per 0.1 g) |
|-------------------|--------|----------|---------------------------------------------|-----------------------------|----------------|--------------------|
| **A. Sweet cookies** | Control | Initial | Not detected                                | Not detected                | Not detected   | Not detected       |
|                   |        | 15 days  | Not detected                                | Not detected                | Not detected   | Not detected       |
|                   |        | 45 days  | 200                                         | Not detected                | Not detected   | Not detected       |
|                   |        | 60 days  | 400                                         | Not detected                | Not detected   | 1.0                |
| Test              | Initial | Not detected | Not detected                        | Not detected                | Not detected   | Not detected       |
|                   | 15 days | Not detected | Not detected                        | Not detected                | Not detected   | Not detected       |
|                   | 45 days | 270                                         | Not detected                | Not detected   | Not detected       |
|                   | 60 days | 440                                         | Not detected                | Not detected   | 1.0                |
| **B. Masala cookies** | Control | Initial | Not detected                                | Not detected                | Not detected   | Not detected       |
|                   |        | 15 days  | Not detected                                | Not detected                | Not detected   | Not detected       |
|                   |        | 45 days  | 150                                         | Not detected                | Not detected   | Not detected       |
|                   |        | 60 days  | 370                                         | Not detected                | Not detected   | Not detected       |
|                   |        | 90 days  | 770                                         | Not detected                | Not detected   | 1.0                |
| Test              | Initial | Not detected | Not detected                        | Not detected                | Not detected   | Not detected       |
|                   | 15 days | Not detected | Not detected                        | Not detected                | Not detected   | Not detected       |
|                   | 45 days | 163                                         | Not detected                | Not detected   | Not detected       |
|                   | 60 days | 346                                         | Not detected                | Not detected   | Not detected       |
|                   | 90 days | 750                                         | Not detected                | Not detected   | 1.0                |
| **C. Khakhra**    | Control | Initial | Not detected                                | Not detected                | Not detected   | Not detected       |
|                   |        | 30 days  | Not detected                                | Not detected                | Not detected   | Not detected       |
|                   |        | 60 days  | 100                                         | Not detected                | Not detected   | Not detected       |
|                   |        | 90 days  | 300                                         | Not detected                | Not detected   | Not detected       |
| Test              | Initial | Not detected | Not detected                        | Not detected                | Not detected   | Not detected       |
|                   | 30 days | Not detected | Not detected                        | Not detected                | Not detected   | Not detected       |
|                   | 60 days | 100                                         | Not detected                | Not detected   | Not detected       |
|                   | 90 days | 300                                         | Not detected                | Not detected   | Not detected       |

Table 2. Effect of storage and product groups on moisture, free fatty acids (FFA) and peroxide value (PV) of sweet cookies

| Groups (A) | Duration (D) | Moisture (%) | FFA (%) | PV (meq O₂/kg) |
|------------|--------------|--------------|---------|----------------|
| Control    | Initial      | 2.53 a       | 2.62 c  | 3.55 c         |
|            | 15 days      | 2.74 d       | 3.40 e  | 5.81 b         |
|            | 30 days      | 2.77 cd      | 3.85 c  | 5.66 b         |
|            | 45 days      | 2.88 b       | 6.29 a  | 7.73 a         |
| Test       | Initial      | 2.71 a       | 2.67 f  | 3.51 f         |
|            | 15 days      | 2.74 d       | 3.46 c  | 4.81 b         |
|            | 30 days      | 2.81 c       | 3.72 d  | 5.70 b         |
|            | 45 days      | 3.04 a       | 6.17 b  | 7.63 a         |

Analysis

- **F test**: * * * NS * * NS
- **SEM±**: 0.013 0.018 0.026 0.013 0.018 0.026 0.177 0.250 0.354
- **CD at 5 %**: 0.036 0.051 0.072 - 0.051 0.072 - 0.693 -

Means in the same column followed by different superscript letters differ significantly.

