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Microbiological status of fine wheat bran collected from different mills and bakeries at Sohag government, Egypt

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Abstract: This study is designed to investigate the microbial community of twenty randomly selected fine wheat bran samples collected from different mills and bakeries at Sohag Government, Egypt. Investigated microbial groups include fungi, yeast and actinobacteria. The moisture content of the tested samples were fluctuated between 6 – 18% and the moisture values of great majority (75%) of samples tested were less than 13% and fluctuated between 10 – 12 %. The pH values were near neutral, slightly acidic or slightly alkalin. The great majority values of samples (95%) were near neutral and fluctuated between 6.5 - 6.9. The organic matter values of samples examined were fluctuated between 13 – 54% and the great majority (60%) of samples tested were less than 28% and fluctuated between 13 – 27%. In this study**,** 30 genera, 75 species and 2 species-varieties of microbiota were isolated from the tested samples on four different media. From Actinobacteria (1 genus and 6 species), fungi (21 genera, 55 species + 2 species varieties) and from yeast (8 genera and 14 species). Most of the recorded microes were isolated for the first time from fine wheat bran in Egypt.
Keywords: Wheat bran, Fungi, Yeast, Actinobacteria, Chemical analyses.

1. Introduction

Cereals and their products are of the most important food products [1]. Wheat (Triticum aestivum L.) is a principal food and an important crop worldwide [2]. According to the Food and Agriculture Organization of the United Nations (FAO), an estimated 794 million tons of wheat will need to meet the global demand for this crop [3].

Wheat bran consists of the layers of the wheat kernel separated during the milling process and is rich in fiber, minerals and organic matter for human consumption. However, surface-attached contaminants such as fungi, yeasts and actinobacteria are concentrated in the product bran [4].Wheat bran is primarily used as animal feed, but is also used in human cooking due to its high fiber content [5] Therefore, wheat bran use to produce specialized products, aleurone layer from one of the bran layers, which also contains two other components: the outer layer and the middle layer [6]. Typical bran produced from wheat flour milling consists of approximately 6–23% glumes, 6–30% outer and inner glumes, 33–52% aleurone, and 9–35% starchy endosperm. The bran estimated about 15–25% of the weight of the wheat grain [7].

The worldwide production of wheat bran is estimated about 150 million tons per year [8].Wheat bran major components are fiber (45% – 63%) as cellulose (6.5–9.9%); hemicellulose (20.8–33.0%); lignin (2.2–9.0%), protein (9.6 –22.0%), ash (4.0–7.0%), and fats (4.8–2.9%). As well as, phytic acid (~4.2%), polyphenols (~1.1%), vitamins (~0.04%), minerals (~3.4%), phytosterols (0.16-0.18%), and alkylresorcinols (~0.3%) represented wheat bran bioactive compounds [5, 9-11]. Wheat bran contains two types; soluble
and insoluble fiber according to its water solubility. The soluble fiber in wheat bran accounts less than 5% of the total fiber, composed of glucans and xylans [12]. Dietary fiber is defined as the edible residues of polysaccharides, lignin and other substances in plant cells that can't be hydrolyzed, enzymatically digested in the upper gastrointestinal tract. They are indigestible carbohydrates of plant origin with a heterogeneous chemical structure that resist digestive enzymes in the human intestine [13]. Wheat bran is a cheap and rich source of fiber, play an important role to improve intestinal health and the prevention of colon cancer and cardiovascular diseases [14]. In addition, fiber increases intestinal viscosity, reduces postprandial blood sugar response, has a laxative effect and reduces blood cholesterol levels [15]. The microbial contamination of cereals related to the handling, growing and processing conditions. As well as, cereals microbial contamination can occur from several sources, such as air, dust, soil, water, insects, rodents, animals, birds, humans, and the containers used for storage and transport. In addition to the environmental factors involved rainfall, drought, humidity, temperature, solar radiation, soil, wind, harvesting equipment, handling, and storage conditions are just some of the factors influence microbial contamination of cereals [16]. However, fungi and yeast are the mean source of contamination of cereals and cereal products, which lead to spoilage of cereals and their products and mycotoxins production [17, 18]. Yeasts present in many different environments such as soil, on the leaves, flowers, and fruits of plants, and they are frequently isolated from different materials with high sugar content and they can lead to the spoilage of food items [19, 20 and 21].

