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Morphological Properties and Shelf Life Estimation of Teff Fortified Wheat Flour Tortillas

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A B S T R A C T

In the present study tortillas were prepared using flour blends of Eragrostis tef.(Teff seed flour) and Wheat flour using a 10:90 ratio and its morphological as well as microbiological properties in terms of overall product acceptance was evaluated with tortillas made from 100% wheat flour which is also a staple food in Indian households. Results showed that incorporation of teff contains affected the structure and morphology of the tortillas and also tortillas fortified with teff had a longer shelf life.

Keywords: Morphology, SEM, FTIR, Shelf Life

1. INTRODUCTION

Due to its substantially higher nutritional value than most other cereal grains, teff (Eragrostis tef), an ancient grain extensively cultivated in locations like Eritrea and Ethiopia, provides fascinating new food uses.

The grass that grows every year Eragrostis tef, also known as teff, Williams love grass, or annual bunch grass, is a love grass native to the Horn of Africa, specifically Ethiopia. It is cultivated for its edible seeds, commonly known as teff. (1) Teff was one of the earliest plants to be tamed. It is one of the most important staple crops in Ethiopia and Eritrea. Eragrostis tef is a self-pollinating tetraploid annual cereal grass. Teff is a hybrid between a tropical and temperate grass and is a C4 plant, which means it can fix carbon more efficiently under drought and high weather. Teff is thought to originate from the Amharic word teffa, which means "lost." This is most likely due to its tiny seeds, which have a diameter of less than 1 mm.

Teff is a fine-stemmed, tufted grass with massive crowns and numerous tillers. Its weak roots grow into a huge fibrous rooting system. The plant's height is determined by the cultivar and the surrounding environment. Teff, like many ancient crops, is highly versatile and can survive in a wide range of conditions; for example, it can be grown in both dry and wet areas on marginal soils. Teff was one of the earliest plants to be tamed. Teff is estimated to have originated in Eritrea and Ethiopia between 4000 and 1000 BC. According to genetic research, E. pilosa is the most likely wild ancestor.

The authenticity of a 19th-century identification of teff seeds from an ancient Egyptian site has been called into question; the seeds in question (which are no longer available for research) are more likely to be of E. aegyptiaca, a common Egyptian wild grass. (2) The primary goal of this research is to study the physical as well as its antioxidant properties of wheat flour tortillas fortified with different formulations of teff seed flour and to also estimate shelf life of the developed food product. Teff grain may be used in the future to create unique super-foods as a vegan, plant-based replacement.

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2. Material and Methodology

Wheat flour (Triticum aestivum L.,) commonly used in bread making was procured from local market of Lucknow , Uttar Pradesh, Country India, Tef seed flour (Eragrostis tef.) was obtained from a food product supplier named lil goodness based in Bengaluru, Karnataka country India

2.1 Sample Preparation

Eragrostis tef flour was mixed with various proportions of wheat flour to which 2% baking powder, 5% shortening was added. The control sample used for this study was prepared by using whole wheat flour, 2% baking powder and 5% shortening. The quantity of water used to prepare the dough was kept constant. After preparing blends of flours with different ratios, dough was manually prepared by adding constant volume of water and was kneaded for almost 10 minutes. The dough was then set to rest for almost 15 minutes and was then weighed, divided into equal parts, rolled out in equal round sizes and cooked on gas stove for about 2-5 minutes.

Fig 1:- Final Product and its packaging

2.2 Sample Size

Any research work has sampling procedure to conduct the research work. Sampling consists of selection of sample and sampling technique (i.e., step by step procedure.)

2 Different Samples were prepared consisting of different flour Ratios. The level of fortification (in Percentage %) has been mentioned below:-

Sample A:- Control Sample consisting of 100% Whole Wheat Flour

Sample B:- 10% Teff Fortified Sample.

3. Evaluation of Morphological structures (Physical Properties)

3.1 SEM WITH EDS (Scanning Electronic Microscopy With Energy Dispersive Spectroscopy)

The morphological structure of tortillas was studied using a Scanning Electronic Microscope. By scanning a focused electron beam across a surface, a scanning electron microscope provides a picture. The electrons in the beam interact with the sample and produce a number of signals that can be used to figure out the surface's topography and composition. It can be used at both low and high vacuums, depending on the type and nature of the specimen. The equipment came with an optional attachment, EDS, that expanded the application range by allowing any metal connected to biological material to be detected and analyzed, whether in isolation or as an alloy.

