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Morphological Analysis and Shelf-Life Estimation of Functional Baked Products (Wheat Base) Enrich by fox Nut and Palm Date

Manvi Sinha[1], Dr. Anu Ram Kailash Mishra[2]

[1]PG Scholar, Department of Food and Nutrition, Food Science and Technology School for Home Science, Babasaheb Bhimrao Ambedkar University, Lucknow-226025, Uttar Pradesh
[2]Associate Professor, Department of Food and Nutrition, Food Science and Technology School for Home Science, Babasaheb Bhimrao Ambedkar University, Lucknow-226025, Uttar Pradesh

ABSTRACT
This study aims to produce and standardise baked items that have been enhanced with Fox nut (Makhana) and Palm Date by evaluating their Morphological analysis and Shelf life estimation.The morphological characteristics of the biscuits and muffins were analysed by scanning electron microscopy (SEM). The SEM images were used to study surface morphology and microbial growth on the sample surface. The elemental composition of the food products was analysed through SEM imaging. The shelf-life of the samples were evaluated by assessing the shelf life of the products. For shelf life analysis agar plate were used and the created product's business values were thus determined through it.

Keywords: SEM, Nutritional, Shelf Life.

1. INTRODUCTION

Baked items are a staple in diets worldwide due to its nutrients preserving and providing healthy life choices. In contrast, traditional formulations sometimes miss out on important functional features that might boost their health benefits and nutritional worth. An increasing interest in the topic has been spurred by the creation of functional baked goods, which are enhanced with substances that have certain health-promoting benefits. Among these components, Makhana—also called fox nuts or lotus seeds—is becoming more popular in the food sector. An exceptionally healthy variety of lotus flower, the Makhana seed comes from the Nelumbo nucifera plant. Functional foods benefit greatly from its inclusion due to its abundance of protein, fibre, and bioactive components. The date palm tree, or Phoenix dactylifera, produces palm dates, a natural sweetener, which are harvested from its fruit [1]. Because of its high sugar content and unique flavour character, it is famous as an excellent ingredient for boosting the taste of baked goods. Baking with Makhana and Palm Date provide a great chance to make functional foods that offer multiple health advantages in addition to satisfying hunger [2]. What a fantastic chance this combination gives. The purpose of this research is to produce and standardise baked items that have been enhanced with Makhana and Palm Date by evaluating their sensory, nutritional, and physicochemical qualities. Baking items' nutritional profile and health benefits can be greatly enhanced with the use of functional additives. The substantial protein, fibre, and antioxidant benefits of makhana—also called fox nuts or lotus seeds—have contributed to its rising popularity as a nutritious ingredient [3]. According to studies,

Makhana is rich in bioactive compounds such as alkaloids and flavonoids, which have anti-inflammatory and anti-aging effects [4].

The date palm tree produces palm dates, which are a functional ingredient known for their high nutritional content and natural sweetness [5]. A great source of fibre, vitamins, and minerals, the palm date is an exceptionally healthy fruit. Baking with it is a great idea because of this [6]. You can use palm dates as a natural sweetener because of their high sugar content, which means you won't need as much sugar in baked items [7]. Makhana and palm date, when added to baked goods, can make functional foods with better nutritional content and health-enhancing characteristics. According to previous studies, these ingredients can be added to a variety of foods and drinks. This includes snacks, breakfast cereals, and drinks. However, studies focusing on the development of useful baked goods improved with Makhana and Palm Date are few [6]. Developing a standard morphological analysis for the baked items is a primary objective of this study. Finding the shelf lives for the products is another aspect of this complete baking process.

2. MATERIALS AND METHOD

2.1 Raw Material

The concentrations of various substances in the control and experimental samples, expressed as a percentage per 100 grammes. The main ingredient, whole wheat flour, is used in varying amounts across the samples. In comparison to the reference amount of 100g, test samples 1, 2, and 3 each contain 50g, 60g, and 70g of whole wheat flour, respectively. Unique to the samples used for testing are the ingredients Makhana powder and palm date sugar powder. In the first test, we used 25 grammes of each ingredient; in the second, we used 20 grammes; and in the third, we used 15 grammes. All examples use the same amounts of common components, such as custard powder, milk, salt, baking soda, and yoghurt, thus the recipes are consistent in those areas.

