Research Article

Study on microbial profile of bread during storage

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Abstract

Cereals as well as bakery products are valuable sources of nutrients in human diet. Most of our food calories and half of our protein requirements are provided by them. Bread is one of the important bakery products which is widely consumed. A variety of microorganisms are found during the storage of bread. The microbial flora which colonizes a particular food depends highly on the characteristics of the product and the way it is stored. Food composition is a critical intrinsic factor that influences microbial growth. If a food consists primarily of carbohydrate, fungal growth predominates and spoilage does not result in major odors. Thus foods such as bread first show fungal growth during spoilage. This study investigated the microbial profile of bread stored for 5 days at 29-31˚C and 70% relative humidity. The microorganisms found in bread during storage were isolated and identified based on the standard keys available. After the initial fungal growth mainly *Mucor* sp. and *Rhizopus* sp. after three days, the soft texture of the bread changed and became hard during which the microbial load was dominated by bacteria. The bacterial species identified were *Bacillus cereus* and *Bacillus subtilis*.   The pH of the bread (5.7) increased during the first 5 days of storage and then decreased continuously. The change in micro flora may be due to the change in the intrinsic factors in the food as well as the extrinsic parameters like temperature and RH.

Keywords: bread, microorganisms, food.

Introduction

Cereals played an important role in human diet and were consumed long before bread making process was developed. The consumption of bread and other bakery products has increased within the past decades. Next to bread, biscuit is one of the most commonly consumed baked snacks in Nigeria (Ogunjobi and Ogunwolu, 2010). In India, breads and biscuits constitute over 80 percent of total bakery products produced in the country. The urbanization has resulted in increasing demand for ready to eat food items at reasonable price. Although bakery products were considered as poor man's diet earlier, they have now become essential food items in the day-to-day life of a large majority of human population. Previous studies done by Oluwajoba et al (2012) have shown bread as a good source of nutrients namely macronutrients

(carbohydrate-52%, protein-9% and fat-1%) and micronutrients that are important for health. Wheat has been the major raw material used in the manufacture of bread for a long time. But in most of the developing countries wheat flour is imported to satisfy local flour needs for making bakery products. Several researches have shown that bread can be made by using composite flours in which flour from other sources replace a portion of wheat flour to be used in bread. This in turn decreases the demand for imported wheat and produces nutrient-enriched bread. Study on soy flour supplemented wheat flour in making bread was done by Sanful and Darko (2010). Another study was done in cookies made of a blend of wheat, yam and soybean (Apotiola and Fashakinly, 2013). The microbial flora which colonizes a particular food

depends highly on the characteristics of the product and the way it is stored. The parameters affecting proliferation of microorganisms in food can be intrinsic parameters, extrinsic parameters and modes of processing and preservation (Mossel et al.,1995). Information regarding the microbiological profile is important to determine appropriate processing parameters and conditions of storage required to prevent the proliferation of microorganisms. The microorganisms associated with the storage of biscuit produced from composite flour of 60% maize and pigeon pea have been studied (Olunlade et al., 2013). The aim of this study is to determine the microorganisms that are associated with the storage of bread.
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Materials and Methods

Ten loaves of bread used in this study were purchased from different shops in Jaffna, Sri Lanka. The samples collected were brought in a sterile polythene bag to the laboratory for analysis.

Storage

The loaves of bread were stored for 5 days at 29-31 ˚C and 70% relative humidity. The pH of the samples was measured.

Nutritional analysis

The nutritional composition of the bread was studied according to the methods of AOAC (2005).

Microbiological analysis

The media used were Potato Dextrose Agar (PDA) and Nutrient Agar. NA was prepared by adding 7g of agar into a 500ml conical flask and dissolving it with 250ml distilled water. The conical flask was plugged with cotton wool wrapped with aluminium foil and the mixture was warmed on a heating mantle to homogenize. Then the medium in the flask was sterilized in an autoclave at 121 ˚C for 15 mins.

PDA was prepared by weighing 9.75g of the agar into a 500ml conical flask and dissolving it with 250ml distilled water. Then, chloramphenical was added aseptically to molten PDA at 45 ˚C in order to retard bacterial growth. The isolation of microorganisms was done by using the pour plate method.

Serial dilution was done as follows. 1g of each sample was added into test tubes containing 10ml sterile water and these were used as stock solutions. 1ml was removed from each of the solution and added to another set of test tubes containing 9ml sterile water which made 10^{-1} dilution. The same procedure was repeated to make 10^{-4} dilution. Then, 0.5ml of the 10^{-4} dilution was added into sterile petridishes and sterile molten agar was poured into the plates. The inoculated plates were allowed to set and incubated. The plates were kept in an inverted position to avoid the condensation of water vapour on the plate cover from dropping on the culture. The NA plates (bacterial cultures) were incubated at 37 ˚C for 24 hrs while PDA plates (for fungal cultures) were incubated at 25 ˚C for 72 hrs. The number of colonies found on each media was counted.

