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Isolation and Characterization of Microbial Consortium in Wheat Flour Stored Under Different Conditions in Maraba Market

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Abstract **Original Research Article**

Flours or powders are made from edible seeds, roots, tubers, fruits among other raw materials. Twenty samples were used in this study. Fungi was isolated using Potato dextrose Agar while Bacteria were isolated using Nutrient Agar. Biochemical test were also done to confirm the bacteria isolated. The highest fungal load was 2 while that of bacteria was 2.5 at 35-38^{0}C while the highest fungal load at 25-28^{0}C was 3 while the highest bacterial load was 1.6. Fungal genera isolated were *Aspergillus* while the bacterial genera were *Escherichia* and *Bacillus*. The microorganism's isolated show that contamination can occur at any point in the production chain and even in storage, microbes can grow when the temperature is favorable for their growth.

Keywords: *Aspergillus*, Flours or powders, bacteria isolated, Fungal genera isolated, Microbial Consortium.

BACKGROUND OF THE STUDY

Flour is a powder made by grinding raw grains, roots, beans, nuts, or seeds. Flours are used to make many different foods. Cereal flour, particularly wheat flour, is the main ingredient of bread which is staple food for some cultures. Flour contains a high proportion of starches, which are a subset of complex carbohydrates also known as polysaccharides. The kinds of flour used in cooking include all-purpose flour, self-rising flour, and cake flour including bleached flour. The higher the protein content the harder and stronger the flour, and the more it will produce crusty or chewy breads. The lower the protein the softer the flour, which is better for cakes, cookies, and pie crusts.

Wheat flour is powder obtained from the milling of wheat grains, which is the main raw material for cereal-based food products. The quality of wheat flour, which will directly affect the appearance, taste, and texture of flour foods, is a function of many factors including wheat variety, processing technology, and storage conditions. Currently, flour quality is typically evaluated by measuring the chemical compositions (protein, gluten, starch, and damaged starch content), dough rheological properties or directly investigating the performance in food making (steaming, boiling, and baking). The quality of wheat flour is fundamentally determined by its chemical composition. The major components of wheat flour are protein 10%-12% and starch 70-75%, and the minor components are polysaccharides 2-3% and lipids 2%. Wheat grains are generally oval shaped, although different wheats have grains that range from almost spherical to long, narrow and flattened shapes. The grain is usually between 5 and 9mm in length, weighs between 35 and 50mg and has a crease down one side where it was originally connected to the wheat flower. The wheat grain contains 2-3% germ, 13-17% bran and 80-85% mealy endosperm. The bran is made up of several layers, which protect the main part of the grain. Bran is rich in B vitamin and minerals; it is separated from the starchy endosperm during the first stage of milling. In order to protect the grain and endosperm material, the bran comprises water-insoluble fibre. More than half the bran consists

of fibre components (53%). Chemical composition of wheat bran fibre is complex, but it contains essentially, cellulose and pentosans, polymers present in the cell walls of wheat and layers of cells such as aleuronic layer.

The endosperm is surrounded by the fused pericarp and seed coat. The outer endosperm, the aleurone layer, has a special structure: it consists of single layer of cubic shaped cells the aleuronic is rich in proteins and enzymes, which play a vital role in the germination process. The inner endosperm, i.e the endosperm mainly contains food reserves, which are needed for growth of the seedling. It is rich in energy-yielding starch. Apart from carbohydrates, the mealy endosperm contains fats (1,5%) and protein (13%): albumins, globins and the major proteins of the gluten complex-glutenins and gliadins- proteins that will form the gluten at dough making. The contents of minerals and of dietary fibres are low: 0,5% and 1,5%, respectively.

The germ lies at one end of the grain. It is rich in proteins (25%) and lipids (8-13%). The mineral level is also rather high (4,5%). Wheat germ is available as a separate entity because it is an important source of vitamin E. Wheat germ has only one half the glutamine and proline of flour, but the levels of alanine, arginine, asparagine, glycine, lysine and threonine are double.