Development of Fox nut (Makhana) powder :

Makhana pods are sourced from the local market. Quality and freshness are ensured during procurement. The purchased Makhana pods are heated in a pan at a moderate temperature to remove excess moisture and improve shelf life. The heated Makhana pods can cool down to room temperature before further processing. The cooled Makhana pods are ground into a fine powder using a grinder or milling machine. The ground Makhana powder is passed through a sieve to remove coarse particles or impurities.

Development of Palm date Fruit powder:

Purchase fresh date fruits from the market. Thoroughly wash the date fruits to remove any dirt or debris. Cut the pitted date fruits into small pieces. This facilitates the drying process. Spread the cut date pieces evenly on oven trays Preheat the oven to 65 ° C (149 ° F) and place the trays inside. Allow the date pieces to dry in the oven at this low temperature for 24 hours. Once dried, remove the date pieces from the oven and let them cool. Transfer the cooled date pieces to a grinder or food processor. Grind the dried date pieces into a fine powder. Store the packaged date fruit powder in a cool, dry place away from direct sunlight.

Making of muffins:

Whole wheat flour, makhana powder, and custard powder were sieved and combined in a large bowl. Refined oil, palm date sugar, and milk and Curd were combined into another large bowl. To produce the batter, the dry ingredients were progressively poured into the wet ingredients bowl and gently combined with a mixing spoon. The batter was then placed gently in the muffin liners and heated between 120-150 Celsius in the oven.

Making of Biscuits:

Into a large bowl desi ghee and palm date sugar were mixed by a whisker. Whole wheat flour, salt, and baking powder were added. Then, milk is poured into the mixture in the large bowl and mixed properly to form a dough. The dough was kneaded and rolled with a roller and cut using a biscuit round cutter. Then the biscuits were placed in an oven tray and then they were placed in 120-150Celsius in microwave oven.

2.2 Morphological analysis

SEM

SEM images were used to study surfacer morphology and microbial growth on the sample surfacer. Morphology of the biscuits and muffins were analysed by scanning electron microscopy. Both samples were taken at three magnifications such as X200, X1000, X2000 at 15kv. Morphological study of a wide range of materials, including biological samples, is made possible by the powerful scanning electron microscopy (SEM) technology. An explanation of how SEM works is as follows:

1. Getting the Sample Ready:

Proper imaging in the scanning electron microscope (SEM) relies on well-prepared samples. Frequently, this requires drying, coating, and fixing. The process of fixation involves cross-linking proteins or other components to maintain the sample's structure. In order to keep the sample from distorting when imaged, dehydration is used to eliminate water from it. Coating with a thin coating of conductive substance (like carbon or gold) improves picture quality and decreases charging effects [7].

2. Sample Loading:

Using conductive adhesive or carbon tabs, the prepared sample is attached to a sample holder or counterfoil. Make sure the sample is firmly fastened so it doesn't move around while imaging.

3. Chamber Preparation

After that, the SEM chamber is filled with the sample holder containing the mounted sample. Electron beam imaging requires a vacuum, which can only be achieved by emptying the chamber [8].

4: Imaging using Electron Beams

The scanning electron microscope operator chooses the accelerating voltage, beam current, and working distance, among other imaging settings, after the chamber is prepared. After that, the sample's surface is scanned with a concentrated electron beam. The interaction between the sample and the electrons causes the generation of several signals, such as characteristic X-rays, secondary electrons (SE), and backscattered electrons (BSE). Specialised detectors pick up on these signals, and the scanning electron microscope (SEM) uses their intensity and spatial distribution to create high-resolution images.

5: Acquiring Images:

The scanning electron microscope (SEM) operator uses specialised software to control the scanning operation and acquire images. To get the right pictures of the sample's morphology, it's possible to take many shots at various magnifications and angles [9].

6. Analysis of Data

The morphological characteristics of the sample are characterised by analysing the SEM images that are acquired after image acquisition. Size, form, surface roughness, and dispersion throughout space are some of the characteristics that might be measured in this process.

7. Analysing and Communicating Results:

The last step is to understand the significance of the research or application by looking at the SEM data. Scientific journals, technical papers, or other written works may contain the results. The scanning electron

microscope (SEM) is an invaluable tool for researchers in fields as diverse as nanotechnology, materials science, biology, and morphology due to the high resolution morphological information it gives [10].

