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A comparative study on preservation of soy flour (*Glycine max.(L.) Merr.*) using sodium benzoate, a chemical preservative and *Aframomum melegueta* (grains of paradise), a natural preservative.

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Abstract

This investigation has explored the possibility of using ethanolic extract of seeds of *A. melegueta* at concentration of 1 mg/10g soy flour and sodium benzoate at a concentration of 1 mg/10g soy flour for preservation of soy flour over a period of sixty days. It has been shown that the pH of the samples treated with sodium benzoate was in the range of 6.50-6.80 whereas for samples treated with seeds of *A. melegueta* was in the range of 6.62- 6.86 over sixty days period of storage. The pH of untreated samples was in the range of 6.40-6.55 over the periods of storage. The bacterial and fungal isolates identified on 60th day of storage from the untreated samples were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. and *Candida* sp. whereas the organisms isolated from the samples treated with sodium benzoate were found to be *B. subtilis*, *B.cereus*, *Candida* sp. and *Aspergillus* sp. The organisms isolated from samples treated with extract of seeds of *A. melegueta* were *B. subtilis*, *B.cereus*, *S.aureus*, *Rhizopus* sp. and *Aspergillus* SP. The total bacterial counts for the untreated samples, samples treated with sodium benzoate and samples treated with seeds of *A. melegueta* were $9.9x10^{10}$, $7.5x10^{8}$ and $6.0x10^{8}$ respectively on 60th day of storage. The total fungal count for untreated samples, samples treated with sodium benzoate and samples treated with seeds of *A. melegueta* were $9.9x10^{10}$, $9.0x10^{6}$ and negligible. The protein content (25.1%) and the fat content (18.0%) for untreated samples were less than those of samples treated with sodium benzoate (32.0% and 19.0%) at 1.0 mg/10g soy flour and samples treated with seeds of *A. melegueta* (35.0% and 19.2%) at 1.0 mg/10 g soy flour.

Key words: soy flour, preservative, storage, protein and fat content.

Introduction

Flour is generally regarded as a microbiologically safe product and it is a low water activity commodity. Although the growth of pathogenic bacteria may not be supported under such conditions, pathogens that contaminate flour may survive for extended periods. There are few reported incidents of food poisoning resulting from contaminated flour. Australian, European and United States of America studies indicate that *Salmonella* sp., *Escherichia coli*, *Bacillus cereus* and spoilage microorganisms are present in wheat flour at low levels (Cicognani *et al.*, 1975; Ottogali and Galli, 1979; Richter *et al.*, 1993). In 1952, an outbreak of salmonallosis caused by *Salmonella paratyphi* B phage type I occurred in New South Wales, Australia where flour was implicated, but the organism was never isolated from the suspected flour (Dack, 1961).

Soybean is a good source of lecithin and it is widely used in making pastries and sauces. Soy flour has many applications worldwide namely Com-soy milk (CSM), wheat soy blend and soy sauce (Obatolu *et al.*, 1993).

There is little information on the nutritional and microbial changes of soy flour during storage and its preservation using preservatives. Many spices have been reported to possess antimicrobial properties and have been successfully used as preservatives (Shelef, 1983). *Aframomum melegueta* Roscoe (Hausa name: Chita mai koko) is a perennial herb cultivated in tropical Africa and the seeds are used as a condiment or spice. It belongs to the family of Zingeberaceae. Ginerols, shagaols and paradols have been isolated from the grains of *A. melegueta*. The crude extracts showed considerable bactericidal activities against *E. coli*, *P.aeruginosa*, *B. subtilis*, *P. vulgaris*, *K. pneumoniae* and *S. marcescens* and fungicidal activities against *C. albicans*, *T. mentagrophytes*, and *A. niger* (Oloke and Kolawale,1987). In this study, an attempt was made to preserve soy flour using extracts of seeds of *A. melegueta* and compare the preservative effect of seeds of *A. melegueta* of paradise with that of sodium benzoate, a chemical preservative.