Isolation of microorganisms was done. Sterile molten agar (NA and PDA) was poured into petridishes and allowed to solidify. Different colonies were taken from the mixed culture plates and streaked on plates separately. The streaked plates were incubated at 25 ˚C for 72 hrs for fungal growth and at 37 ˚C for 24 hrs for bacterial growth. Similar subculturing was done until pure cultures were obtained. The pure cultures were stored in Mc Cartney bottles. PDA and NA were prepared in Mc Cartney bottles and sterilized in an autoclave at 121˚C for 15 mins. Then, the media were allowed to set in an incline position to prepare agar slants. The pure fungal isolates were inoculated into PDA slants and incubated at 25 ˚C for 48 hrs and NA slants containing bacterial isolates were incubated at 37 ˚C for 24 hrs. The fungal isolates were identified by microscopic examination and cultural characteristics and confirmed by using the keys available. The bacterial isolates were identified by gram staining, motility, cultural characteristics and biochemical tests (Catalase, oxidase, starch hydrolysis, sugar utilization, urease and citrate tests).

Microbial load estimation for the bread samples was carried out for fresh as well as 3, 5 days old samples.

Results and Discussion

The nutritional composition (nutrients per 100g) of the bread is given in Table I

Table I nutritional composition of the Bread (nutrients per 100g)

Carbohydrate, g (% of energy)	52
Proteins, g (% of energy)	9
Fats, g (% of energy)	1
Dietary fibre, g	3
Vitamin B1 (Thiamine), µg	83
Vitamin B2 (Riboflavin), µg	58
Iron, mg	0.6
Sodium, g	0.8
Magnesium, mg	23

The determination of microflora carried out in this study is necessary in safeguarding public health. This study therefore provides basic information about the microflora in bread likely to cause food-borne disease when present in bread which is a ready-to-eat type of food. Table II shows *Rhizopus* sp and *Mucor* sp were associated with the storage of bread after 3 days of storage. This was also observed in our previous study (Nirmala et al., 2016). Saranraj and Geetha (2012) in their study showed that *Rhizopus* sp, *Aspergillus* sp, *Penicillium* sp, *Mucor* sp and *Eurotium* sp are the most common fungi found in bakery products. In this study the total fungal count ranged from 2.5×10^5 to 3.0×10^5.

Table II Cultural and morphological characteristics of identified fungi

Fungal Isolate	Cultural Characteristics	Morphological Characteristics
Mucor sp.	Large white colonies which turns into black later.	Erect sporangiophores are formed. Sporangiophore swells ate the tip to form sporangia which are globular shaped. Columella is present.
Rhizopus sp.	White cottony mycelia, with black dots and covers the entire plate.	Sporangiospores are produced inside a spherical sporangium. Columella is present on the top of the sporangiophore. Root-like rhizoids are found.

Nirmala et.al., 2016.

On the 4th day of storage the texture of the bread became hard. This may be due to the ropiness caused by bacteria. The bacterial load in the bread samples was relatively high on the 4th day and afterwards. It showed that there were some microorganisms which were heat-resistant may have been associated with the bread dough and subsequently found in the bread. This was confirmed to be the *Bacillus* sp. It has the ability of forming endospores which are known to be heat-resistant. In this study, *Bacillus cereus* and *Bacillus subtilis* were recorded in the bread samples. The microbial load increased during storage. (Table III)

Table III Bacterial count in bread during the first five days of storage (cfu/g)

Sample	Day 1	Day 3	Day 5
1	No growth	3×10^5	4×10^6
2	No growth	2.2×10^5	4.8×10^6
3	No growth	2.8×10^5	5.0×10^6
4	No growth	2.3×10^5	3.9×10^6
5	No growth	3.2×10^5	4.7×10^6
6	No growth	2.9×10^5	3.2×10^6
7	No growth	3.3×10^5	4.5×10^6
8	No growth	2.7×10^5	3.4×10^6
9	No growth	2.4×10^5	3.7×10^6
10	No growth	3.1×10^5	4.9×10^6

This was shown earlier by Nagi et.al (2012). From this study, it was observed that the microbial load increased during storage as the storage condition was favorable to microbial growth. It is preferable to consume bread within three days of storage (29-31˚ C and 70% RH) as the bacterial load falls within the safe limits recommended by the Food and drug Administration or else suitable packaging materials and storage conditions should be determined to improve the quality parameters and shelf life of bread.

The presence of microflora in bread is mostly due to the contamination in the baking environment, contamination by handlers and market sellers. The personal hygiene of the food handlers in bakeries should be thoroughly maintained.

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