* Significant at 5 % level  NS Non significant

3.3.1 Sweet cookies

Table 2 represents the effect of storage duration and product groups (Control & Test) on moisture, FFA and PV of sweet cookies. Moisture content significantly (P=.05) increased from 2.53 (Initial) to 2.88 per cent and 2.71 (Initial) to 3.04 per cent at 45 days in control and test cookies respectively. Moisture content was not significant between the storage duration 15 days and 30
days in case of control cookies, whereas in test cookies between fresh and 15 days statistical analysis of variance revealed a significant (P= .05) difference with product groups, storage duration and interaction.

Storage of cookies for 45 days at ambient temperature significantly (P= .05) increased the FFA content from 2.62 (Initial) to 6.29 per cent in control cookies and 2.67 (Initial) to 6.17 in test cookies at 45 days. Statistical analysis of variance revealed a significant difference with product groups, storage duration and interaction.

Peroxide value (PV) of extracted lipids increased significantly (P= .05) from 3.55 (Initial) to 7.73 (45 days) meq O₂ per kg in control cookies and 3.51 (Initial) to 7.63 (45 days) meq O₂ per kg in test cookies. PV between storage duration 15 days and 30 days was not significant in both product groups. Analysis of variance test indicated that storage duration, product groups and interaction were found to have significant (P= .05) effect on all parameters studied.

### 3.3.2 Masala cookies

Table 3 represents the effect of storage period and product groups (Control & Test) on moisture, FFA and PV of masala cookies. Moisture content significantly increased (P= .05) from 3.02 to 4.26 per cent in control cookies and 3.13 to 4.33 per cent in test cookies at initial and 60 days after storage respectively. FFA value significantly increased from 2.12 to 3.04 per cent in control cookies and 2.03 to 2.95 per cent in test cookies at initial and 60 days of storage. Significant increase in PV recorded with the lengthening of storage duration from 3.06 (Initial) to 8.37 (60 days) meq O₂ per kg in control cookies and 3.08 (Initial) to 8.44 (60 days) meq O₂ per kg in test cookies. Analysis of variance test indicated that storage duration, product groups and interaction were found to have significant (P= .05) effect on all parameters studied.

Table 3. Effect of storage and product groups on moisture, free fatty acids (FFA) and peroxide value (PV) of Masala cookies

| Groups (A) | Duration (D) | Moisture (%) | FFA (%) | PV (meq O₂/kg) |
|------------|--------------|--------------|---------|---------------|
| Control    | Initial      | 3.02         | 2.12    | 3.06          |
|            | 15 days      | 3.26         | 2.33    | 3.71          |
|            | 30 days      | 3.53         | 2.49    | 5.21          |
|            | 45 days      | 3.79         | 2.54    | 6.80          |
|            | 60 days      | 4.26         | 3.04    | 8.37          |
| Test       | Initial      | 3.13         | 2.03    | 3.08          |
|            | 15 days      | 3.32         | 2.32    | 3.89          |
|            | 30 days      | 3.67         | 2.41    | 5.27          |
|            | 45 days      | 4.05         | 2.54    | 6.86          |
|            | 60 days      | 4.33         | 2.95    | 8.44          |

Analysis
- F test: A D AXD A D AXD A D AXD
- SEm±: 0.008 0.013 0.018 0.008 0.013 0.018 0.008 0.013 0.018
- CD at 5 %: 0.023 0.036 0.051 0.023 0.036 0.051 0.023 0.036 0.051

* Significant at 5 % level

Means in the same column followed by different superscript letters differ significantly
Table 4. Effect of storage and product groups on moisture, free fatty acids (FFA) and peroxide value (PV) of Khakhra