Materials and Methods

Collection of materials

One thousand grams of soybean seeds, TGX 536-021, was collected from the Crop Production Department in the School of Agricultural Engineering, Federal University of Technology, Minna, Nigeria. The seeds of *A. melegueta* were purchased from Minna main market and were sun dried and kept in Laboratory cabinet for further use. Sodium benzoate was purchased from BDH (England).

Preparation of materials

(A) Soy flour and Sodium benzoate

Preparation of soy flour from soy seeds started with cleaning and scouring of seeds to separate and remove non-seed material. Then the seeds were washed and oven dried at 55°C for 24 hours. The dried seeds were then ground into fine powder using a milling machine as described by Konan and Agbo, 1997. After sieving, the powdered soybean was kept in a sterile container for further experiments.

(B) Extract of seeds of *A. melegueta*

Dried seeds were ground into powder using an electric blender. Then 100 g of dried powdered samples was extracted with 400 ml of ethanol in a 2 litre conical flask for 24 hours. The extract was then recovered by filtration using Whatman no. 1 filter paper. A rotary evaporator was used, in vacuo, at 40°C to concentrate the extract. The dried extract (12.1 g /100 g of grains of paradise) was used for preservation purpose.

Treatment of soy flour with sodium benzoate.

Fifty milligrams of sodium benzoate was dissolved in 5 ml of acetone (concentration 10 mg/ml) for this purpose. One milliliter of this solution was added to 100 g of soy flour (concentration 1 mg/10g) and mixed with the flour using a sterile spoon. Soy flour s

prepared was distributed in 10 polythene bags each containing 10 g and the bags containing the samples were sealed and stored at room temperature for 60 days. The samples were withdrawn at regular intervals for microbiological and biochemical analysis.

Treatment of soy flour with the seed extract

Thirty milligrams of extract of seeds of *A. melegueta* was dissolved in 3 ml of acetone to obtain a stock solution of 10-mg/ml concentrations. To 100 g of soy flour 1 ml of this solution was added to get a concentration of 1 mg/10g soybean flour and mixed with the flour vigorously using a sterile spoon. The treated soy bean flour was dispensed in 10 polythene bags each containing 10 g and were sealed with an electric sealer and kept for 60 days at room temperature. Soy flour without any extract was used as control and also kept for 60 days for comparison. The samples were withdrawn at regular intervals for analysis.

Quality assessment of treated and untreated samples

(a) Determination of pH

One gram of each of the treated and untreated samples was added to 10 ml distilled water and after vigorous shaking, the pH was measured using a pH meter (Micro pH 3310 Crison).

(b) Isolation and enumeration of fungal and bacterial isolates

Each of the samples (0.1 g) was added to 9.9 ml of distilled water and using this as a stock solution, serial dilution up to 10^{-6} were made following the procedure of Fawole and Oso (1988). An aliquot (0.1ml) of each dilution was introduced onto agar medium (for bacteria, Nutrient agar, NA and for fungi, potato dextrose agar, PDA, was used) and the plates were incubated at 28°C and 37°C for PDA and NA plates respectively. The bacterial isolates were identified using the procedure of Hudson and Sherwood (1997) and fungal isolates were identified using the procedure developed by Smith (1977).

(c) Determination of moisture, fat and crude protein content

The moisture content (% of flour) was determined using an electric protimeter grain master 2000. The fat content (%) of the treated and untreated soy flour was determined by soxhelet extraction using petroleum ether as solvent. Two grams of soy flour for each sample was used for this purpose (A.O.A.C, 1980). The total nitrogen content of the samples were determined by Kjeldahl method (Bermner,1965) and then the crude protein content of soy flour was determined by multiplying the total nitrogen content by a factor of 6.25. Two hundred fifty milligrams of soy flour was used for determination of crude protein.

Results

Quality assessment of treated and untreated samples

(a) Determination of pH

For sodium benzoate treated samples and for seeds of *A. melegueta* treated samples, the pH range was 6.50-6.80 and 6.60-6.90 respectively whereas for the untreated samples the range was 6.35 to 6.65.

