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Soy Flour as Alternative Culture Media for Yeasts

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Abstract- The high cost of readymade media like Potato Dextrose Agar, Nutrient agar, Peptone yeast Extract agar and media alike has deprived the use of these in laboratories with low facilities. Legume seeds and products have been found to be a very good protein source. The present study deals with the feasibility of using soy flour as an alternative culture media to grow yeasts. Soy flour has several functional properties other than its high protein content which has been reported as 50%. As the starch content is very low it has a higher dissolving property and it solidifies easily due to its gelling ability. Therefore soy flour can serve as a good nutrient source as well as to replace agar to some extent due to its solidifying property. It was found in this study that soy flour had shown to be a simple, cheap source which can replace peptone in the conventional medium.

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Soy Flour as Alternative Culture Media for Yeasts

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Abstract- The high cost of readymade media like Potato Dextrose Agar, Nutrient agar, Peptone yeast Extract agar and media alike has deprived the use of these in laboratories with low facilities. Legume seeds and products have been found to be a very good protein source. The present study deals with the feasibility of using soy flour as an alternative culture media to grow yeasts. Soy flour has several functional properties other than its high protein content which has been reported as 50%. As the starch content is very low it has a higher dissolving property and it solidifies easily due to its gelling ability. Therefore soy flour can serve as a good nutrient source as well as to replace agar to some extent due to its solidifying property. It was found in this study that soy flour had shown to be a simple, cheap source which can replace peptone in the conventional medium.

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I. Introduction

The preservation and maintenance of stock cultures of yeast is important in laboratories to use in various studies involving identification of yeast strains and protein expression studies etc. A recent study done by Arunava et.al (2014) showed a cost effective technique to maintain stock cultures of yeast in laboratories with less facilities. The pure cultures can be prepared from the stock cultures.

Subculturing in media is necessary to test the viability and structural properties. The use of legume seeds as alternative culture media for fungi and bacteria has been studied (Arulanantham et.al, 2012 and Ravimannan et.al., 2014). Antony et.al. (2014) reported the use of soychunk extract agar as a bacteriological medium. Ojokoh and Ekundayo (2005) used sweet potato agar as a medium to culture yeasts. Peptone yeast extract agar (PYEA) has been used extensively for growing yeasts on regular basis for streak plate and spread plate experiments. This is a commercially available medium which is very costly. The present study is aimed at replacing the nutrient source peptone in the conventional media by soy flour. The medium is a combination of soy flour with a little amount of agar or Soy flour-Agar medium (SA). Recent research papers have been focused on the possibility of using natural plant materials as alternatives to conventional media because of their exorbitant price. Soy flour is a finely ground product processed from full-fat cotyledons or defatted flakes of soybeans. Soybeans and soybean products have been the chief source of protein for millions of people in the Orient (Waggle and Kolar, 1979). Whole soybeans are an excellent source of protein (Nelson et.al., 1978). About 40% of dry matter in soybeans is protein so the quantity is high. Soybean is a profitable crop, grown commercially for human consumption. At present soybean is one of the five major legumes cultivated in Sri Lanka the others being cowpea, mungbean, black gram and groundnut. Soy bean protein is considered as a protein of high quality as it supplies most of the essential amino acids required by the human body. The soybean breeders say that it produces the highest yield of protein per unit area of land. It has been found to be the richest, cheapest and easiest form of protein for a very long time. The protein contents in products made from soybean vary widely due to the processing conditions. Soy flour and TVP (Textured Vegetable Protein) which is a processed product from soy flour has been used as some of the protein sources to formulate alternative culture media to grow bacteria and fungi (Uthayasooriyan et.al., 2016).