2.3 Test stability and Shelf life

Microbiological examination of food products involves the application of biological, biochemical, molecular, or chemical techniques to identify, detect, or count microorganisms present in a substance, such as food, beverages, environmental samples, or clinical samples. It is commonly used to describe bacteria that cause sickness and spoilage [8]. For the purpose of assessing the shelf-life of the sample, I performed a microbiological examination on my product. Specifically, I conducted a colony-forming unit (CFU) count using Nutrient agar media.

CFU= Colony forming unit

Calculation of CFU per ml is: CFU/ml=number of colonies (whole plate)* dilution factor/volume of culture plate.

Process

When testing the microbiological stability and longevity of food items, the agar diffusion method is a typical tool in the toolbox. A general outline of its potential uses is as follows:

1. Making Agar Plates:

Consider the microbes that are intended to study before deciding on an agar medium. Nutritional agar, Sabouraud agar for fungus, and selective media for certain bacteria are common options. Before pouring the sterilised agar into petri dishes to set, make sure you follow the manufacturer's instructions for preparation.

2. Vaccinating Agar Plates

To get the test sample ready for analysis, it can be poured straight over the agar surface or dilute it to get the right amount of microbes. Distribution of the sample uniformly across the agar plates using sterile procedures is required

3. Incubation:

Placing the inoculated agar plates in an incubator set up to promote the growth of the microbes of interest. Time required for incubation could change based on the microbe under examination.

4. Evaluation of Microbiological Development:

Checking the agar plates for the growth of microbes once the incubation period has passed. Visible spots or colonies on the surface of the agar will be caused by colonies of bacteria, yeast, or mould. Locating the antimicrobial agents in the sample and determining their inhibition zones' diameters. Preservatives and other inhibitory compounds in the product can be better understood with this information.

5. Analysis of Data:

This process can be done by taking note of the data, including the microbial count and species composition, and any inhibitory zones that were detected and finding out how long the product will last by comparing the amount of microbes that have grown on it. The end of shelf life can be determined by looking at the moment at which the development of microorganisms becomes intolerable.

6. Analysis and Final Thoughts:

Drawing inferences regarding the product's microbiological stability and shelf life from the results of the microbiological analysis. To increase the product's microbiological quality or lengthen its shelf life, one may need to suggest changes to the formulation or packaging. For businesses looking to make sure their products stay safe and stable for the duration of their shelf life, the agar diffusion method is a great tool to

have at their disposal. It gives important information regarding the microbiological quality of food products.

Total Plate count Microbial Shelf life

- Dilution series from a sample up to 10-4
- There was 100 amount of the appropriate desired dilution series on to the centre of the surface
- The glass spreader was flamed over a lamp and the sample was spread out evenly by using sterilise glass spreader while the petri dish underneath was rotated carefully
- Incubation occurred at 37 degree celsius for 24 hours
- After incubation a petri plate that contains at least 30 to 300 colonies were kept while others were discarded
- The number of bacteria was calculated using CFU per ml of gram of sample by dividing the number of colonies multiplied by dilution factor and divided by the amount of specimen added to liquified agar over the period of day 1, 3, 4.

3. RESULTS AND DISCUSSION

3.1 Stability Test

- **Sample 1**

Product: Test sample sample 1 represents biscuits produced from 50% whole wheat flour, 25% Makhana and 25% palm date

Palm-Date Makhana Biscuits

	Product (p)
Total colonies of TPC	**2235.5 cfu/gm**
Total colonies of coliform	**Absent/gm**
Salmonella	**Absent/gm**
Total colonies of yeast and moulds	**Absent/gm**

Table 1: Observation of shelf life for biscuits

Remark: Sample validity before 3 months from the date of manufacturing

- **Sample 2:**

Product: Test sample sample 2 represent muffins produced from 60% whole wheat flour, 20% Makhana and 20% palm date

Palm Date Makhana Muffins

	Product (p)
Total colonies of TPC	**5180 cfu/gm**
Total colonies of coliform	**Absent/gm**
Salmonella	**Absent/gm**