Decaying plant materials is an important microhabitat for several yeast species [22]. Conversely, bacterial infections considered a serious global public health concern represent

approximately 17 million deaths per year [23]. Actinobacteria are free-living, saprobic, and filamentous Gram-positive bacteria.Which are distributed throughout various environments.

The largest group of actinobacteria is ***Streptomyces***, which comprises 50% of the entire population of soil actinobacteria**.** They involved more than 500 species occurring mainly in rhizospheric soil, marine and in land water [24]. Unfortunately, there is a spare information available dealing with the mycological, actinobacteria and yeast assessment of quality of the cereal bran proper. Therefore, this study have been designed to investigate some randomly selected samples of wheat bran collected from different mills and bakeries at Sohag Government, Egypt in order to assay the overall fungal, yeast and actinobacteria contamination as a source of toxigenic and pathogenic strains.

2. Materials and methods

I- Samples collection:-

Twenty fine wheat bran samples were collected from millers and bakeries about 150 g each from different places at Sohag Governorate. Each sample was packed in two sterile polyethylene bags, transported to the Mycology laboratory in icebox, and kept at 4ºC for microbial surveys and chemical analysis.

II- Chemical analyses: -

II-1-Determination of Moisture Content (MC %)

Moisture content (MC) was determined by drying the wheat bran sample (100 g) in an oven at 105^{O}C for 24h., and the percentage (MC %) was calculated according to the equation [25]:

$$MC\ \% = \frac{W_1 - W_2 \times 100}{W_1}$$

W_1 = initial weight (100 g) of wheat bran W_2 = dry weight of wheat bran

II-2-Determination of Organic Matter Content (OM %)

The wet digestion method, developed Walkelya *et al* [26] was used to measure organic carbon. This involved oxidizing the carbon from wheat bran with an acid dichromate solution.

II-3-Determination of pH Value:

The pH-meter (EUTECH instruments PH 510/mV/ºC meter) was used for the determination of pH of wheat bran. The pH was measured potentiometrically in a suspension of 10 g wheat bran in 100 ml sterile dist. water. The electrodes were immersed in the wheat bran suspension with a ratio 1:10 to avoid the error arising through higher dilution [27]

III-Cultivation and Isolation of *microorganisms*

III-1-Media:-

These media were used for cultivation and isolation of different species of actinobacteria, yeast and fungi and they were as follows:-

III-1-1-Malt extract Glucose Peptone Yeast extract medium (MGPY) for yeast isolation:

This medium was employed Sastry et al [28] which composed of (g/L); peptone, 5.0; malt extract, 3.0; yeast extract, 3.0; dextrose, 10.0; agar, 20.0 pH 6.2± 0.2 (25°C). Medium was sterilized autoclaving at 121°C and 1.5 atmosphere for 15-20 min.

III-1-2-Starch Casein Agar:

The second medium was starch casein agar. The composition of the medium is as follows (g/L): 3.0 of yeast extract, 17.5 of casein, 1.5 of starch, 20 of agar, with a pH of 7.3 ± 0.2 [29].

III-1-3-Czapek' s-Glucose Ager Medium for fungi isolation

The third medium was Czapek's-glucose ager medium, composed (g/l) of: glucose, 10; $NaNO_3$, 2.0; KH_2PO_4, 1.0 ; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; agar, 20 and pH 6.4±0.2 [30, 31] .

III-1-4-Mannitol Soya Flour medium for actinobacteria isolation:

The medium composed (g/l); of; mannitol, 8.0; soya flour, 8.0; $CaCl_2$, 40 ml /l of 1 M (111 g/l); pH 6.2±0.2 [32].

III-1-5- Nutrient Agar medium for actinobacteria isolation:

The medium as described Harrigen et al**.** [33] was used for cultivation and purification of isolated Streptomyces species, and composed of (g/l): peptone, 5.0; meat extract, 1.0; yeast extract, 2.0; NaCl, 5.0; agar, 20.0; pH 7.2.

