PRESERVATION AND MAINTENANCE OF IMPORTANT FUNGI RELATED TO FOOD PRODUCTS

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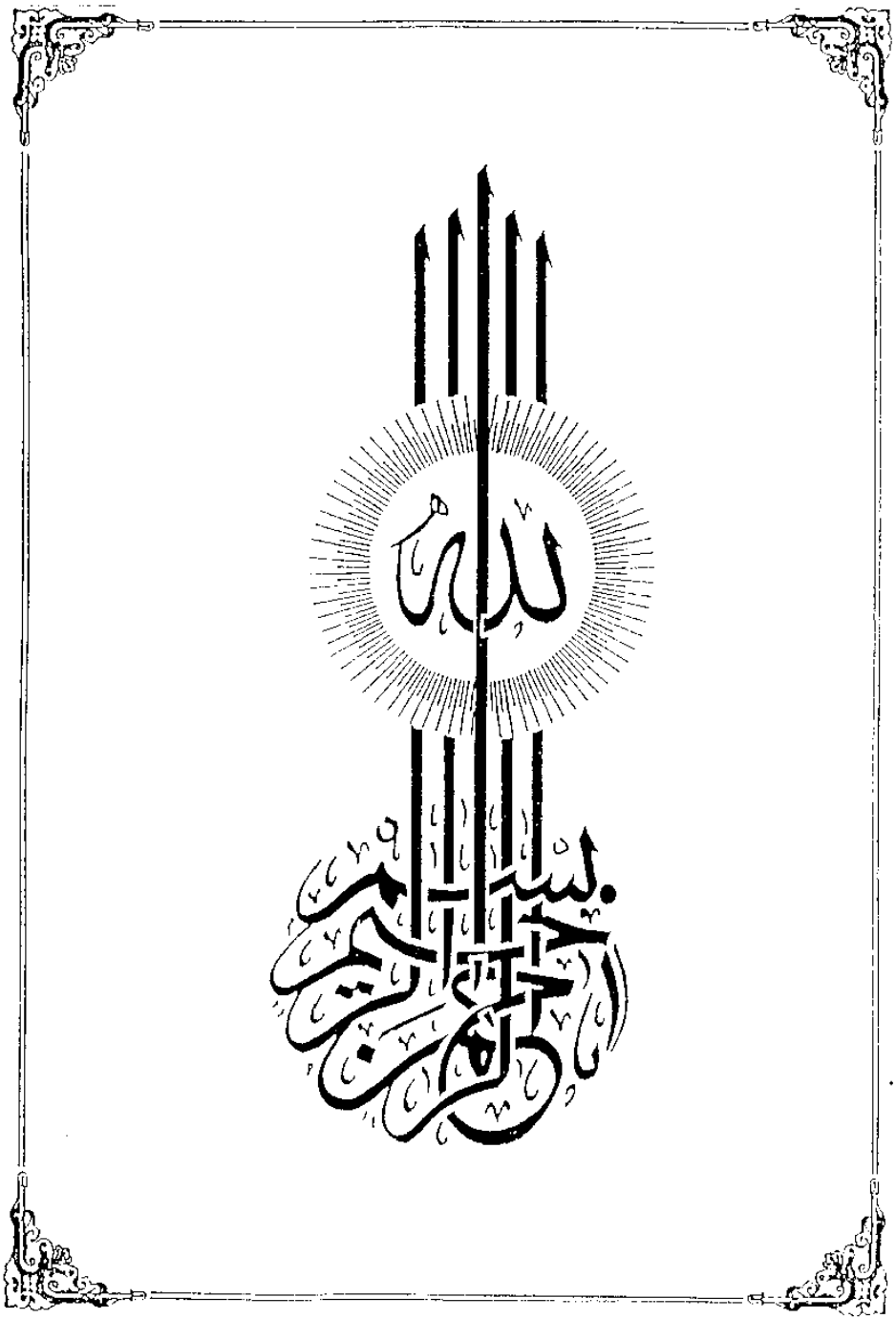

C O N T E N T S

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INTRODUCTION

Fungi have been involved in the production of a wide variety of foods and beverages for several centuries. Commercial interest in some fermented products has catalysed research in those products with the result that the major fermented food products and alcoholic beverages have received most attention. Most of the major manufacturers of these products have highly refined the methods used and produce products with their own particular aromas and flavours.