Wheat and Reasons for Its Consumption

Wheat is one of the world's most commonly consumed cereal grains. It comes from a type of grass (*Triticum*) that is grown in countless varieties worldwide. Wheat is highly controversial because it contains a protein called gluten, which can trigger a harmful immune response in predisposed individuals. Wheat is mainly composed of carbs but also has moderate amounts of protein, calories 340, water 11%, protein 13.2 grams, carbs 72 grams, sugar 0.4 grams, fiber 10.7 grams, fat 2.5 grams . The health effects of starch mainly depend on its digestibility, which determines its effect on blood sugar levels. Wheat is high in fiber but redefined wheat contains almost none. The fiber content of wheat is 12-15% of the dry weight. Protein make up 7-22% of wheat's dry weight. Gluten a large family of proteins, accounts for up to 80% of the total protein content. It's responsible for the unique elasticity and stickiness of wheat dough. Wheat harbors significant amount of fiber, which may aid your digestion. Wheat is a good source of several vitamins and minerals which include, selenium which has various function in the body, manganese which is found in high amount, phosphorus it plays an essential role in the maintenance and growth of body tissue, copper, folate one of the B vitamins also known as vitamin B9 or folic acid it's particularly important during pregnancy. Enriched wheat flour maybe a good source of iron, thiamine, niacin, calcium and vitamin B6, in addition to the other nutrients. Eating wheat in place may lower the risk of type 2 diabetes, in part of this because fiber-rich grains can also help with weight control and prevent obesity.

Mechanisms of Contamination of Wheat Flour

Wheat flour is a raw agricultural product and thus, is subject to contamination from soil, animal feces, insects, diseased plants, and other agents [1]. Historically contamination has been a little concern because it was assumed that the low water activity of wheat flour cannot support bacterial growth and that any pathogenic contaminant would be destroyed during baking, frying, or cooking of flour used in final product processing. It has discovered, however that although wheat flour cannot support bacterial growth at normal stage moisture contents, bacteria can survive in a dormant state for extended periods in dry flour. As a raw agricultural product, wheat carries with it contaminants from the environments it is exposed to during production, including harvest, storage, and transport. As might be expected, microbial contaminants are found mostly on the surface of the grain.

Sources of Contamination of Wheat Flour

The sources of wheat microbial contamination can be found along the crop production chain, including the stages of preharvest, harvest, transportation, storage, and processing [2]. The contaminating microbes can be carried by different elements such as animals, air, water, dust, and contaminated equipment [1]. Furthermore different weather conditions such as precipitation level and relative humidity level, as well as specific field micro flora can influence the type and amount of microbial load on kernels [3].

Food-borne diseases associated with wheat flour

In recent years wheat flour has been implicated as the root cause of several recalls and outbreaks of foodborne illness. Suspected vehicles include cookie dough, cake batter, and raw wheat flour. Bacteria from animal waste in the field could contaminate the grain, *E.coli* 0121 can cause abdominal cramps and often diarrhea but most people recover within a week. In rare cases it can cause a type of kidney failure called hemolytic uremic syndrome. Young and elderly people and those with weakened immune systems are most at risk of complications.

Epidemiology of food-borne diseases associated with wheat flour

In 2005, 26 people in several states across United States were infected by a single strain of *Salmonella enteric* serotype Typhimurium after eating cake batter ice cream [4]. The cake mix used to prepare the cake batter ice cream was implicated by epidemiologic investigation as the source of salmonella. In 2008, a cluster of salmonellosis cases emerged in New Zealand. These illnesses were attributed to consumption of an uncooked baking mixture containing

flour contaminated with *S. enterica* serotype Typhimurium phage type 42 [5]. An *E. coli* outbreak in 2009 resulted from the consumption of raw cookie dough. This is the first reported enter hemorrhagic *E.coli* (EHEC) outbreak associated with consuming ready to bake commercial prepacked cookie dough [6]. In 2015 and 2016 there was suspected *Salmonella* contamination in bags of wheat flour distributed in the united states and Germany. Although illnesses were not reported in these last two incidents, they resulted in large-scale recalls and significant losses of manufactured products and revenue.