(b)Identification of isolates

The predominant bacterial isolates on 0 day of storage were *B.subtilis* (40%) followed by *S. aureus* (35%) and *B.cereus* (20%). The relative distribution of bacterial isolates on 60th day of storage for control samples were *B. cereus* (70%) followed by *B. subtilis* (20%), *S. aureus* (6%) and *E. coli* (4%). For all the treated samples, the predominant bacterial isolates were *B. cereus* (80-90%) followed by *S. aureus* (5-10%) and *B. subtilis* (3-5 %). The relative distribution of fungal isolates in soy flour on 0 day of storage and 60th day of storage was *Aspergillus* sp. 20- 30%, *Penicillum* sp. 30%-32%, *Mucor* species 20-30%, *Rhizopus* sp. 10%-15% and *Candida* sp. 5%-10%. For the samples treated with sodium benzoate and seeds of *A. melegueta*, the predominant bacterial isolates were *B. cereus* followed by *S. aureus* and *B. subtilis*. The predominant fungal isolates for sodium benzoate treated samples were *Candida* sp. followed by *Aspergillus* sp.

(c) Enumeration

For control samples, the initial bacterial count was $1.1x10^{10}$ and on 60th day of storage the count was $9.9x10^{10}$. For sodium benzoate treated samples the bacterial count and the fungal count were $7.5x10^{8}$ and $9.0x10^{6}$ respectively on 60th day of storage. The bacterial count for grains of paradise treated samples was $6.0x10^{8}$. The results are shown in Table 1.

Table 1: Total number of bacterial and fungal isolates in untreated (UTS) and sodium benzoate (SB) and seeds of *A. melegueta* (AF) treated samples (mean± SEM; n=3).

Time (Day)	UTS		SB		AF	
	B	F	B	F	B	F
0	$1.1x10^8$± 0.3	$1.9x10^8$± 0.2	$1.1x10^8$± 0.1	$1.9x10^8$± 0.2	$1.1x10^8$± 0.1	$1.9x10^8$± 0.2
7	$1.4x10^8$± 0.3	$3.2x10^8$± 0.3	$9.0x10^8$± 0.1	$2.1x10^7$± 0.2	$9.5x10^8$± 0.1	$4.0x10^6$± 0.3
14	$9.0x10^{10}$± 0.3	$4.3x10^{10}$± 0.2	$8.7x10^8$± 0.3	$1.7x10^7$± 0.2	$8.3x10^8$± 0.1	NG
30	$9.0x10^{10}$± 0.2	$5.1x10^{10}$± 0.2	$7.7x10^8$± 0.2	$1.1x10^7$± 0.2	$7.5x10^8$± 0.2	NG
60	$9.9x10^{10}$± 0.2	$9.9x10^{10}$± 0.1	$7.5x10^8$± 0.2	$9.0x10^6$± 0.3	$6.0x10^8$± 0.1	NG

NG-negligible; B- bacterial count (cfu/g); F- fungal count (cfu/g)

(d) Determination of moisture, fat and crude protein content of treated and untreated samples.
The initial moisture count of the samples of soy flour was 17.6%. The moisture content of treated and untreated samples of soy flour on 7^{th}, 14^{th}, 30^{th} and 60^{th} day of storage are listed in Table 2.

Table 2: Determination of moisture content (%) and fat content (%) of treated and untreated samples (mean± SEM; n=3)..

Time (Day)	UTS		SB		AF	
	F	M	F	M	F	M
0	20.0±0.1	16.9±0.1	20.0±0.1	16.9±0.1	20.0±0.1	16.9±0.1
7	19.4±0.2	16.0±0.0	19.6±0.1	17.5±0.2	19.2±0.2	14.2±0.0
14	19.0±0.2	16.1±0.1	19.6±0.2	15.3±0.1	19.2±0.1	15.2±0.1
30	19.8±0.1	16.1±0.1	19.2±0.1	14.2±0.0	19.8±0.0	16.7±0.0
60	18.0±0.0	16.3±0.0	19.2±0.2	14.1±0.1	19.2±0.2	16.8±0.1

UTS- untreated samples; SB- sodium benzoate treated samples; AF- seeds of A. melegueta treated samples;
F- fat content (%); M-moisture content (%)

The fat content of soy flour samples on 0 day was 20.00 (%). The fat content (Table 2) for treated and untreated samples remained almost constant.