| Groups (A) | Duration (D) | Moisture (%) | FFA (%) | PV (meq O₂/kg) |
|-----------|--------------|--------------|---------|----------------|
| Control   | Initial      | 1.95<sup>b</sup> | 0.34<sup>b</sup> | 0.76<sup>d</sup> |
|           | 30 days      | 1.97<sup>c</sup> | 1.37<sup>c</sup> | 0.77<sup>d</sup> |
|           | 60 days      | 2.15<sup>a</sup> | 2.15<sup>b</sup> | 0.86<sup>c</sup> |
|           | 90 days      | 2.49<sup>d</sup> | 2.60<sup>c</sup> | 1.05<sup>b</sup> |
| Test      | Initial      | 2.07<sup>e</sup> | 0.34<sup>f</sup> | 0.76<sup>d</sup> |
|           | 30 days      | 2.22<sup>c</sup> | 1.08<sup>a</sup> | 0.80<sup>d</sup> |
|           | 60 days      | 2.47<sup>b</sup> | 1.22<sup>d</sup> | 0.88<sup>c</sup> |
|           | 90 days      | 2.56<sup>a</sup> | 2.59<sup>a</sup> | 1.23<sup>a</sup> |

Analysis

| F test | AXD A | AXD D | AXD AXD |
|--------|-------|-------|---------|
|        | *     | *     | *       |
| SEM±   | 0.009 | 0.022 | 0.001   |
| CD at 5% | 0.025 | 0.062 | 0.003   |

* Significant at 5% level

Means in the same column followed by different superscript letters differ significantly

3.4 Statistical Analysis on Product Groups with Different Parameters

Table 5 demonstrates independent ‘t’ test results performed to determine statistical significant difference between the means of two product groups during each storage with regard to moisture, FFA and PV.

3.4.1 Moisture

Among masala cookies, test cookies had significantly (<i>P</i>=.05) highest moisture content at all storage durations except 15 days compared to control cookies. Among sweet cookies moisture content was significantly highest in test cookies at initial and 45 days compared to control cookies, however it was not significant at 15 days and 30 days storage.

Within khakhra, moisture content recorded significantly highest in test <i>khakhra</i> among all storage durations compared to control <i>khakhra</i>.

Table 5 was prepared adding native corn or banana starches to wheat flour [16], showing moisture levels of 4 to 5 per cent. Slightly lower values were observed in this study; however products characterized by low water content increases their shelf life.

3.4.2 Free Fatty Acids (FFA)

The acid value is the number of mg of KOH required to neutralize the free acid present in 1 g of oil or fat. A small quantity of free fatty acid is usually present in oils along with the triglycerides. According to Demain [17], acid values are used to measure the extent to which glyceride in the oil has been decomposed by lipase and other actions such as light and heat.

Among masala cookies, FFA found significantly (<i>P</i>=.05) highest in control cookies at initial and 60 days compared to test cookies, however it was not significant at 15 days, 30 days, and 45 days. Per cent increase in FFA content of sweet cookies was found significantly highest in control cookies compared to test cookies at 30 and 45 days. However, FFA was not significant at initial and 15 days of storage duration. Per cent increase in FFA was significantly highest in control <i>Khakhra</i> at 30 days and 60 days compared to test <i>khakhra</i>, however it was not significant at initial. Among all the products stored, control product group showed slightly higher FFA levels compared to test group. This difference may be attributed to the particular formulation of each product, a fact that can be related to the relative abundance of amorphous starch zones in each material, which influences water absorption to a large extent [15]. The gain in moisture content during storage might also be due to storage environment (temperature and relative humidity) and packaging material [8]. A similar cookie was prepared adding native corn or banana starches to wheat flour [16], showing moisture levels of 4 to 5 per cent. Slightly lower values were observed in this study; however products characterized by low water content increases their shelf life.
Table 5. Effect of individual storage duration on moisture, free fatty acids (FFA) and peroxide value (PV) of products