Common Bacteria and Fungi found in wheat flour

The common bacteria found on grains include enteric pathogens such as gram-negative bacteria *Escherichia coli* and *Salmonella* and the gram-positive bacteria *Bacillus cereus*, yeast, and mycotoxin-producing fungi from the genera *Aspergillus*, *penicillium* and *Fusarium*, among others.

Methods of culturing and indentifying Bacteria and Fungi found in wheat flour

Pour plate method is usually the method of choice for counting the number of colony forming bacteria present in a liquid specimen. In this method fixed amount of inoculum from a sample is placed in the center of a sterile petri dish using a sterile pipette. Molten cooled agar is then poured into the petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37ºC for 24-48 hours. Materials and equipment used in this method include:

1. Test sample
2. Nutrient agar
3. Hot water bath
4. Sterile petri dishes
5. Flame
6. Test tube
7. Pipette.

After culturing and there is visible growth a Gram-stain method is been carried out to identify if the bacteria is gram-positive or gram-negative. The cells appear colourless a secondary stain is applied safranin, leaving the gram-negative cells pink and the gram-positive bacteria obtain the primary stain crystal violet.

According to Aryal (2018), uses of potato Dextrose Agar include the detection of yeast and molds in foods. It may also be used for the cultivation of yeast and molds in clinical specimens. The lactophenol cotton blue (LPCB) stain is the most widely used staining solution in the examination of yeast and molds. Its serves as both mounting fluid in wet mounts and as a stain. It is simple to prepare. The preparation of (LPCB) has three components:

1. Phenol, which kill any live organisms including the fungi;
2. Lactic acid which preserves fungal structures and
3. Cotton blue which stains the chitin in the fungal cell walls.

METHOD

Study Area

The area for this research was carried out in Maraba which is located in Nasarawa state central Nigeria. It is a district of Karu Local Government Area. Nasarawa state and is among the towns that make up the Karu urban area, a conurbation of towns stretching to Nigeria's Federal Capital Territory. Its neighboring towns are, Ado, Nyanya, New Nyanya, Masaka and New Karu.

Fig 1: Map showing Maraba, Nasarawa state

MATERIALS AND METHOD

Study Population; A total of 20 samples were be collected. Study Design; the samples were collected randomly and samples were screened for bacteria and fungi.

Media Preparatio: The agar is measured and prepared according to producers instructions. The agar is sterilized in a conical flask in the autoclave at 121ºC for 15 minutes and cooled to 40-50ºC was poured aseptically, in the volume of 20ml each, into sterilized petri dishes and allowed to harden under room temperature.

Serial Dilution of wheat samples

Wheat flour samples from various storage condition were taken and serial dilutions were made. For this 1g of each sample was added into test tubes containing 10ml sterile water and these was used as stock solutions. 1ml was removed from each of the solution and added to another set of test tubes containing 9ml of sterile water which made $10^{-}1$ dilution. The same procedure was repeated to make $10^{-}4$ dilution. Then 0.5ml of each dilution was added into sterile petri dishes containing the prepared agar. The Nutrient Agar plates (for bacterial cultures) were incubated at 37◦C for 24hrs while Potato Dextrose Agar (for fungal cultures) were incubated at 25◦C for 72hrs. The number of colonies found on each media was counted. Isolation of microorganisms is done.

Test Procedure for Identification of Bacteria

A Gram- stain method was carried out by adding a drop of distilled water on clean glass slide and a loop of the bacterial isolate was smeared on the water which was then be allowed to dry. It was passed through flame twice in order to fix it. One to two drops of crystal violet was added and left for 60 seconds before washing it with distilled water. Grams iodine was added and washed away with distilled water after 1 minute. It was then be flooded with alcohol and washed with distilled water after 30 seconds and then adding safranin which was also washed with distilled water after 1minute. The glass slide was left to dry and examined under the microscope. Catalase, oxidase, citrase, amylase, tests were performed in order to isolate the required bacteria.

