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Full Length Research Paper

Mycological and Nutrient Profile of Soymilk

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The mycological profile during soymilk processing, nutrient profile and keeping quality of the milk were studied. Two moulds–*Rhizopus nigricans* and *Neurospora sitophila* were implicated as processing contaminants. The freshly prepared soymilk was rich in iron, calcium and potassium. Conversely, the milk contained very low amounts of total fermentable sugar (0.022%). Gas chromatography analysis showed consequent low alcohol content (4%). An evaluation of the effect of inorganic chemical preservatives on fungal growth and sensory parameters showed that soymilk fortified with calcium chloride significantly (*P*<0.05) enhanced sensory characteristics and exhibited a high inhibitory activity against the moulds–*R. nigricans* and *N. sitophila*, especially at 3.5g/L concentration than other salts. Therefore, calcium chloride may serve as a better inorganic chemical preservative over sodium salts in soymilk processing for large scale commercial purposes since it is capable of suppressing mould contamination at the same time enhancing organoleptic properties.

Keywords: Soymilk; Fungi; Food quality; Preservation; Sensory characteristics.

INTRODUCTION

Soybean (*Glycine max*) is a rich oilseed crop with about 18% fat and 40–48% crude protein It is particularly noted for its richness in lysine, arginine, cysteine, leucine, and methionine (Rastogi and Singh, 1989: Riaz, 1999: (Sharma., et al 1999), minerals and some vitamins like calcium, iron, vitamin A, riboflavin and other trace elements (Cronan and Atwood, 1972). It can be conveniently consumed as powder, milk or tofu (LU., et al 1980). However, some anti nutritional factors like proteinase inhibitors, alkaloids and tannins may sometime weigh down the benefits of its whole unprocessed consumption, as these substances are not well digested by monogastrics like man. Various processing techniques generally are aimed at detoxification and the removal of flatus factors so that enhanced digestibility is achieved. Soybean, an attraction for vegetarians and health enthusiasts, is considered low in saturated fats and cholesterol which may reduce the risk of heart disease and diabetes. It also alkalizes the blood.

During processing into soymilk, which is a traditional non dairy protein food (6), certain microbes are resident and find nutritional support in the matrix (Scott and Aldrich, 1970; Dobson, 1998)). To our knowledge, not much has been done on the mycological profile of soymilk processing. The keeping quality and nutrient profile of the milk, and the cultural conditions of the resident moulds were studied.

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Materials and Methods

Sample collection and processing

Soybean seeds (5kg) were collected from the University's Food Services Department, and processed into the milk by standard conventional method (Berk, 1992: No., et al 2004) with only slight modifications.

Mycological analysis

The indigenous mycoflora at each processing stage was determined by plating out 50 surface-sterilized raw seeds, 50 steeped-peeled seeds, the paste and milk on Malt Extract agar (MEA) and Potato Dextrose agar (PDA) (BDH) containing 0.4g/L of streptomycin sulphate. Surface sterilization of the raw seeds was performed in 3% NaOCl for 2 minutes prior to plating. The direct plating method and pour – plate technique were used for plating the seeds (raw and peeled), and paste and milk respectively. The inoculated plates were incubated at 35 ^{o}C for 24 to 96hrs. The fungal isolates were purified on PDA plates and identified via their macro- and micro-characters and by comparison with illustrations in mycological literature.

Assay of nutrients

The mineral elements contained in the prepared milk were assayed according to (10). Calcium and iron contents were analyzed using the Atomic Absorption Spectrophotometer (AAS) (Cecil) while the potassium content was determined by the Flame analyzer (Gallenkamp). The Q94 Gas chromatography (1996 Jones Chromatograph) was used to determine the total alcohol content; a measure of possible fermentation by indigenous fungi. The crude protein and total sugar contents were determined using the Kjedahl distiller and UV spectrophotometer at 490nm (Cecil CE3041) respectively. The pH of the milk was also determined.

Basal medium

The basal medium for mycological studies contained – NaH_2PO_4, 1.2g; $FeSO_4$, 0.8g; CaCl $_2$, 1.8g; streptomycin sulphate, 0.4g; distilled water, 1 liter (pH 6.5). Inoculation of test fungus was by means of two 5mm agar discs cut from young actively growing region of fungal mycelia on PDA.