| Parameters | Products | Groups  | Initial | 't'      | 15 days | 't'     | 30 days | 't'     | 45 days | 't'     | 60 days | 't'     | 90 days | 't'     |
|------------|----------|---------|---------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| A. Moisture (%) | Masala  | Control | 3.02    | 4.20*   | 3.26    | 1.73<sup>NS</sup> | 3.53    | 2.91*   | 3.79    | 10.69*  | 4.26    | 2.86*   | -       | -       |
|             | cookies  | Test    | 3.13    | 3.32    | 3.67    | 4.05    | 4.33    | -       | -       | -       | -       | -       | -       | -       |
|             | Sweet    | Control | 2.53    | 9.88*   | 2.74    | 0.14<sup>NS</sup> | 2.77    | 1.27<sup>NS</sup> | 2.88    | 3.64*   | -       | -       | -       | -       |
|             | cookies  | Test    | 2.71    | 2.75    | 2.81    | 3.04    | 3.04    | -       | -       | -       | -       | -       | -       | -       |
|             | Khakhra  | Control | 1.95    | 5.45*   | -       | 1.97    | 11.20*  | -       | 2.15    | 10.30*  | 2.49    | 6.93*   | -       | -       |
|             | Test     |         | 2.07    | -       | 2.22    | -       | -       | 2.47    | 2.56    |         |         |         |         |         |
| B. FFA (%) | Masala   | Control | 2.12    | 5.96    | 2.33    | 0.52<sup>NS</sup> | 2.49    | 2.74<sup>NS</sup> | 2.55    | 0.01<sup:NS</sup> | 3.04    | 4.80*   | -       | -       |
|             | cookies  | Test    | 2.03    | *       | 2.32    | 2.41    | 2.54    | 2.95    | -       | -       | -       | -       | -       | -       |
|             | Sweet    | Control | 2.62    | 1.45<sup>NS</sup> | 3.40    | 1.87<sup>NS</sup> | 3.85    | 3.20*   | 6.29    | 4.03*   | -       | -       | -       | -       |
|             | cookies  | Test    | 2.67    | 3.46    | 3.72    | 6.17    | -       | -       | -       | -       | -       | -       | -       | -       |
|             | Khakhra  | Control | 0.35    | 0.23<sup>NS</sup> | -       | 1.37    | 10.28  | -       | 2.15    | 25.57*  | -       | -       | -       | -       |
|             | Test     |         | 0.34    | -       | 1.08    | *       | -       | 1.22    | -       | -       | -       | -       | -       | -       |
| C. PV (meq O₂/kg) | Masala  | Control | 3.06    | 0.76<sup>NS</sup> | 3.71    | 5.20*   | 5.21    | 1.78<sup>NS</sup> | 6.80    | 1.75<sup>NS</sup> | 8.37    | 3.24*   | -       | -       |
|             | cookies  | Test    | 3.08    | 3.89    | 5.27    | 6.86    | 8.44    | -       | -       | -       | -       | -       | -       | -       |
|             | Sweet    | Control | 3.55    | 1.01<sup>NS</sup> | 5.81    | 1.00<sup>NS</sup> | 5.66    | 0.74<sup>NS</sup> | 7.73    | 2.42<sup>NS</sup> | -       | -       | -       | -       |
|             | cookies  | Test    | 3.51    | 4.81    | 5.70    | 7.63    | -       | -       | -       | -       | -       | -       | -       | -       |
|             | Khakhra  | Control | 0.76    | 0.01<sup>NS</sup> | -       | 0.77    | 3.16*  | -       | -       | 0.86    | 0.78<sup>NS</sup> | 1.05    | 6.78*   | -       |
|             | Test     |         | 0.77    | -       | 0.80    | -       | 0.88    | 1.23    | -       | -       | -       | -       | -       | -       |

* Significant at 5 % level, NS Non significant
higher values for FFA (% of oleic acid) compared to test product group at the end of storage period (Table 5). FFA significantly (P=.05) increased as increase in storage duration in all the food products. In general, the product sweet cookies got highest FFA content followed by masala cookies and khakhra. Similar trend of subsequent increase in the FFA content of cereal bran incorporated biscuits and soy fortified biscuits were reported by Nagi et al. [8] and Singh et al. [18] respectively.

Reddy et al. [4] also found an increase in FFA (% of stearic acid) value in all the biscuit samples on storage. The increase was considerably higher in control biscuits (7.1 - 14.10%) prepared without the addition of antioxidant compared to biscuit samples, in which synthetic or natural antioxidants were incorporated (0.34-3.31%). In the present study no synthetic or natural antioxidants were added to the products and FFA values of sweet cookies were comparable with the control biscuits reported in the earlier study. Further, FFA values of Masala cookies and Khakhra were within the ranges reported in the earlier study for synthetic or natural antioxidants incorporated biscuits. For this reason, the products Masala cookies and Khakhra showed longer shelf life (60 days and 90 days respectively) than sweet cookies (45 days).