III-2-Dilution Plate Method

The procedure of this method was essentially as described Johnson *et al*. [34] as follows:-

1. Ten g of wheat bran sample (on a dry basis) was placed in an Erlenmeyer flask. Then, sterilized dist. water is added to the sample to a total volume of 100 ml is reached, shaken well on a shaker (120 rpm) for 15 minutes. **2**. Ten ml of the previous suspension were immediately drawn (while in motion) using sterile [35] dipper and transferred immediately into 3 known volume of sterile water blank until the desired final dilutions are reached (~10^{-1}, 10^{-3} and 10^{-5}). **3**. One ml of the desired dilution was transferred aseptically into each of five Petri dishes (9 cm) contained 12 to 15 ml of each of malt extract glucose peptone yeast extract medium (MGPY), mannitol soya flour medium (MSF), Czapek's-glucose agar medium (Cz) and starch casein agar (SCA) medium. The dishes were rotated by hand in a broad swirling motion, so that the diluted wheat bran is dispersed in the agar. After incubation at 28°C, for 5 - 7 days, the resulting *Streptomyces* colonies are counted. The average number of colonies per dish was multiplied by the dilution factor to obtain the number of colonies per g in the original wheat bran sample. Single colonies of isolated different *Streptomyces* species were re-streaked on to NA medium to ensure their purity, morphological, biochemical identification and kept for nanoparticles biosynthesis.

VI-Identification of *Streptomyces* isolates:-

VI-1-Colony morphological studies:

The separated *Streptomyces* were examined for the presence of aerial mycelium, submerged mycelium, their color, and any pigments that could diffuse. The isolated species were identified using Bergey's Manual of Determinative Bacteriology, Systematic bacteriology, and the International *Streptomyces* Project guidelines [36-39]. According to the colonial morphology, bacteria can be identified as:

Form: This refers to the appearance of the colony, such as circular filamentous, irregular or radiate, etc.

Elevation: it is the cross-sectional shape of the colony, eg, flat, elevated, low convex, convex, and umbonate.

Surface: this describes how the colony's surface looks, for example, smooth, shiny, or rough, dull (opposite of glistening), rugose (wrinkled), etc.
Opacity: can refer to different levels of visibility: for instance, there's transparent (completely clear), opaque (not letting light through), translucent (somewhat clear but blurry, similar to frosted glass), and iridescent (showing shifting colors when reflecting light), among others.
Consistency: mucoid, firm friable, membranous, butyrous, etc. **Chromogenesis:** which relates to color, includes shades like white, buff, red, purple, and various others. **Edges**: entire, ciliated, crenated, lobate, etc.

VI-2-Microscopical examination:-

Smear preparation from bacterial colony culture of isolates, which grow on Nutrient Ager (NA) with age from 12-18 hr. Use Gram's staining techniques, then examined under microscope to show the cell shape, positivity according to reaction with Gram's staining [40,41].

VI-2-1-Staining:

The isolates were subjected for Gram's and acid-fast staining [42, 43]

VI-2-1-a-Gram's Staining:

lugol's iodine (1gm iodine + 2gmKI in 300 ml H_2O) and safranin (1gm + 10ml ethanol +90 ml H_2O).
Smears of tested cultures were prepared and heat-fixed. The heat fixed films were stained with crystal violet for about one minute. Then rinse with tap water. These films covered with lugol's iodine for about 2 minutes, and then rinsed with water. The films were decolorized with 95% ethyl alcohol. Counter staining was performed with safranin about 20-30 seconds and finally these films were rinsed with water and blotted to dry [44].

VI-2-1-b-Acid Fast staining:

The isolates were l mixtusmeared and heat-fixed on clean, grease-free glass slides. Before decolorizing with an acid alcohore, Flood the slide with Carbol fuchsin. Use a Bunsen burner to steam the slide while standing over the sink. Let the slide set for 5 minutes. Rinse with water. Flood slide with Acid Alcohol for 30 seconds. Rinse with water. Methylene blue was used to counterstain the slides for 2 minutes. Water washed the slides after each reagent addition step. After blotting the slides, they were examined under a light microscope with an oil immersion objective [45].