II. Materials and Methods

a) Collection of samples

Soybean seeds were purchased from the sales centre of the Soybean Research Institute, Gannoruwa, Central Province, Sri Lanka.

b) Solid media preparation

The soybean seeds were finely powdered using electric blender and sieved. The powder was stored in sterile containers until its use. 3g of soy flour was taken and mixed with 0.5-2.0g agar (HIMEDIA). The solidification times of each media preparation with different amounts of agar were recorded. Finally 1g of agar was added (as the solidification time was more or less equal to that of peptone yeast extract agar – HIMEDIA) and dissolved in 100ml distilled water with soy flour. The pH of the media was adjusted to 6.8. The standard medium PYEA was prepared by dissolving 3g in 100ml of distilled water.

c) Estimation of composition of soy flour

Moisture content was determined using the procedure in AOAC (2000). Crude protein of soy flour was determined using micro-kjeldahl method (AOAC, 2000). Crude fat, crude fiber and ash contents were determined using the standard methods available in AOAC (2000). Carbohydrate content was calculated by

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getting the difference of the contents of the crude fat, fiber and ash.

d) Inoculation of microbial cultures

The yeast cultures (*Saccharomyces* sp and *Schizosaccharomyces* sp) were collected from the microbial culture collection in the Department of Botany, University of Jaffna. Standardized cultures were made by streaking on slants and maintained in the refrigerator. Aqueous suspensions of yeast cells were prepared from pure cultures of the yeast strains. Serial dilutions were prepared with sterile saline water. 0.1ml aliquots of the suitable dilutions were dispensed into triplicate plates. The prepared SA and PYEA were poured on the plates by pour plate technique. The yeast cultures introduced on PYEA served as control. All the plates were incubated at ambient temperature for 5-6 days. After incubation the plates were observed and viable counts were recorded in both media.

III. Results and Discussion

The proximate composition of soy flour is given below.

Constituents	%
Protein (Nx6.25)	50
Fat	6
Fiber	3.5
Ash	6.5
Carbohydrate	32.3
Moisture	8

a) Solidification time

Different solidification times were obtained when 3g of soy flour was mixed with 0.5-2.0g agar which is shown in Table I

Table I: Solidification times of media preparations

Weight of soy flour (g)	Weight of agar (g)	Solidification time (mins)
3	0.5	40
3	1.0	30
3	1.5	22
3	2.0	19
3 (PYEA)	-	30

1g of agar was added to soy flour (3g) as the solidification time was equal to that of PYEA.

This experiment showed results comparable to conventional PYEA and even more. Fig 1 and 2 show the growth curves obtained when *Saccharomyces* sp and *Schizosaccharomyces* sp were plated on different media respectively.