Lower values of free fatty acids in gluten free biscuits were observed by Caponio et al. [19] and found to be 0.99 per cent (range 0.45–1.50%). This value, however, could not describe the real extent of the hydrolytic degradation because, the type of fat used in the production process of the biscuits examined was represented by lipids deriving from a refining process involving the removal during the neutralization treatment of the free fatty acids.

3.4.3 Peroxide value (PV)

Peroxide value serves as an indicator of the extent of formation of primary oxidation products [20]. The PV method is probably the mostly used one and measures concentration of peroxides formed from fatty acid oxidation. Because of the unstable of peroxides, PV is an approximate indicator of state of oxidation but particularly in the early stage of oxidation it serves as a good tool for the measurement of degree of oxidation [21].

With regard to masala cookies, PV expressed in terms of meq O₂ per kg found significantly (P=.05) highest in test cookies compared to control cookies at 15 and 60 days, however it was not significant at initial, 30 days, and 45 days. Among sweet cookies, PV did not differ significantly between the product groups, whereas in khakhra, it was significantly highest in test khakhra at 30 and 90 days storage duration compared to control khakhra, however no significant difference was observed between the product groups at initial and 60 days storage duration. In general, the product sweet cookies got highest PV content followed by masala cookies and khakhra.

AOAC specification for crude oil is less than 10 meq/kg [21]. In the present study the peroxide values were within the AOAC specification. PV significantly increased in all the food products as the storage duration increased. Similar trend in increasing PV in stored biscuit samples ranged from 0.7 to 2.6 g equiva. of O₂ per 100 g was observed [4].

The oxidative degradation of the lipid fraction of the gluten-free biscuits examined [19], the mean value of peroxide value was 10.3 meq O₂/kg (range 5.8–22.2 meq O₂/kg), indicating only a moderate extent of oxidative degradation. However the European law admit a maximum level of 20 meq O₂ per kg [22]. There are many factors influencing the rate of autoxidation, including manufacturing, storage condition, saturation degree, content of unsaturated fatty acid, and double bond position in the molecules [23,24]. Even in present study, similar factors might have contributed for storage quality of products.

4. CONCLUSION

Storage quality attributes in sweet cookies, masala cookies and khakhra developed by using modified rice starch-a functional ingredient was carried out. Storage duration significantly affected the microbial load, free fatty acid, and peroxide value of products prepared by incorporating modified rice starch. Total bacterial colony count increased as the storage duration increased in all the stored products. No colony counts for E. coli were detected. The fungi colony counts were detected at 60 and 90 days of storage duration in sweet cookies and masala cookies respectively, whereas no fungi counts were detected in product Khakhra. Moisture, free fatty acid (FFA) and peroxide value (PV) of stored products significantly increased as the
storage duration increased, however they were acceptable at the end of storage duration. The products such as sweet cookies, *masala* cookies and *khakhra*, developed by incorporating modified rice starch were acceptable up to 45, 60 and 90 days respectively. From the findings, it is concluded that, free fatty acids, peroxide value and microbial load were in permissible limit. Hence, modified rice starch based products be popularised as an alternative to existing products. These can be used as products of functional properties to avail better nutritional benefits.

5. RECOMMENDATIONS

Modified rice starch is a functional ingredient, possess lot of nutritional and health benefits. Bakery and snack foods can be successfully developed from incorporating modified rice starch. There is lot of scope exists for further research on process variables and packaging materials to extend the shelf life of developed products.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Champagne ET. Rice structure: Composition and characteristics. Cereal Food World. 1996;41:833–838.

2. Juliano BO. Rice starch: Production, properties and uses. In: Whistler RL, Bemiller JN, Paschall EF, editors. Starch: chemistry and technology. Academic Press, Inc, New York; 1984.

3. Shu X, Jia L, Ye H, Li C, Wu D. Slow digestion properties of rice different in resistant starch. J Agric. Food Chem. 2009;57:7552-7559.