Total colonies of yeast and moulds	**Absent/gm**

Table 2: Observation of shelf life for muffins

Remark: Sample validity 7 days from the date of manufacturing

3.2 SEM

Sample 1- Palm date Makhana Biscuit

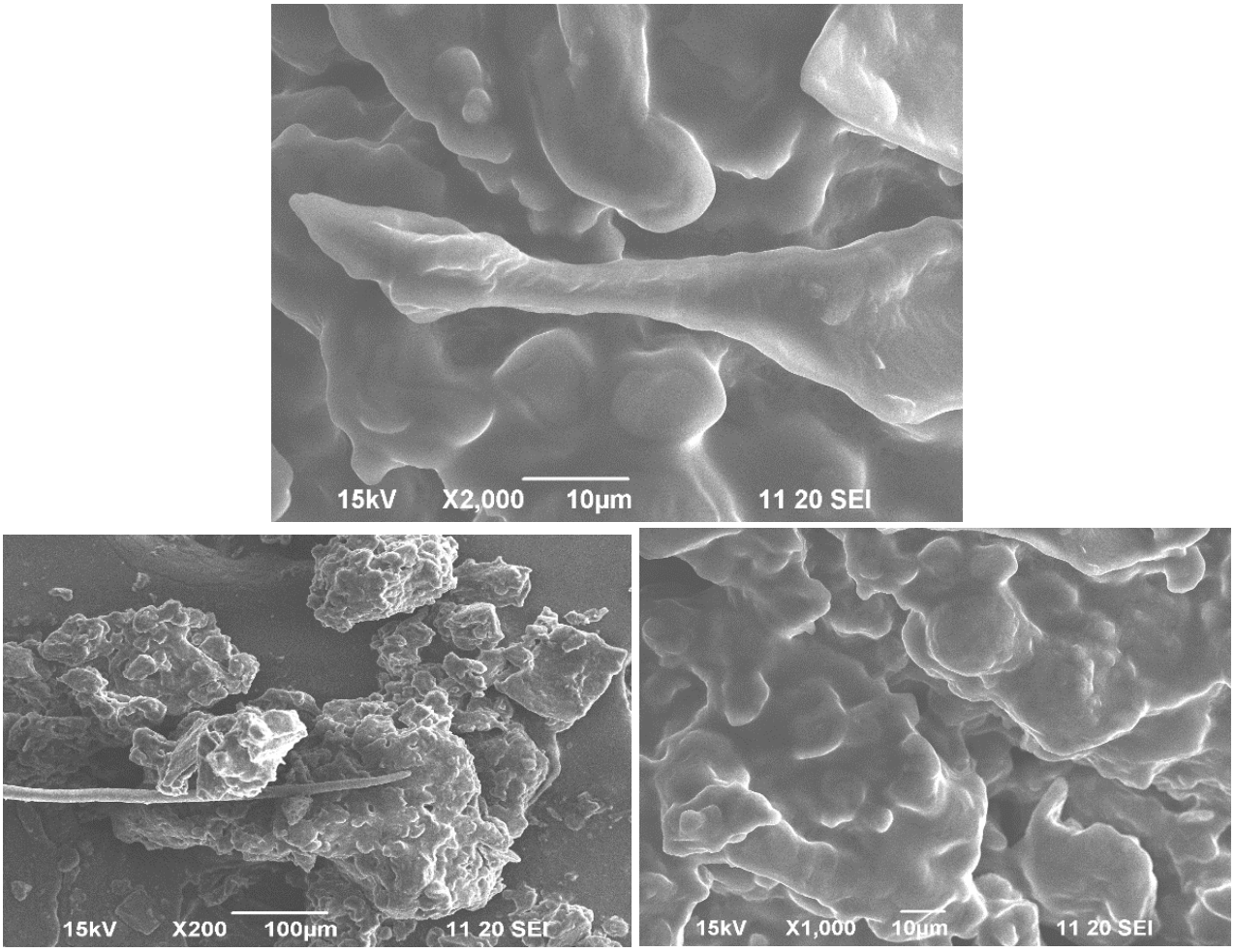
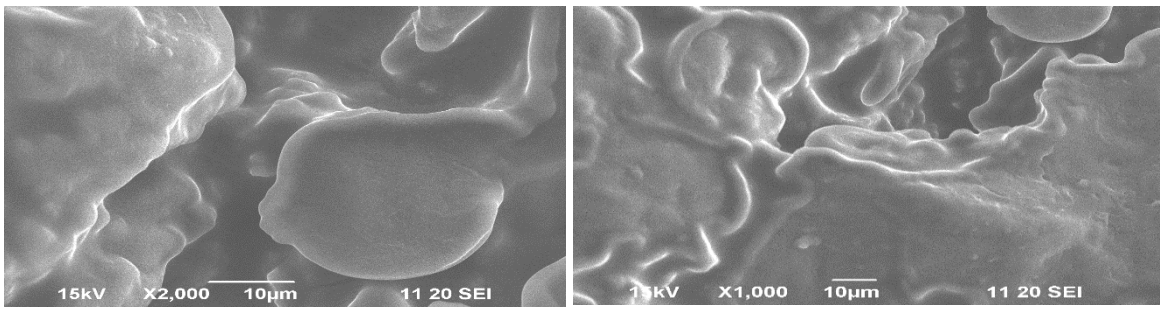