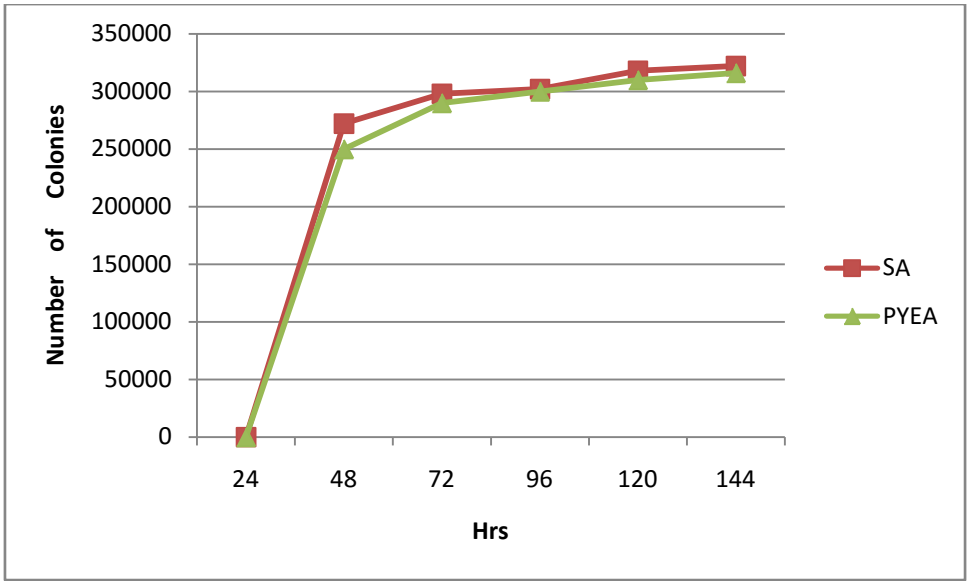

Fig. 1: Growth of *Saccharomyces* sp on SA and PYEA

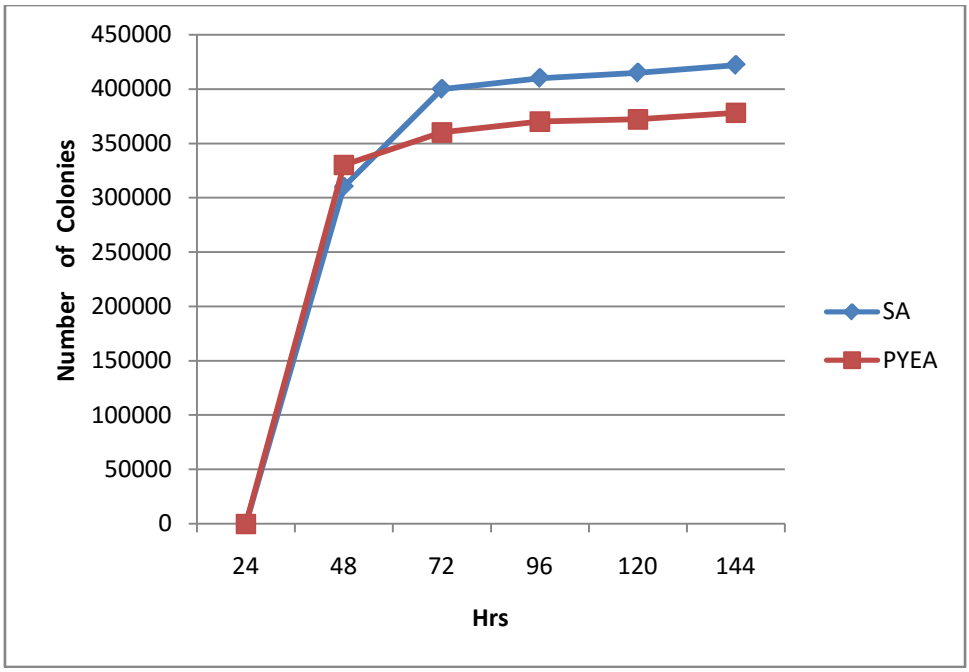

Fig. 2: Growth of *Schizosaccharomyces* sp on SA and PYEA

In both cases, growth of yeasts in terms of No of colonies was higher than the conventional medium PYEA. The medium (SA) in which soy flour was added instead of PYE was very effective on the growth of yeasts. Previous study shows that mature soybean contains little or no starch (Wilson et.al, 1978).

Generally yeasts do not require starch for their growth. But in fact sugars in the form of sucrose or glucose enhance the growth of yeasts. It is interesting to note that due to the gelling property of soy flour it easily solidifies. Further studies are required to confirm its gelling property and future uses in the preparation of media. In this study it was found that SA medium gives a transparent golden brown color which is lighter than the PYEA and the colonies could be observed clearly (Fig 3) though the colony contrast is more clear in the PYEA.

Fig. 3: Growth of *Saccharomyces* sp on SA medium

Due to this easily solidifying property of soy flour it can therefore be used to replace agar which is a common constituent in all the culture media as a solidifying agent. For e.g. In this experiment only 1g agar is used in the preparation of SA medium. The average colony diameter of *Saccharomyces* sp was

higher (2.5mm) in SA than that in PYEA (1.5mm). Similarly the average colony diameter of *Schizosaccharomyces* sp was also higher (2.0mm) in SA than that in PYEA (1.5mm). From the results, it could be seen that the growth of *Saccharomyces* sp in terms of average colony diameter was higher than that of *Schizosaccharomyces* sp on SA. From this study it can be said that soy flour has shown to be a simple efficient and cost-effective medium which can effectively replace the peptone based conventional media. Soy flour which is obtained by grinding soy beans is 50 times cheaper than peptone yeast extract agar. Also it can be stored in dried form in containers for one year. It is readily available and economically feasible which can be used to culture yeasts in laboratories with less facilities. This SA medium can be introduced to developing countries where the conventional medium cannot be purchased due to high cost.

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