Role of nutrient sources on fungal growth

The effect of carbon and nitrogen sources on fungal growth was determined after 6 days of incubation at 35 ^{o}C as static cultures. Sucrose, glucose and mannitol as carbon sources and isoleucine, alanine and glycine as nitrogen sources were each appropriately added as 1.2g/L to the basal medium. Two control flasks were used: control flask 1 (C1) with no sugar or amino acid treatment and control flask 2 (C2) with appropriate sugar or amino acid but not inoculated with test fungus. The mycelial dry weight of each fungus was taken by drying the mycelia mats on Whatman #1 filter paper to constant weight using oven and weighing with Ohaus Portable Advance balance. Results were recorded as means of triplicates.

Effect of metal salts on fungal growth

The following inorganic salts and their concentrations were used—sodium carbonate, sodium bicarbonate, and calcium chloride as 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5g/L. It is known that these salts are sometimes used as additives designed to regulate food taste, pH and at times spoilage (Coral., et al 1988). The test moulds were freshly maintained on PDA from where 3 mm disc each was used as inoculum on a modified water agar. The water agar was constituted by dissolving 1.5% agar (BDH) in distilled water and fortified with 7.5g sucrose. The plate was prepared by adding 2ml of each salt concentration into the 1.5% water agar as pour plate (Alvindia., et al 2004). Incubation was carried out in a transparent illuminated incubator at 35 ^{o}C for 5 days. Growth was recorded by visual observation and recorded in factors---- 1, 2, 3, 4; 1 being very slow growth, indicating very high antifungal ability and 4 indicating luxuriant growth meaning low antifungal ability. Results were reported as means of 3 replicates.

Sensory evaluation of soymilk samples

Sensory assessment was performed by adopting the Hedonic scoring scale having a 5 – scoring range; 1 as the lowest/worst and 5 as highest/best. A 6-member panel was used to evaluate odour, taste and appearance. The soymilk samples used for the sensory evaluation included the freshly prepared soymilk without any preservatives and those fortified with 3.5g/L (the best salt concentration for antifungal activity) of the individual chemical preservatives: sodium carbonate, sodium bicarbonate and calcium chloride.

Statistical analysis

The One-way ANOVA and DMRT were used for the separation of means and test for significance in this study. The sensory assessment, influence of nutrient

Table 1. Some mineral elements in freshly prepared soymilk samples

	Mean (SD) concentrations of some nutrients in soymilk					
	Calcium (ppm/ml)	Iron (ppm/ml)	Potassium (ppm/g)	Alcohol (%)	Crude Protein (%)	Total Sugar (%)
Soymilk	79.00 (0.03)	1677.5 (3.15)	15.00 (0.00)	4.00 (0.02)	7.99 (0.02)	0.022 (0.01)

SD: Standard deviation

Values are means of triplicate samples

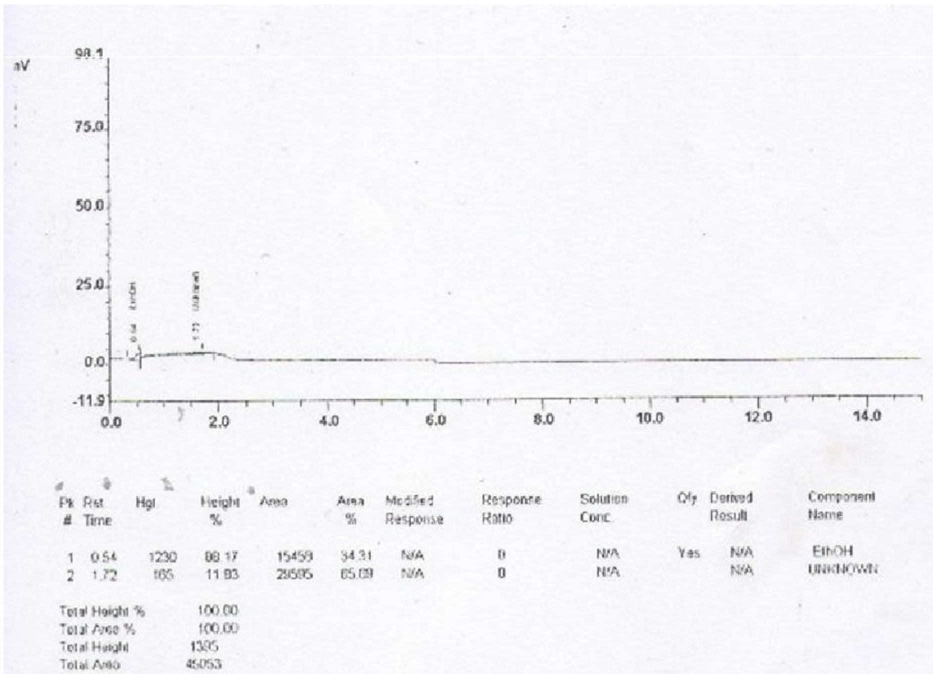

Figure 1. Gas chromatogram of soymilk sample for alcohol content

sources on fungal isolate growth and inhibition rate of salts against the moulds were all tested at $P = 0.05$ using the SPSS® 15.0 version.