VI- 3-Biochemical Tests:

Biochemical tests viz., Starch hydrolysis, Casein hydrolysis, Citrate test, Catalase test, vogues-proskauer test, and carbohydrate fermentation test were carried out [42, 43]

VI- 3-1-Catalase Test [46];
Medium used: Nutrient ager.
Reagent used: Hydrogen peroxide 10%
Procedure: A loopful of young tested bacterial culture (8-12hr) cultivated on nutrient agar medium was emulsified with a drop of H_2o_2 on a glass slide. The effervescence caused by liberation of free oxygen as gas bubbles, indicated the presence of catalase in the tested culture.

VI- 3-2-Starch Hydrolysis [47];
Medium used: Starch agar medium composed of peptone 5.0g: beef extract 3.0g; NaCl 5.0g; soluble starch 0.20%; agar 20g; distilled H_2O 1 L; Ph adjusted to 7.2. Sterilization carried out at 121° C for 15 min.
Reagent used: Lugol's iodine solution.
Procedure: Starch hydrolysis tested conveniently using nutrient agar supplied with 0.5% soluble starch. The starch agar plates inoculated with tested bacterial isolates, and then incubated at 37°C for 24h. After growth of tested isolates, the plates were flooded with lugols iodine solution. Areas in which starch has not been hydrolyzed were blue stained, while areas in which starch has been hydrolyzed remain colorless.

VI- 3-3-Citrate test ;
Medium used:
Method of Simmons[48], prepare the following medium (per liter of distilled water): $(NH_4)H_2PO_4$, 1.0 g; NaCl, 5.0 g; sodium citrate, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; bromothymol blue, 0.08 g; and agar, 15.0 g; pH 6.9.; distilled H_2o 1 L. Sterilization was carried out at 121oc for 15 min.
Reagent used: bromothymol blue indicator.
Procedure: The slope cultures were streaking over the surface with loopful of diluted inoculum under aseptic conditions, and then incubated at the optimum temperature of each isolate for up to 24hr. Utilization of citrate results in changing the color of bromothymol blue indicator from **green to bright blue color**.

VI- 3-4- voges-proskauer reaction [49]:
Medium used:
Voges- proskauer broth medium composed of peptone 7.0g; glucose 5.0 g; sodium chloride 5.0g; distilled H_2O one liter; pH adjusted at 6.5 sterilization was carried out at 121°C for 15 min.
Reagents:
-Sodium or potassium hydroxide solution (40%wt. / vol).
-Alpha-naphthol (5%).
-Methyl red indicator.
Procedure:
A-Acetyl methyl carbinol production:
Tubes of vogues-proskauer broth inoculated with tested bacterial isolates. After 24-48hr. of incubation, test for acetyl methyl carbinol production was performed by adding 0.2ml of 40% sodium or potassium hydroxide and 0.6 ml of 5% alpha-naphthol, shaking well and letting stand exposed to air the appearance of red to rose color in 5 to 20 minutes indicates the production of acetyl methyl carbinol.
B-Final pH produced in vogues proskauer broth:
The pH was determined as follow: few drops of methyl red as indicator were added to a portion of culture. The color of methyl red indicator becomes orange or red below pH 5.9 and yellow color at alkaline medium.

VI- 3-4-Acid and /or gas production from Carbohydrate utilization test [50]:
Medium used:
Glucose medium composed of A. Basel medium; diammonium hydrogen phosphate 1.0g/l; KCl 0.2g/l; Magnesium sulphate 0.2g/l; yeast extract 0.2g/l; distilled H2O one liter; pH adjusted at 7. Before adding 15 ml of 0.04% solution of bromocresol purple as indicator the medium was distribute in test tubes (10 ml each) then inverted Durham tube was inserted in each tube contain medium. B. Glucose solution, 10g glucose/ l. the basal medium (A) and glucose solution (B) were autoclaved separately at 121°C for min.
Procedure: Tube of glucose medium were inoculated with the tested bacterial isolated, making sure the inverted Durham tube is full with medium and incubated at 30°C. The growth and

production of **acid yellow color and gas bubbles** in Durham tube were observed.