Likewise Test Procedure for Identification of Fungi were performed

A primary classification of the colonies was carried out, based on the colony characteristics (pigmentation and shape) and then a microscopic examination of wet mounts of the fungal isolates were carried out as follows; A portion of the isolated fungi was collected using sterile needle and placed on the microscope slide having two drops of lacto phenol cotton blue could penetrate into the cells of the fungi. Later the slide was covered with slips and viewed under x40 magnification.

RESULTS

Number of colonies formed

Colonies formed from every sample were counted and results were represented in Table 1.

Table 1: Number of colonies formed in samples stored at temperature of 35ºC-38ºC and 25ºC-28ºC

Wheat flour samples stored under different temperature	Number of colonies formed
35ºC-38ºC:	
Sample 1	2
Sample 2	1
Sample 3	1
Sample 4	2
Sample 5	1
Sample 6	1
Sample 7	1
Sample 8	2
Sample 9	1
Sample 10	1
25ºC-28ºC:	
Sample 1	1
Sample 2	3
Sample 3	1
Sample 4	1
Sample 5	3
Sample 6	1
Sample 7	1
Sample 8	2
Sample 9	1
Sample 10	1

As shown in Table 1 above sample 1,4 and sample 8 had the highest number colonies from all sample stored in the refrigerator at temperature of 35ºC-38ºC. From samples stored at normal room temperature of 25ºC-28ºC sample2, and sample 5 had the highest number of colonies.

Suspected Fungal Species Isolated

The fungi grown were stained on a slide using lacto phenol cotton blue stain and were viewed under the microscope. The suspected fungal species identified based on their morphological characteristics were recorded in table 2 below:-

Table 4: Suspected fungi species and their macroscopic and microscopic characteristics

Fugal species	Macroscopic features	Microscopy features
Aspergillus flavus	Colour of colony formed was green forming a wrinkled pattern.	Have conidial heads, thick walled and vesicle bearing.
Aspergillus niger	Colour of colony formed was dark brown.	Have filament consist of conidiophores and spores.
Aspergillus candidus	White cottony colony was formed.	Have conidial heads, thin walled white spores.

Suspected Bacterial Species Isolated

A gram stain was carried out for the bacteria grown then biochemical test (catalase, oxidase, citrate, amylase) was carried out to identify the specie of organism. The suspected bacterial species identified based on their gram reaction and biochemical test were recorded in Table 3 below.

Table 3: Suspected bacteria species and their gram reaction and biochemical test

Bacteria species	Gram stain	Catalase	Oxidase	Citrate	Amylase
Escherichia coli	−ve bacilli	+ve	−ve	−ve	−ve
Bacillus cereus	+ve bacilli	+ve	+ve	+ve	+ve

DISCUSSION

As shown in Table 1 the samples stored in the refrigerator at temperature of 35ºC-38ºC had the lowest number of colonies. Wheat flour when stored in an air tight container in the refrigerator tends to have a longer shelf life of one year, and lesser microbial activity which makes it less contaminated. The samples stored under room temperature of 25ºC-28ºC had the highest number of colonies, wheat flour when stored at room temperature have a short shelf life of 6 months, which makes it easy for microbial activity and contamination, which when consumed can cause food borne diseases. The results in Table 2 show the suspected list of fungal species that were indentified by their macroscopic and microscopic characteristics, however the suspected fungi species namely *Aspergillus niger* causes black mold in wheat flour, *Aspergillus candidus* when consumed causes respiratory diseases. The suspected bacterial species that were indentified in Table 3 based on their gram stain and biochemical test namely *Escherichia coli* and *Bacillus cereus* when consumed in products made with wheat flour when consumed causes gastrointestinal illness.

CONCLUSION

The samples used in this work were stored at different temperature level of 35ºC-38ºC in the refrigerator and 25ºC-28ºC at room temperature for a period of 30 days which indicates that the microbial contamination of wheat flour during storage varies in the temperature level.

RECOMMENDATIONS

Long storage of wheat flour should be done in an air tight container and kept in the refrigerator.

1. Wheat flour when stored at normal room temperature should be consumed within the period of 6 months to avoid too much microbial activity that causes food borne illness when consumed.

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