However, burgeoning local populations, the tourist industry itself and migration of people to other countries have broadened world interest in formerly indigenous products and consequently the demand for these products increased. Small scale production can no longer satisfy demand and emphasis is now turning to larger scale production and increase in efficiency in modes of manufacture of these products. With this comes the demand for more knowledge of the specific microorganisms or group of microorganisms which are involved in the production of these foods, methods for improving the strains used and a more detailed knowledge of the biochemical process.

Foremost among areas in developing countries

where intensive research is being conducted at present into indigenous foods is south East Asia and North Africa, in both of which the United Nations agencies (UNEP and UNESCO) have established Microbiological Research Centers (Mircen) to act as centralised bases for the collection, testing and distribution of selected cultures of microorganisms Mere deposit of such strains is not enough. Unlike most plants and animals microorganisms are subject to rapid and natural mutational changes which may affect the specific property for which it was selected. Consequently such centers are continually engaged in studies of means of preservation of strains and periodic checking of properties.

In these studies we have tried to found a simple and suitable method to preserve some fungal strains which are important in food industries. The studied methods are drying in gelatin discs and preservation in sterile distilled water in comparison with lyophilization. There is another aim in this study which is trial to produce one of the indigenous Asian remented food which is Tempeh. The Tempeh was prepared from fermented soybean and fermented broad bean and was compared with Taameia.

2. REVIEW OF LITERATURE

2.1. Preservation of Microorganisms:

There are many methods for the preservation of microorganisms. We will attempt to summarize information on the various methods used to preserve microorganisms. There are many factors to be considered and there is no single method applicable to all situations.

2.1.1. Direct transfer or periodic subculture:

Perlman and Kikuchi (1977) stated that maintenance of cultures by infrequent transfers on agar slants and storage of these slants either in freezers or under oil has been practiced for many years. Hekly (1978) reported that agar slants are the most common method for maintaining the working inoculum, but stab or broth cultures are preferred by some workers, particularly for anaerobic organisms. Martin (1964) reported that the storage of conventional agar slants or stab cultures at refrigerator temperatures (Ca 5°C) has long been used as means of reducing the metabolic activity of cultures and thereby increasing their longevity. However, on theoretical grounds, this

is the least satisfactory method of preservation because the cultures are in a growing state and thus subject to degeneration and variation.

Labage and Redway(1974)mentioned that cultures of many bacteria inoculated by stab into meat extract agar and sealed with wax corks will survive for many years in a refrigerator at (4-6°C).

Boesewinkel(1976)reported that the serial transfer of cultures is laborious and subjects them to contamination and change of characteristics.

Investigation by Atkin,et al.(1949)and Reusser (1963) showed that subculturing of brewing yeasts at regular intervals, usually less than 4 months, is necessary and with repeated serial subculture, genetic variants occur.

Lapage and Redway (1974) reported that the frequency of subculture can be reduced by keeping the organism in a minimal of nutrients.

Conder (1972) mentioned that the storage of cultures on agar slants has the advantages that no special equipment is needed, that it is simple to operate and that cultures are at all times available for immediate use or subculture. On the other hand the dis-

advantages of the method include the relatively limited period for which cultures of many fungi remain viable, the tendency of high yielding strains of industrially important fungi to loss their productive capacity on repeated subculture and the liability of cultures particularly when stored at ambient temperature to be attacked by mites.

Lapage and Redway (1974) mentioned that the disadvantages of this method include also mislabelling contamination and inoculation with wrong organism.

It is suggested by Dade (1960) as a rough guide that cultures should be transferred to fresh medium at intervals of about 6 months.

