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Microbiology of dough leavening

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I N T R O D U C T I O N

In almost all-over the world, especially in developing countries of Africa and Asia, bread is considered the chief food, if not the principal one for human-beings. About 70 % of the nutritional energy necessary for personnel consumption is taken from cereals, of which bread represents 70 % of total energy obtained daily.

In bread-making, bread yeasts and the other actively gas-forming microorganisms, play a very essential rôle. The functions of these organisms in bread-making are to leaven and lighten the dough, as well as to impart a characteristic aroma and flavour to it.

Generally, flour of which bread is done, loads a lot of organisms with an extremely heterogenous flora present. Yet, kinds and numbers of these organisms in flour and consequently in the dough vary widely depending on storage conditions, moisture content, milling procedures, handling as well as sanitary practices of processing.

To the ancient Egyptians who recognized leavening action and by 2600 B.C. practiced bread making by methods akin to those followed in modern times. Generally, bread was extensively used by the early Egyptians and there is

some evidence that White-bread was appreciated by the wealthier classes. Leavend bread was said to have been known in Egypt since 2000 B.C. In its early days, under the rule of the pharaohs, Egypt was the largest wheat-growing and bread-consuming country in the world.

This investigation aimed to study the microbiological flora of local and imported flours found in Mansoura area. Microbiological flora of the above samples, changes occurring in the resembling doughs during the fermentation period was also studied. Yeast strains found in the studied samples, were isolated, identified, then screened according to their activities in dough leavening. The effect of the most active isolated strain of Saccharomyces cerevisiae, on dough fermentation, compared with other local strain was also investigated.

Such points are worthy of study especially that bread-making is one of the important food industry in Egypt, where methods used might be considered still primitive, since most of the processes involved are carried out by hand.

REVIEW OF LITERATURE

I. The microbial flora of flours :

Wheat flour invariably has a variable population, either in counts or in the dominant groups of microorganisms, derived from the outside sources especially from the grain during its milling.

A. Microbial counts of flours :

As for counts, Kent-Jones and Amos (1930) found that constant bacterial counts of wheat and flours were not obtained until they adopted the practice of adding sterile sand to saline in which the grist or flour was shaken. The counts of bacteria obtained depended upon the medium and the temperature of incubation; the lower the temperature, the higher the figures obtained. In flours the same grist, the lower the grade of flour, the greater is the bacterial population. They also showed that the storage of flours considerably decreased the bacterial populations. The relative humidity had a greater influence on the rate of decrease than the temperature of storage. Furthermore, bacterial counts of flour increased with the increase in percentage of extraction.

Amos (1931) found that the counts of B. subtilis in flour ranged between 10 - 160 spores per gram.

Holtman (1935) observed that the presence of moisture and favourable storage temperature tended to increase the number of microorganisms in flours. Barton-Wright (1938) expressed that the relative humidity affected the bacterial numbers more than the temperature of storage did; when the flour was dampened to 18 % moisture, the bacterial population decreased at a faster rate during storage. He attributed this effect to the rapid reduction in the pH of the flour.

James and Smith (1948) found that the bacterial count varied according to the ash content of the flours, figures of 2010, 6600, 13900, 8700, and 16000 bacteria per gram were obtained where the ash content of the flours were 0.32, 0.35, 0.44, 0.53, and 0.60 % respectively. On bromocresol purple agar acid producers ranged from 39 - 1760 per gram.

Bacterial decrease in flour during storage was later confirmed by Teunisson (1954), and Hesseltine and Graves (1966).

According to Weiser (1962) apparently, moisture content will determine the number and kinds of organisms present. The bacterial content of flour may range from 20,000 to 5,000,000 per gram with an extremely heterogeneous flora present; this

may include secondary invaders as well as the epiphytic flora of grain.

Frazier (1967) stated that numbers of bacteria in flour vary widely from a few hundred per gram to million. Most samples of white wheat flour from the retail market contain a few hundred to a few thousand bacteria per gram, and average about twenty to thirty bacillus spores per gram. He also stated that patent flours usually give lower count than straight or clear, and numbers decrease with storage of the flour. Higher counts usually are obtained on prepared flours, and still higher (8,000 to 12,000/g. on the average) on graham and whole-wheat flours which contain also the outer parts of the wheat kernel and are not bleached. So corn meal often contains from 5,000 to 70,000 bacteria/g. Malts, because of incubation in a moist condition, contain high numbers of bacteria, usually in the million/g. He mentioned that white wheat flour, however, usually is bleached by an oxidizing agent, such as oxide of nitrogen, chlorine, nitrosyl chloride, or benzoyl peroxide, and this process serves to reduce microbial numbers and kinds.