3. Results and Discussion:

I: Chemical Analysis:-

I-A-Moisture content of different fine wheat bran samples:

The moisture content of fine wheat bran samples tested were fluctuated between 6 – 18 %. The great majority (75%) of samples tested, their moisture contents were less than 13% and fluctuated between 10 – 12 %. The lowest values of moisture content were recorded in samples number 17 only (6 %MC) and number 14 (8 %).Whilest the richest values of moisture content were (14 % & 18 %) as recorded in samples number 10 (14% MC), while 18% appeared in samples number (4 & 11) as shown in Table (1). The previous results were in full agreement with that recorded by Magan, Tančinová and Manali [51, 52, 53]. In addition, Radenkovs and Klava [54] on their research of physical-chemical characterization of industrial wheat bran from Latvia reported that moisture content varied from 10.01±0.51percentage to 11.61±0.47percentage. According to the information from fourth edition of 'Wheat chemistry and technology' [55], the moisture content increases in outer layers. The rise in bran likely happens due to water acting as a plasticizer, indicating a change in the cell walls, especially in the areas that are most hydrophobic**.** Which are rich in cutin [56]. The moisture content of most samples was below than 17%, which limit and inhibit fungal growth of wheat bran samples analyzed in this study which in full agreement with that recorded by **Magan** and **Tančinová** [51, 52].

I-B- pH values of different fine wheat bran samples:

The pH values were near and ranged between slightly acidic to slightly alkalin. The great majority values of samples (95%) were near neutral and fluctuated between 6.5 - 6.9, while only pH value of one sample (No, 4) was 7.4, which represented 5% of samples tested. The lowest value recorded in sample (11), while the maximium value recorded from sample (19), whilst, most pH values ranged between 6.6 – 6.8 in 18 samples out of 20 tested as shown in Table (1). These results were in harmony with that obtained by Radenkovs and Klava [54] who recorded that the pH value of wheat bran ranged from 6.31±0.61 to 6.80±0.05. As well as, the obtained results agreed with that obtained by different researchers in other countries that the pH value in wheat bran is approximately pH 6.90 [57].

I-C-Organic matter of different fine wheat bransamples:-

The organic matter values of wheat bran samples tested were fluctuated between 13 – 54%. The great majority (60%) of samples tested, their organic matter values were less than 28% and fluctuated between 13 – 27%. The lowest values of organic matter recorded in samples number 8 only (0.13%OM), number 2, 5, 11, 14 & 20 (0.14%) and number 1,7,10, 12, 16 &18 (0.27%). Whilst the richest values of organic matter were (0.40 % & 0.54%) and listed in samples number 4, 6, 15 & 19 (40% OM), while 0.54% OM recorded in samples number 3, 9, 13 &17 as shown in Table (1).

Table (1): Moisture contents (MC %), pH values and organic matter (OM) of different wheat bran samples collected from different mills and bakeries at different places in Sohag Governorate.

Sample No	Collection Place	MC %	pH	OM%
1	Gerga mill	12%	6.6	0.27
2	Gerga city (Bakery)	10%	6.6	0.14
3	Gerga city(Bakery)	10%	6.7	0.54
4	Gerga city(Bakery)	18%	7.4	0.40
5	Gerga city(Bakery)	12%	6.7	0.14
6	allam village(Bakery) Bayt	12 %	6.7	0.4
7	Allam village (Bakery) Bayt	10%	6.6	0.27
8	El balyana Mill	12%	6.7	0.134
9	Bardis village (Bakery)	12%	6.6	0.54
10	Sohag city (Bakery)	14%	6.6	0.27
11	Sohag city (Bakery)	18%	6.5	0.14
12	Sohag city (Bakery)	12%	6.8	0.27
13	Sohag city (Bakery)	12%	6.7	0.54
14	As Sala village (Bakery)	8%	6.7	0.14
15	Akhmim mill	12%	6.7	0.4
16	Akhmim city (Bakery)	10%	6.8	0.27
17	Al-Masaid village (Bakery)	6%	6.8	0.54
18	Al-Zouk village (Bakery)	10%	6.6	0.27
19	Al- Birba village (Bakery)	10%	6.9	0.4
20	Al-Minshah village (Bakery)	12%	6.6	0.14

