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Microbiology and Nutritional Quality of Stored Soya Oil

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Abstract: Soyabean cultivars 1681-3f, 1740-2F, 1448-2F and 1440-1E were obtained from the Seed Health Unit of the International Institute of Tropical Agriculture (IITA) Ibadan Oyo State, Nigeria. The microbiology and nutritional qualities of the extracted stored soyaoil at 29±2°C in January, 2006 and at 23±2°C in June, 2006 were investigated. Seven microorganisms were isolated from the soyaoil samples when stored for 4 weeks, at which time the oil samples had gone rancid with offensive odour. The bacterial isolates include *Bacillus subtilis* and *Staphylococcus aureus*, while the fungi isolated were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium poae* and *Rhizepus oryzae*. The total microbial viable counts decreased in the order of 1681-3F soya oil >1440-1E soy oil>1740-2F soy oil>1448-2f soya oil > refined soya oil, respectively. Physicochemical analysis of the soya oil revealed significant increases ($p = 0.05$) in the percentage of free fatty acid and peroxide value both in January and June 2006. The iodine value also increased significantly ($p = 0.05$) in January, but decreased in June. On the other hand, the moisture content increased significantly ($p = 0.05$) in the month of June but decrease in January. Soya oil samples are therefore, better stored in the month of January than June. Aflatoxin was not detected in any of the oil samples, despite the isolation of *A. falvus*.

Key words: Microbiology, nutritional, soya oil, bacterial, microorganisms, peroxide

INTRODUCTION

Soyabean (*Glycine max* (L.) Merril), an important oil seed, is a native of China as far back as 2800Bc in which it is mentioned as one of the principal and sacred crops (Blackman *et al*., 1992). It occupies a premier position as a world crop due to its high and virtually unrivalled protein content and because it is a rich source of edible CHO, vitamins and minerals. Commercial varieties are usually spherical and yellow, although black, green and brown soyabean exists. Nigeria and simbabwe are the leading producers of soyabean in Africa (Omikunle, 1999 and Lang, 2006). Soyabean was introduced into Nigeria in 1908 with limited production within a localized area of the country (Wudiri, 1989). It is cultivated in regions with hot,damp summer weather and an evenly distributed rainfall during the growing period (Lang, 2006). It is successfully planted in Benue, Kaduna, Niger, Federal capital territory, Jos and derived savanna of Oyo state (Root *et al*., 1987). Soyabean provides the most inexpensive source of high quality protein and edible oil. It contains about 40% protein and 20% oil (Liu, 1997 and Lang, 2006). It is probably the most important legume in the world. The leaves are used for hay, silage and fodder and green manure. The unripe seeds are cooked and eaten as vegetable. The dry seeds may be eaten when sprouted, fermented, boiled whole or split or may be used to make soyamilk or soyacheese (Susan and Anne, 1988; Liu, 1997). The consumption of soya oil in various facet of life represents about 72% of all the uses. It has also been known to be of great significance in other industrial fields whereby it could be used in medicinal purpose for its vitamin content as well as its other valuable elemental properties (Douglas *et al*., 1987 and Symolon *et al*., 2004). Hence, the objectives of this study are to isolate and characterize microorganisms associated with the spoilage of stored Soya oil evaluate the effect of the associated microflora on the shelf-life of the oil and study the effect of storage during dry and wet seasons on the nutritional and microbial quality of the extracted oil.

MATERIALS AND METHODS

Collection of sample: Clean healthy-looking seeds of soyabean cultivars 1681-3F, 1748-2F, 1448-2F and 1440-1E were obtained from the Seed Health Unit of the International Institute of Tropical agriculture (IITA), Ibadan in December 2005.

Biochemical analysis: The soybeans were cleaned, dried and surface sterilized with 70% ethanol, rinsed several times with sterile distilled water and then flaked using a

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household warring blender. Oil was extracted from 50 g soybean flake for 7 h with n-hexane in a soxhlet extractor using standard methods of A.O.A.C (1990). The solvent was evaporated by heating at 40°C leaving only the oil in the flask, which was then transferred into sterile specimen bottles. The five oil samples were stored for four weeks in a cupboard. The first storage experiment was done in January 2006 (dry season) and the second in June 2006 (wet season). The microbial load of the Soya oil was determined weekly. Moulds on the soy oils were characterized as described by Barnett and Hunter and Nelson while the Bacteria were characterized and identified according to Cowan and Steel (1985). Physico-chemical parameters of the soy oils were also determined using standard methods of A.O.A.C. (1990). The oils were also screened for aflatoxin using standard methods of A.O.A.C. (1975) of thin layer chromatography plates. The data obtained were suspected to statistical analysis f variance and means were separated using multiple range test.