Two major devices were used to facilitate sample preparation for the SEM facility: a JEOL Sputter Coater (JFC 1600, Auto Fine Coater) and a Quorum Technology Emitech K 850 Critical Point Dryer (CPD). A powdered sample of 2-4 mg was obtained and used to extract the morphology of the sample for morphological examination. The samples were mounted onto the aluminum stubs with carbon tape, and all of the samples were conductively coated using a sputter coater. Using a high resolution field emission Scanning Electron Microscope, all of the materials were examined at 10 KV (JSM 6490)

3.2 Fourier Transmission Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) analysis is a spectroscopic method that uses the electromagnetic spectrum's naturally occurring wavelengths, which range from 2,500 to 25,000 nanometers. Overall, FTIR analysis has advantages in that it provides speedy analytical data for better decision-making in food and agriculture production processes. It's particularly useful for analyzing liquid samples such as milk or wine. In comparison to traditional analysis

processes, it requires minimal or no sample preparation and no reagents or consumables. It's non-destructive, simple to operate, fast, trustworthy, and precise. The Fourier transform infrared spectroscopy (FTIR) is a technique for obtaining an infrared spectrum of organic and inorganic materials' absorption or emission.

The prepared food sample was analyzed using the FTIR Nicolet 6700. The basic idea behind FTIR is to use a broad-band light source to shine light across the whole spectrum of wavelengths to be measured by an interferometer.

The interferometer modifies the light in such a way that the data may be processed afterwards. The sample is passed over the beam, creating absorption that is sample-dependent. A computer detects and analyses the light. The computer analyses all of the data to determine absorption at each wavelength and generates a spectrum that matches the data using the Fourier Transform method. A small amount of Kbr was added to the finely pulverised material (0.1-2 percent) The sample was attached to the sample holder, and the sample was analysed against a Kbr background in the spectral region 4000-400 cm-1.

4. SHELF LIFE ESTIMATION (MICROBIOLOGICAL ANALYSIS) OF THE PRODUCT

Microbial spoilage is undesirable because it can produce gas production, discoloration, altered flavour and aroma, and visible colonies on the product, as well as posing a health concern to customers. Underbaking tortillas is crucial because the less the tortilla is baked, the less microorganisms are inactivated, and under baked tortillas are more prone to contamination due to a higher water activity than properly baked tortillas.

Flour-making grains are frequently contaminated with bacteria, yeasts, and filamentous fungus in addition to bacteria, yeasts, and filamentous fungus. The ability of these bacteria to grow in the final food product is influenced by a number of factors. Intrinsic characteristics include water activity, pH, redox potential, availability of nutrients, and inhibitory compounds, whereas extrinsic parameters include relative humidity, gas composition in the packaging and process, and storage temperature.

4.1 Sample Preparation for CFU (Colony Forming Units) Count.

The microbiological analysis was performed by calculating the growth of Total colony forming Units (CFU) on specified media (shelf life estimation). To identify bacteria, scientists employ specialised media that are augmented with colours, pH indicators, or medicines. The media utilised were Nutrient Agar Medium (NAM), Lactobacillus MRS Agar powder (LMRS), and Violet Red Bile Agar Media (VRBA).
To generate a homogenous sample, crushed tortilla samples were employed. Autoclaving was used to sterilize NAM, LMRS, and VRBA media, as well as distilled water and petri plates. To keep the medium from becoming contaminated, it was autoclaved and then placed onto petri plates using laminar Air Flow. We took all of the appropriate safety procedures. To firm the media plates, they were subjected to UV light for 15 minutes. After that, the homogenous crushed and powdered tortilla sample was mixed with distilled water and diluted serially. Samples were decimally diluted and 0.1 ml diluent was spread out on the matching media for total bacterial count determination. The spread samples were then grown in an incubator for 24-48 hours at 37°C, and the colonies were counted in cfu/gm.

5. RESULTS AND DISCUSSIONS

5.1 SEM WITH EDS (Scanning Electronic Microscopy With Energy Dispersive Spectroscopy)

Tortillas with varied percentages of flour mixtures were created for SEM analysis to examine the microscopic structure of the tortilla samples. SEM was used on just two of the four samples, Control (Sample A) and Fortified (Sample B). Tortillas made with 100 percent wheat flour displayed a distinct structural form as compared to the enhanced sample. The gluten network's topology was altered by the addition of Teff flour, resulting in a discontinuous structure in both the gluten and protein networks.