II: Microbiota Recovered in the Present Investigation:-

Microorganisms play a significant role in deterioration and spoilage of commodities depending on moisture content, storage conditions and organic matter content. Food safety is a global public health threat with frequent incidents of food borne diseases [58-60]. This study designed to determine the microbiological status and the distribution of microorganisms in fine wheat bran samples collected from different mills and bakeries in Sohag governorate, Upper Egypt. In this study**,** 30 genera, 75 species & 2 species-varieties of microbiota isolated from 20 wheat bran samples on different media used. From Actinobacteria (1 genus & 6 species), fungi (21 genera, 55 species + 2 species- varieties) and from yeast (8 genera & 14 species) as stated in Table (2). Most of the preceding microorganisms were isolated for the first time from fine wheat bran in Egypt.

These results were in full agreement with that obtained by Youssef *et al*. [5]. In their study on 20 coarse wheat bran samples collected from different mills and grainers in Sohag governorate reported that forty-nine species and 3 species-varieties belonging to 15 genera were isolated and identified. Thirty-eight species and 3 species-varieties appertaining to 9 genera of fungi, while from yeast; ten species belonging to 5 genera, whilst from actinobacteria, only one species (*Streptomyces coelicolor*) was isolated and identified In addition, fungi represent the main source of contamination and spoilage of cereals and cereal products as reported by de Souza [18].

As well as, data in Table (2) revealed that malt glucose peptone yeast extract agar medium was the best favorable medium for cultivation and isolation of microbiota from 20 wheat bran samples followed by soya flour agar, Czapek's-

glucose agar and casein agar medium. As well as, the total counts of microorganisms, number of genera and species were in harmony with the previous result that 40225, 23 & 47, 27375, 20 & 47, 19100, 16 & 33 and 18825CFU/g, 11genera & 31 species on different media, respectively. Isolated fungi recorded in high frequency of occurrence (100% of the samples) on different media tested, whilst, yeast appeared in high occurrence (75%) on malt glucose peptone yeast extract agar medium, moderate (50%) on mannitol soya flour agar and low in both Czapek's-glucose agar and casein agar media.

Aspergillus and *Mucor* were the most prevalent genera in all media.

Aspergillus and *Mucor* were the most prevalent genera in all media. *Aspergillus* isolated in high frequencies of occurrence in all media used, appeared in 24800 colonies per g & 61.6% on malt glucose peptone yeast extract agar medium, 15175 colonies/g & 55.4% on mannitol soya flour agar, 14575 & 76.3% on Czapek's-glucose agar medium followed by 13150 & 69.8% on casein agar medium, respectively. *A. fumigatus, A. fumigatus* var. *ellipticus, A. flavus, A. niger* and *A. ficuum* were the most dominant species. *A. fumigatus* var. *ellipticus* recorded in high occurrence in all media except malt glucose peptone yeast extract agar medium that appeared in moderate with different counts. *A. flavus* appear in high occurrence on malt glucose peptone yeast extract agar medium, while moderate on other media used. While *A. niger* observed in high occurrence on malt glucose peptone yeast extract agar medium, whereas, moderate in both of Czapek's-glucose agar and mannitol soya flour agar media, and in rare on casein agar media. In addition to, *A. ficuum* isolated in high occurrence on Czapek's-glucose agar, whereas moderate on both of malt glucose peptone yeast extract agar medium and mannitol soya flour agar media, whilst rare occurrence on casein agar medium.

As well as *Mucor* recorded in high occurrence on three media out of four used; casein, malt glucose peptone yeast extract and mannitol soya flour agar media, whereas in moderate occurrence on Czapek's-glucose agar medium.

Eight species of microbiota recorded on casein agar medium and completely missed on the three other different media and these were; from actinobacteria: *Streptomyces fradiae and Streptomyces lydicus* and from fungi; *Aspergillus amstelodami, A. aenus, A. tubingensis, Geosmitha lavendula, Penicillium palitans* and *Talaromyces luteus*.