4. Reddy V, Urooj A, Kumar A. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. Food Chem. 2005;90:317-321.

5. Tate RL. Soil Microbiology. John Wiley and Sons, New York; 1995.

6. Bligh EG, Dyer WJ. Estimation of total lipids. Can J Biochem Physiol. 1959;37: 911.

7. Raguramulu N, Madhavan Nair K, Kalyanasundaran S. A manual of laboratory techniques, NIN Press, Jamal-Osmania, Hyderabad; 2003.

8. Nagi HPS, Kaur J, Dar BN, Sharma S. Effect of storage period and packaging on the shelf life of cereal bran incorporated biscuits. Am J Food Technol. 2012;7(5): 301-310.

9. Giwa OE, Ibrahim TA. Microbial, physical and sensory attribute of cookies produced from wheat flour fortified with *Termitomyces robustus* and spiced with (*Allium sativum*). J Pharm Biomed Sci. 2012;18(18):1-4.

10. Emmanuel GO, Olubunmi BR, Atanlogun KS. Microbial, physical and sensory attribute of cookies produced from wheat flour fortified with *Termitomyces robustus* and spiced with curry leaves (*Xylopia aethiopica*). J Nat Sci Res. 2012;2(3):40-46.

11. Unhale DS, Sakhal BK, Ranveer RC, Pawar VD. Studies on shelf life extension of sorghum roti. Int. Res. J. 2012;19(2): 733-736.

12. WHO. Guideline value for food and drinking water. World Health Organization, Geneva; 1994.

13. Rahman MSM, Labuza TP. Water activity and food preservation. In: Rahman MS, Dekker M, editors. Handbook of Food Preservation. New York; 1999.

14. Aparicio-Saguilia’Na A, Sonia GS, Yago-Ayerdib A, Vargas-Torresa A, Tovar J, Ascencio-Oterob TE, Bello-Pereza LA. Slowly digestible cookies prepared from resistant starch-rich lintnerized banana...
starch. J Food Compost Anal. 2007;20(3-4):175-181.

15. Slade L, Levine H. Beyond water activity. Recent advances based on an alternative approach to the assessment of food quality and safety. Crit Rev Food Sci Nutr. 1991;30:115–360.

16. Bello-Perez LA, Sayago-Ayerdi SG, Mendez-Montealvo G, Tovar J. In vitro starch digestibility of banana starch cookies. Plant Food Hum Nutr. 2004;59:79-83.

17. Demain MJ. Principles of Food Chemistry. 2nd ed. Van Nostrand Reinhold International Company Limited, London, England; 1990.

18. Singh V, Okadome H, Toyoshima H, Isobe S, Ohtsubo, K. Thermal and physicochemical properties of rice grain, flour and starch. J Agric. Food Chem. 2000;48:2639-2647.

19. Caponio F, Summo C, Clodoveo ML, Pasqualone A. Evaluation of the nutritional quality of the lipid fraction of gluten-free biscuits. Eur Food Res Technol. 2008;227:135–139.

20. Anwar F, Anwar T. Mehmoody Z. Methodological characterization of rice bran oil from Pakistan. Grasas Aceites. 2005;56:126-127.

21. Mildner-Szkudlarz S, Zawirska-Wojtasiak R, Obuchowski W, GoSli’Nski M. Evaluation of antioxidant activity of green tea extract and its effect on the biscuits lipid fraction oxidative stability. J Food Sci. 2009;74(8):362-370.

22. Euro communities regulation. Off J Eur Communities. 295 of November 13, EC Regulation; 2003.

23. Gordon MH. The development of oxidative rancidity. In: Pokorny J, Yanishlieva N, Gordon HM, editors. Antioxidants in food—practical applications. CRC Press, Washington; 2001.

24. Wasowicz E, Gramza A, Hes M, Jelen HH, Korczak J, Maleckam, Mildner-Szkudlarz S, Rudzinska M, Samotyja U, Zawirska-Wojtasiak R. Oxidation of lipids in food. Pol J Food Nutr Sci. 2004;13:87–100.