Novikova and Lebed (1980) studied the efficiency of storage of 300 fungal cultures on agar salnt with repeated passages. They showed that the repeated passages for a prolonged period of time observed in some cultures lower levels of sporulation, sclerotia formation and pigmentation.

2.1.2. Storage of culture under mineral oil:

Storage of cultures under oil was first extensively used by Bull and Weston (1947). Hesseltine, et al. (1960) reported excellent revivals after long

periods of storage. Onions (1971) observed that only a loss of 45 strains out of 2000 stored over ten years.

Onions (1971) mentioned that the oil used should be of good quality; British Pharmacepoeia medicinal paraffin oil of specific gravity 0.865-0.890 is quite satisfactory.

Fennell (1960) insisted that the oil must be autoclaved at 15 lb/in^2 for 2 h and dried in the oven at 170°C for 1-2 h.

Dade (1960) suggested that when using 1 oz Universal bottles for oil covered slopes it is advisable to use caps from which the rubber wads have been removed to prevent the oil becoming contaminated by materials disolved from the rubber.

Heckly (1978) reported that some cultures that did not survive lyophilization well were maintained under oil in the Agricultural Research Service (ARS) collection.

Perrin (1979) reported that contaminating fungi and mites are discouraged from entering the cultures by the oil covering.

Considerable loss of viability after 15 years of storage has been reported by Braverman and Grosier, 1966; and Gutter and Barkai-Golan, 1967).

Alves (1963) mentioned that after 7 years of storage, many strains of polypore cultures have failed to survive.

Onions (1971) reported that this method of preservation is cheap, easy, requires no special apparatus or skills and the oil cultures can be stored at room temperature or in a refrigerator.

Martin (1964) mentioned that this technique had the advantage of greatly lengthening the transfer interval, where, over-layering with oil prevented dehydration and slowed down metabolic activity by reducing oxygen availability.

Heckly (1978) reported that the greatest disadvantage of using oil-covered slants was that it is a messy method.

Perlman and Kikuchi (1977) mentioned that a 4-years study showed best results with the oil treated agar slants stored at 4.5°C in a comparison of storage of amylase producing fungi under oil or by lyophilization.

Lapage and Redway (1974) reported that the disadvantages were the inconvenience of liquid paraffin, the dangers of contamination if only single tubes of each strain were maintained, and the storage space required if multiple tubes of each strain were kept.

2.1.3. Storage in sterile distilled water:

Investigation by Castellani (1939; 1967) showed that maintenance of several cultures of human pathogenic fungi and yeasts in sterile distilled water for 12 months without any apparent changes in their morphology or physiology.

Hejtmankova-Uhrova (1969)reported successful maintenance of 73 strains of fungi belonging to 13 genera in sterile distilled water for 12 months.

A slightly modified version of Castellani's method, wherein physiological salt-solution was substituted for distilled water and screw-capped bottles were used in place by Benedek (1962).

Boesewinkel (1976) found that 650 plant pathogenic and saprophytic fungi, obtained during plant disease diagnostic work, were successfully stored in water at room temperature for periods up to seven

years without loss of viability or change of morphological features.

Work by Ellis (1979) showed that water storage of nonsporulating strains appears to be an acceptable means of preservation in many instances and also can be a good reserve procedure to supplement a monthly or semiannual transfer routine.

McGinnis et al. (1974) mentioned that four hundred and seventeen isolates of 147 species belonging to 56 genera of filamentous fungi, yeasts, and aerobic actinomycetes were maintained in sterile distilled water at room temperature over periods ranging from 12 to 60 months in four independent experiments. Of the cultures, 389 (93%) survived storage in sterile distilled water.

Heckly (1978) found that both Saccharomyces cerevisiae and Sarcina lutea survived well when suspended in dilute phosphate buffer at 4°C for 4 months, and even after 1 years 2-19% survived.

Strange et al. (1961) indicated that coliform organisms have not survived in distilled water at room temperature, while Berger (1970) reported the successful storage of Pseudomonas solanacearum under the