While, only ten species of microorganisms isolated from malt glucose peptone yeast extract agar medium and completely disappeared in the other three media used. These species were; actinobacteria: *Streptomyces albidoflavus* and from fungi; *Absidia corymbifera, Aspergillus foetidus, A. ochraceus, A. silvaticus A. sydowii, verticillium terrestre,* and from yeast; *Brettanomyces acidodurans, Candida tropical* and *Trichosporon asteroids*.

While, only six species of microbiota recorded on casein agar medium and completely missed on the three other different media and these were; from actinobacteria: *Streptomyces lydicus* and from fungi; *Aspergillus amstelodami, A. aenus, Geosmitha lavendula, Penicillium palitans* and *Talaromyces luteus*. Whereas four fungal species only appeared on Czapek's-glucose agar medium and completely disappeared on the three other media used, and these were *Aspergillus carbonarius*, *Curvularia lunata, Paecilomyces variottii* and *Penicillium oxalicum.* Whilst, seven microbiota species isolated only on soya flour agar medium and completely hidden on the other media used and these were; from Actinobacteria; *Streptomyces smyrnaeus,* from fungi; *Cunninghamella elegans*, *Penicillium chrysogenum, Penicillium viridicatum* and *Scopulariopsis brevicaulis* and from yeast; *Pichia fermentans* and *Saccharomyces uvarum* as shown in Table (2).

These results were in full harmony with that obtained by Youssef *et al*., [5] who reported that *Aspergillus, Penicillium, Saccharomyces* and *Talaromyces* were quite the most common genera contributed the broadest spectra of microbial species. *Aspergillus* was represented by 20 species in addition to three species varieties which belong to seven groups of the 18 as described by Raper and Fennell [61], Pitt [62, 63] As well as, Berghofer *et al*. [4]who investigated wheat flour and bran and reported that the most dominant fungi isolated were *Aspergillus*, *Penicillium*, *Cladosporium* and *Eurotium* spp. As well as, the genera of *Saccharomyces, Debaryomyces, Kluyveromyces, Pichia, Candida,* and *Zygosaccharomyces* are involved in spoilage in cereal and cereal products in rare case [17].

In addition, the obtained data are in full harmony with that recorded by Tančinová and Labuda [52], who reported that the most prevalent genera isolated from wheat bran samples collected from Ivanka pri Nitre mill (Slovakia) were *Penicillium (*20 spp.) followed by *Aspergillus* (10 spp.) and *Cladosporium* (3 spp.) with 100%, 89% and 72% frequencies of occurrence, respectively. As well as, Saleemi *et al.*, [64] determined mycological contamination of 67 samples of wheat and 17 samples of wheat bran collected from Faisalabad district of Pakistan. *Aspergillus* was the most common (44.77%) genus followed by *Penicillium, Fusarium* and *Alternaria.* This is the first report on microbiota associated with fine wheat bran in Egypt.

4. Conclusion

This is the first report of the different microbiota associated with fine wheat bran samples in Sohag, Upper Egypt. Wheat bran is used in bakery process, in addition to animal feeds. This study clarified that wheat bran samples tested had different spectrum of fungi, yeast and actinobacteria. The samples tested had low the moisture content less than 19% which very safe to limit and inhibit fungal growth, so, it is very well for using it in food industries such as bakery process. As well as the isolated actinobacteria especially *Streptomyces albidoflavus* will be used in future studies for silver nanoparticles synthecizing.

CRediT authorship contribution statement:

"Conceptualization, M. S. Yand Z. A. E.; methodology, Z. A .E. software, M. M. A.; validation, M. S. Y, M. M. A and Z. A. E.; formal analysis, Z.A.E.; investigation, M. S. Y and Z. A .E .; resources, M. S. Y.; data curation, M. M. A.; writing—original draft preparation, Z. A. E.; writing—review and editing, M. S. Y and M. M. A.; visualization, M .M. A.; supervision, M. S. Y .; project administration, M. S. Y.; funding acquisition, M. S. Y. All authors have read and agreed

to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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