Morad (1968) stated that the main total counts of microorganisms in flour ranged between 40,000 and 695,000

cells/g. He also stated that the total counts of bacteria in flour varied from 12,500 to 405,000 cells/g.

Awad, Yvonne (1969) in her study on Balady bread-making at Cairo, stated that flours of higher extraction generally yield higher microbial contents than flours of lower extraction. The latter type is imported to this country and seems to be produced under more strict supervision than the other type as indicated by its lower moisture content. Naturally such low moisture content would depress microbial activity and thus, the characteristics of flour will remain in good condition. Moreover, the exclusion of higher percentages of bran and middling during milling will aid in reducing the microbial content. She attributed the significant differences recorded between samples to many factors; chief among them would be moisture content, relative humidity and temperature of storage, age of flour, and the type of wheat used. The increase of temperature in summer would explain the higher microbial population detected in these months than during winter.

With respect to yeasts in flour, Nikolayev (1935) stated that the average yeasts represented 1/30 of the total bacteria present in wheat.

James and Smith (1948) found that the counts of yeast on Czabek's medium incubated at 25°C ranged from 12 to 268/g of flour.

Morad (1968) found that yeasts occurred in lower numbers in the samples of flours (12,000 - 140,000 cells/g.).

Among the moulds in flour, studies of Soenen and Pingualr (1937) and Barton-Wright (1938) on the fungus content of flours indicated that the counts ranged between 125 - 19,982. Whereas Morad (1968) found that mould counts in flour were higher (6,000 - 100,000 cells/g.).

Frazier (1967) stated that wheat-flour samples contain fifty to one hundred mould spores per gram, so corn-meal often contains from 1,000 to 400,000 moulds. He also mentioned that a moisture content of flour of less than 13 percent has been reported to prevent the growth of all microorganisms. Other workers claim that 15 % permits good mould growth and over 17 % the growth of both moulds and bacteria. Therefore, slight moistening of white-flour brings about spoilage by moulds.

According to Seed et al., (1969) moulds can grow on flour with 16 % water content after a storage time of

more than 20 days, so that mycotoxins can penetrate into foods. At a water content of 14 % such a danger not exist.

B. The dominant microorganisms in flours and their effect on the baked-product quality :

The bacteriological studies on flour of Kent-Jones and Amos (1930) showed that Bacillus subtilis was found in all samples, other species of the bacillus group detected were B. mesentericus vulgaris, B. mesentericus ruber and B. mesentericus fuscus. The micrococcal group was dominating in most of the samples. A strain similar to, if not identical with M. ureae liquefaciens was invariably present at 37°C. Flavobacterium and Achromobacter, members of the epiphytic microflora, were always present in small numbers. A coccobacillus organism was introduced in most of the samples. E. coli (E. coli) was always present in flours and doughs but its density did not follow the total bacterial counts, i.e., some samples of high counts had lower E. coli counts than others of low total counts.

The findings of Kent-Jones and Amos (1930) were further confirmed by Amos (1931). He described the physiological characters of the microorganisms previously isolated and found, in addition, that S. faecalis was detected in many of the flour samples.

Nikolaev (1935) isolated, of 28 samples of wheat, three strains of Lactobacillus panis acidii, and Streptococcus lactis acidii (Bacterium lactis acidii) and a similar organism but which cannot coagulate milk.

Amos (1942) and Kent-Jones and Amos (1957) isolated from wheat flour, B. perfringens (Cl. perfringens), B. coli (E. coli), B. subtilis, several strains of Bacillus mesentericus and three unknown species which they designated as Nos. 3, 4 and 11. They identified these unknown organisms as belonging to the genera Micrococcus, Flavobacterium and Achromobacter respectively. The numbers of B. coli and B. mesentericus were small in normal wheat flour, but the occurrence of B. subtilis was common. Moreover, strains of B. mesentericus and B. subtilis were present in most wheat flour. The microorganism No. 3 (Micrococci) constituted the majority of the organisms present in flour while No. 4 (Flavobacterium) and No. 11 (Achromobacter) were usually present in smaller numbers than Micrococcus spp.

James and Smith (1948) found that flour harboured mesophilic acid-producing bacteria, thermophilic flat-sour spores, aerobic thermophilic spores, spores of rope-producing bacteria and yeasts.