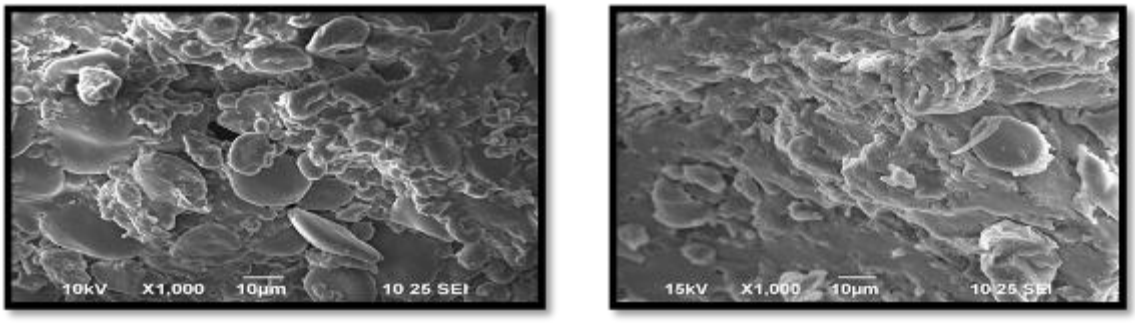

Figure 3:- Morphological Structures of Sample A & B

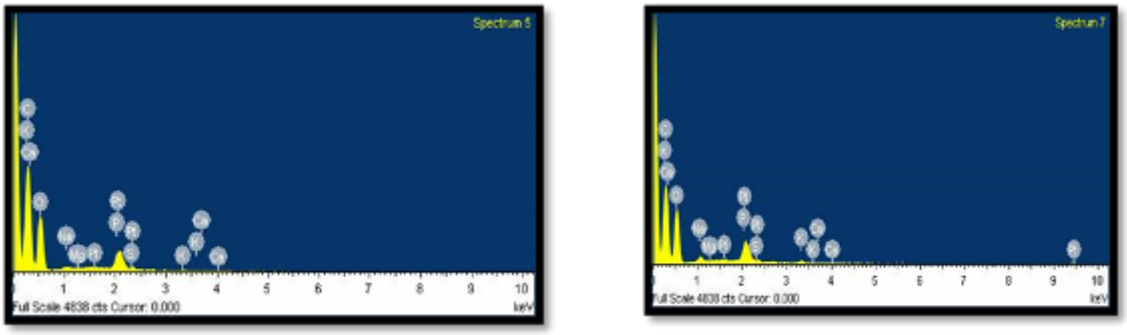

Figure 4:- EDS GRAPH OF SAMPLE A& B

5.2 Fourier Transmission Infrared Sectroscopy (FTIR)

The FTIR results revealed that the infrared spectra of Sample B (Teff Fortified) and Control (Wheat Flour) are displayed in the graph below. The existence or absence of a functional group is determined by the infrared spectrum. The structural similarities and differences between Sample B and Control were discovered by comparing their infrared spectra. The structures of the two samples are dissimilar. The CH3 stretch of sample B is represented as a prominent peak at 2924 cm-1 for sample A. This finding can be linked to a nearby peak at 2855 cm-1. At 1745 cm-1, the presence of an ester group is confirmed by the C=O vibration and the presence of a prominent peak at 1004 cm-1. Control, on the other hand, has a similar structure, but the peak smoothens off at 3360 cm-1. However, in Control similar structure exists but smoothening of the peak occur at 3360 cm-1. Similar observation was seen at wavenumber 2300 to 2100 cm-1.

It can be concluded from FTIR spectrum that, millet is presence in tortilla mix and smoothening of peaks in tortilla mix had altered the properties of final product.