RESULTS AND DISCUSSION

Two moulds—*Rhizopus nigricans* and *Neurospora sitophila* were isolated in this study. They were identified using already known parameters (Domsch., et al 1980: Koneman., et al 1992 : Alexopoulos., et al 1996). *R. nigricans* was found to colonize more of the raw seeds than the past and milk in contrast to *N. sitophila* which inhabited the paste and milk samples only.

The isolation of *Rhizopus* sp. was disturbing since many cases of zygomycoses are now living with man in diverse forms e.g. through *Rhizopus oryzae* (Artis., et al 1982), *R*. *microsporus* (Verdonck., et al 1993)) and *Mucor* sp. (Boelaert., et al 1993). In addition, this mould has been reported to liberate a toxic secondary metabolite – rhizonin A (WILSON., et al 1984). Every attempt should therefore be made to eliminate any fungal spore during processing of this milk, because once they are ingested in any form, their growth is enhanced by body fluid like serum (Verdonck., et al 1993 : (Gifford., et al 2002)).

An evaluation of the mineral element content in the freshly prepared soymilk sample showed that it is rich in iron, calcium and potassium (Table 1). Conversely, the milk contained very low amounts of total fermentable sugar (0.022%) and consequently low alcohol content (Table 1 and Figure.1), a major reason for its consumption by diabetics. This may however have accounted for the very low incidence of fungal contaminants in the milk. Although the cultural conditions for its growth were studied (Figure.2 and 3), the presence of *Neurospora* sp. is not a cause for concern. This is because certain innate attributes make it unlikely that this fungus will incite adverse effect on man. For example, it is an obligate aerobe, which cannot grow in the gut, bladder, or other tissues. It is also not a toxin producer and neither is it capable of forming ‘fungus ball’ in the lung as *Aspergillus* sp. do. Despite many opportunities of human exposure, e.g in bakeries (Yassin and Wheals,

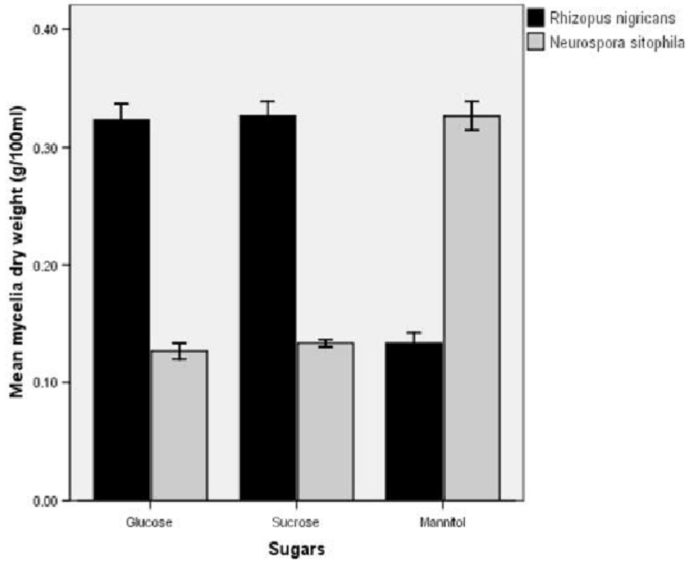

Figure 2. Mycelia growth pattern on basal medium fortified with three different sugars.

Table 2. Sensory characteristics of soymilk samples fortified with various chemical preservatives

	Mean sensory characteristics from 6-member panelist			
	Odour	**Taste**	**Appearance**	**pH**
Control soymilk	4.33±0.52[a]	4.67±0.52[a]	5.00±0.00[a]	6.75
$CaCl_2$ fortified soymilk	4.17±0.41[a]	4.50±0.55[a]	5.00±0.00[a]	6.81
Na_2CO_3 fortified soymilk	2.67±0.52[b]	2.17±0.41[b]	4.00±0.00[b]	7.40
$NaHCO_3$ fortified soymilk	2.50±0.55[b]	2.17±0.41[b]	4.00±0.00[b]	7.97

Means with different alphabet in a column indicate significant difference in $P < 0.05$

1992), sugar cane fields (Pandit and Maheshwari, 1996) and burnt fields (Perkins and Turner, 1998), no disease or infection has been linked to it. Indeed it is on record that it is been put to use as a food or in processing foods and beverages like soybean-based cake, in Java and oriental consumables like *koji* (Shurtleff and Aoyegi, 2000) and Roquefort cheese in France (perkins Davis, 2000). The uninvited presence of *Neurospora sitophila* in this work is therefore capable of enhancing the nutritive value of the milk.