Figure 1: Stages of SEM for sample 1, at 2000, 1000 and 200 unit magnification

Sample 2- Palm date Makhana Muffins



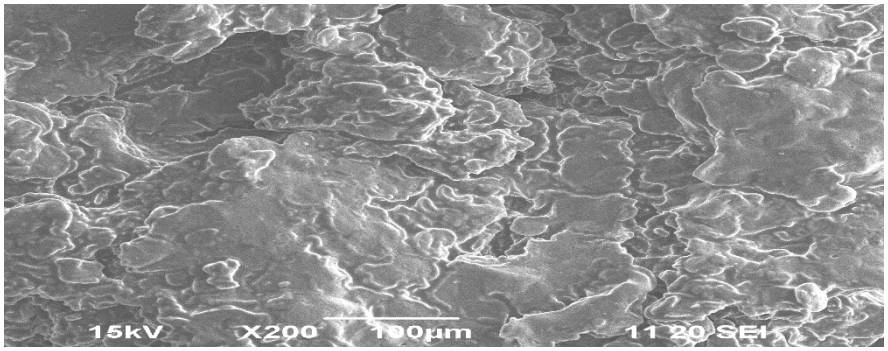


Figure 2: Stages of SEM for sample 2, at 2000, 1000 and 200 unit magnification

It seems that the SEM method is quite an interesting aspect for the research. The surface topography and morphology of samples can be studied in great depth with scanning electron microscopy (SEM) examination. Principal component analysis (PCA) yielded the following insights:

When it comes to surface morphology, scanning electron microscopy (SEM) provides a high-resolution image of a sample's surface characteristics, textures, and structures. Surface coatings, imperfections, cracks, pores, and roughness are all part of this.Grain boundaries, crystalline structures, and phase distributions can all be seen in SEM images of a material's microstructure. Nanoparticles, powders, or aggregates can have their size and form determined by scanning electron microscopy (SEM).

Reconstructing three-dimensional (3D) pictures of samples is now possible with the use of state-of-the-art scanning electron microscopy (SEM) methods including focused ion beam (FIB) milling and electron tomography. This provides a more thorough picture of the internal structure and morphology of the material.

Surface Modification: SEM is able to show how coatings, treatments, or modifications to the surface affect the morphology of the sample. For research into surface engineering procedures and evaluation of surface treatments, this is helpful.

4. CONCLUSION

Total Plate Count (TPC): The TPC count is significantly higher in muffins (5180 cfu/gm) compared to Palm-Date-Makhana Biscuits (2235.5 cfu/gm). This indicates a higher overall microbial load in muffins.

Coliform and Salmonella: Both products show absence of coliform bacteria and Salmonella, which is a positive indicator of food safety.

Yeast and Moulds: Both products show absence of yeast and moulds, suggesting that neither product has contamination from these microorganisms.

In summary, while both products demonstrate absence of coliform bacteria, Salmonella, yeast, and moulds, Muffins exhibit a higher total plate count compared to Palm-Date Biscuits, indicating potentially higher microbial contamination or growth. This could be due to differences in ingredients, processing, or storage conditions between the two products. The higher shelf life is achieved for muffins though.

The study provides important data on the shelf life and SEM analysis of Makhana-date-based products and highlights the nutritional differences among them. Optimising formulation and processing procedures to improve these products' nutritional composition and sensory features should be the priority of additional research and development efforts. In the long run, this will create snack options that are both healthier and more appealing to customers.

The use of scanning electron microscopy (SEM) in the study of cells, tissues, organs, and microbes allows

researchers in the field of biological and life sciences to better understand the microscopic structure and morphology of these samples. Pathological characteristics, tissue architecture, and cellular morphology can all be better understood with its help.

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