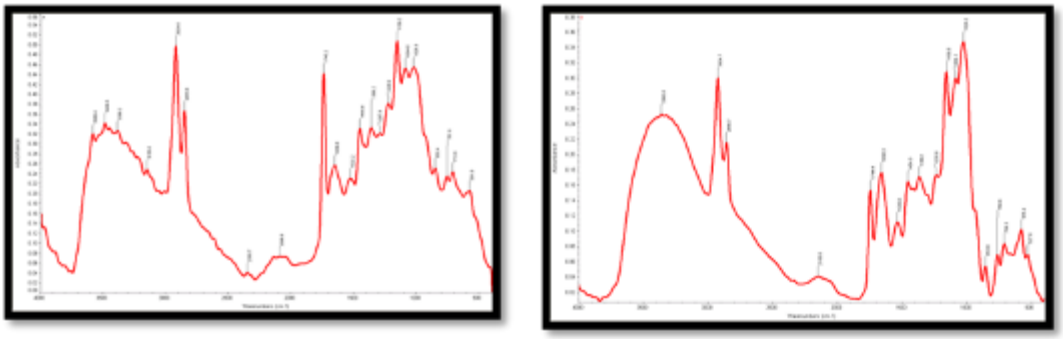

Figure 5:- FTIR Peaks of Sample A & B

5.3 Shelf Life Estimation (Microbiological Analysis) of the product

5.3.1 Nutrient Agar Media

On day 0, colonies form in NAM after a 48-hour incubation period, whereas colonies form after 10 and 15 days of sample preparation. The temperature of the incubation chamber was fixed at 37°C. As a result, after 24 hours, we may infer that the samples were clear of any microbial burden. After 10 days of storage in refrigerated conditions, the samples were deemed to be safe for eating since their microbial load was under control; but, after 15 days, the samples were no longer safe to consume. As a consequence, we may conclude that fortification increased the product's shelf life, with the Control sample being unfit for consumption after 10 days and the Fortified sample being acceptable. Sample D showed highest shelf life of 15 days under refrigerated conditions.

Table 1:- CFU/Gm in Nutrient agar media at different intervals

SAMPLES	No. of Days	After 24 hours of Inoculation
Sample A	0 days	2.68×10^5
	10 days	3.40×10^5
	15 days	$1.5x\ 10^5$
Sample B	0 days	$1.28x\ 10^5$
	10 Days	3.12×10^5
	15 days	1.2×10^5

5.3.2 Lactobacillus MRS media

This media is used to isolate and enumerate lactic acid bacteria especially all bacillus species from milk and milk product. This is the main reason of spoilage of the milk and milk product. Samples were inoculated after 3 days of sample preparation while samples were stored in refrigeration condition at 4˚C. Results show it fit for consumption.

Table 2:- Showing CFU/Gm in MRS media after 24 and 48 hours.

SAMPLES	CFU count per gram after 24 hours of inoculation	CFU count per gram after 48 hours of inoculation
Sample A	3.0×10^4	$6.0x10^4$
Sample B	5.0×10^4	$8.0\ x\ 10^4$

5.3.3 Violet Red Bile Agar Media

It is used for selective isolation , detection and enumeration of coliform in water, milk and other dairy products. Samples were inoculated after 5 days of preparation. Growth was observed at 24 hours and 72 hours.

Table 3:- CFU/Gm in VRBA media at 24 and 48 hours

SAMPLES	CFU per gm after 24 hours	CFU per gram after 72 hrs
Control	1.72×10^5	2.20×10^5
Sample B	9.6×10^5	1.36×10^5

4. CONCLUSION

The purpose of this study was to understand the morphological and microbiological analysis of tortilla prepaed from from mixing wheat flour with teff flour. Teff flour is a very good source of protein and essential trace elements. The tortilla was developed using different percentage of flour blends and then the prepared samples were subjected to analyse the morphological,, microbial evaluation. The morphological properties of tortilla showed that addition of teff flour affected the gluten structure and protein structure of the tortilla.

Further the samples were subjected to FTIR testing to assess the presence of organic or inorganic materils present through infrared spectroscopy

Shelf Life estimation was carried out on different specific medias and it was concluded that the sample was fit for consumption upto 15 days when stored under refrigeration at 4˚C.

Many countries are increasingly interested in using teff to make gluten-free foods because to its medical properties. Teff consumption is already common in various parts of the world, including Ethiopia, the Netherlands, and North America. Processors will have no trouble introducing this cereal to other parts of the world.As a result, Teff has a broad application. Teff as a weaning food is the subject of research. Preservation of Teff products, decrease of anti-nutritional components, and more extruded Teff products are only a few of the topics that have yet to be thoroughly investigated. Also, using Teff with various amounts and ratios of composite flour in baked goods such as cakes and bread, the most difficult aspect of teff's future is developing plant-based protein foods and vegan alternatives. To summarise, teff is a promising new superfood for the next generation. [6]

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