In this study, three commonly used chemical preservatives in food industries were evaluated for their contribution to the enhancement of sensory parameters of the milk as well as their efficacy in inhibiting the moulds from soymilk. The soymilk sample fortified with calcium chloride had comparable odour, taste and appearance with the control soymilk (freshly prepared) while both samples showed higher significant ($P < 0.05$) values over those samples that were fortified with sodium carbonate and sodium bicarbonate (Table 2).

In addition to the enhancement of sensory characteristics, calcium chloride exhibited a high inhibitory activity against both tested moulds isolated in this study. Its antifungal activity was significantly ($P <$ 0.05) higher than the other two sodium salts especially at 3.5g/L concentration against both moulds (Figure. 3 and 4). This is similar to the reports of (Samapundo., et al 2010), which showed that calcium chloride exhibited the largest antifungal activity in a sodium chloride replacement test for chemical food preservatives including magnesium and potassium salts. Conclusively, our findings therefore suggest that calcium chloride may serve as a better chemical agent of preservation over sodium salts in soymilk processing for large scale commercial purposes since it is capable of suppressing mould contamination at the same time enhancing organoleptic properties.

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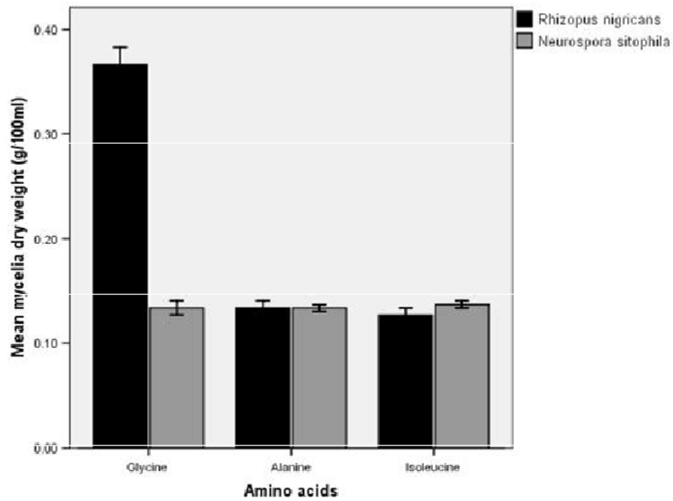

Figure 3. Mycelia growth pattern on basal medium fortified with three different nitrogen sources.

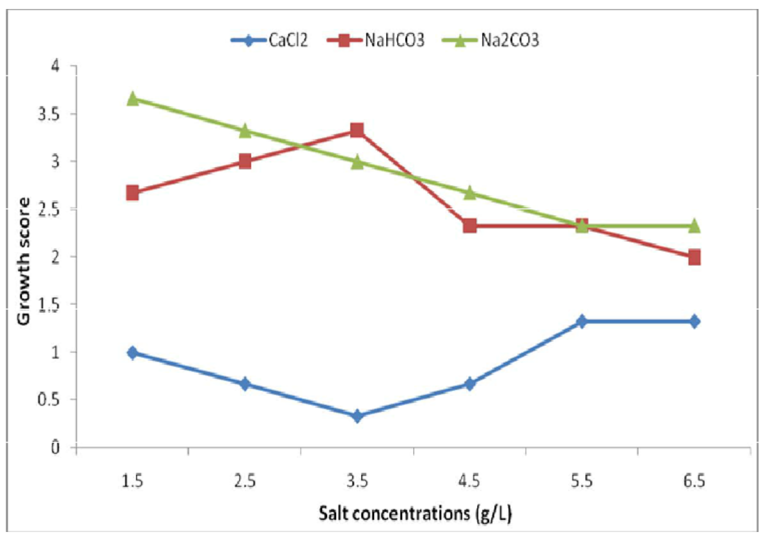

Figure 4. Inhibition pattern of different concentrations of calcium and sodium salts against *Neurospora sitophila*

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