The mean reduction in crude protein (%) /day for the untreated samples was 0.34 whereas for the sodium benzoate treated samples and seeds of *A. melegueta* samples the respective values were 0.21 and 0.15. The results are shown in Table 3.

Table 3: Determination of crude protein content (%) of treated and untreated samples (mean± SEM; n=3).

Samples	* % Crude protein at different time interval (days)									
	7		14		30		60		MCP	TMRP
	CP	MRP	CP	MRP	CP	MRP	CP	MRP		
UTS	35.00±0.1	0.71	33.00±0.2	0.29	29.26± 0.0	0.23	25.10±0.2	0.12	30.59	0.34
SB	36.25±0.2	0.54	35.26±0.1	0.14	32.90±0.1	0.15	32.50±0.2	0.01	35.40	0.21
AF	36.25±0.1	0.54	36.00±0.2	0.04	36.00±0.0	0.00	35.10±0.0	0.03	36.70	0.15

*CP- crude protein content, MRP- mean crude protein content per day, MCP- mean crude protein over sixty days, TMRP- mean crude protein content per day over sixty days period of storage

Discussion

Whole soy flour used in this study contains 40% protein and 20% fat (dry weight). This agrees with the crude protein (42 %) and fat content (20 %) of the whole soy flour prepared by Bressani (1981). This study revealed that there are three types of bacteria present in soy flour namely *B. cereus, S. aureus* and *B. subtilis*. The fungi isolated on 0 day of storage were species of *Aspergillus* , *Penicillium*, *Mucor*, *Rhizopus* and *Candida*. The initial bacterial count was $1.1x10^8$ whereas the count was $9.9x10^{10}$ on 60 days of storage for control samples. Microbiological guidelines for flour have been proposed for various countries (Potus and Suchet, 1989; Richter et al., 1993). A survey by Richter et al. (1993) found US flour contained mean counts of 10^3-10^4 cfu/g, depending on wheat type. Spicher (1986) reported German flour contained mean counts of 10^4 cfu/g, coliform counts of 10^2 cfu/g and mould counts of 10^3 cfu/g. Potus and Suchet (1989) detected 10^4 cfu/g total aerobes and 10^3 cfu/g moulds in French flour. Berghofer *et al.* (2003) detected the presence of *B. cereus* in Australian wheat flour but at low levels. The initial fungal count was $1.95x10^{10}$ and on 60^{th} day of storage the count was $9.90x10^{10}$. Some workers claim that 15% or above moisture permits good fungal growth. Aflatoxin is not considered a problem in soybean storage. Hesseltine *et al.* (1966) were unable to detect aflatoxin on soybeans inoculated with *A. flavus* although Farag *et al.*(1986) detected aflatoxin in sterilized and non sterilized soybeans inoculated with *A.parasiticus*. *E.coli* was detected in untreated soy flour samples on 60th day of storage. Graves *et al.* (1967) found *E.coli* in only 1 of 16 US flour samples but Richter *et al.*(1993) reported 12.8% of US flour contained *E.coli*.

Sodium benzoate was selected to preserve soy flour because it is very effective at acidic pH and it has been used extensively as an antimicrobial agent in foods (the maximum level of benzoic acid permitted in food is 0.1%). It has been shown that the seed extract inhibited the growth of *B. theobromae*, *A. niger*, species of *Mucor*, *Rhizopus*, *Fusarium* and *A. flavus* (Oloke and Kolawole, 1987). The mean reduction in crude protein (%) for the control over 60 days of storage was 0.34 whereas for sodium benzoate it was 0.21 whereas the value was 0.15 for seed extract treated samples. For seed extract treated samples the fungal count is negligible but the bacterial count was in the order of 10^8. This value is high compared to the standard of quality flour set by Berghofer *et al.* (2003). *A. melegueta* may be a useful

preservative for soy flour though the presence of *B. cereus* is a cause of concern because this might cause food poisoning and it also can withstand heat (Blakey and Priest, 1980).

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