RESULTS AND DISCUSSION

The results of the microbial total viable counts and spore counts are shown in Table 1 and 2. The refined oil had a lower microbial load and significantly different ($p \leq 0.05$) from the crude soy oil cultivars. This may be because of environmental microbes in the research laboratory where the extraction was carried out. Among the four cultivars, 1681-3F had the highest microbial count ($p \leq 0.05$) both in January and June. This was followed by 1440-1E, 1448-2F and 1740-2F, respectively. The differences in the microbial load in the oils could be attributed to the differences in the elements present in the soybeans which enhance the growth of microbes and the level of contamination because the same process was involved in the production of the oils. In all, two bacterial and five fungi species were isolated from the soy oil samples. Table 3 shows the microbes associated with each oil sample. Table 4 shows the changes in physico-chemical properties of the soy oil samples. Sample 1681-3F had the highest M.C and significantly difference ($p \leq 0.05$) from the other while refined oil had the lowest moisture content. There was a gradual decrease significantly ($p \leq 0.05$) in M.C in January but a gradual increase ($p \leq 0.05$) in June. The percentage free fatty acid slightly increased significantly ($p \leq 0.05$) throughout the storage period sample 1440-1E had the highest FFA value and refined oil had the lowest FFA-value. The changes in iodine value refined oil had the highest value while 1681-3F soy oil had the lowest value. There was slight increase ($p \leq 0.05$) in PV both in January and decrease ($p \leq 0.05$) in June. Peroxide value 1681-3F soy oil had the highest value ($p \leq 0.05$) and refined oil had the lowest value ($p \leq 0.05$). There was a gradual increase ($p \leq 0.05$) in PV both in January and June. Aflatoxin was not detected in all the oil samples screened.

The isolation of *S. aureus* is indicative of human contamination as *S. aureus* is a normal flora of the skin and mucous membranes of humans (Jensen, 1997; Brooks *et al*., 2001) another probable source is air. Isolation of *Bacillus subtilis* could be due to contamination by soil. *Bacillus* sp. is often associated with food poisoning as reported by Antai (1988). *Bacillus subtilis* is capable of producing hydrolytic enzymes, for the breakdown of polymers (CHO, crude proteins and fats/oils) as reported by Ensari *et al*. (1995). The endospore of bacillus are resistant to heat, chemical and radiation (Olutiola *et al*., 2000) thus its isolation in the oil. The increase in FFA could be due to the presence of fungi with lipolytic enzymes that hydrolyze the oil

Table 1: Total viable bacterial counts (cfu $mL^{-1} \times 10^1$) of soya oil samples in January and June 2006

Period of storage (weeks)	Total viable bacterial count per sample				
	Refined	1681-3F	1740-2F	1448-2F	1440-1E
1	0.0 (0.0)	0.5 (0.1)	0.1 (0.0)	0.1 (0.0)	0.4 (0.1)
2	0.1 (0.0)	0.7 (0.2)	0.2 (0.0)	0.1 (0.0)	0.6 (0.1)
3	0.1 (0.0)	0.9 (0.2)	0.2 (0.1)	0.2 (0.0)	0.8 (0.2)
4	0.1 (0.0)	1.0 (0.3)	0.2 (0.1)	0.3 (0.1)	0.8 (0.2)

Figures in parenthesis represent data for June, 2006

Table 2: Total mould counts spore $mL^{-1} \times 10^1$ of soya oil samples in January and June 2006

Period of storage (weeks)	Total mould count per sample				
	Refined	1681-3F	1740-2F	1448-2F	1440-1E
1	0.0 (0.2)	0.6 (1.3)	0.2 (1.0)	0.1 (0.5)	0.5 (1.3)
2	0.0 (0.3)	0.9 (1.7)	0.3 (1.2)	0.1 (0.6)	0.7 (1.7)
3	0.1 (0.5)	1.1 (2.5)	0.5 (1.3)	0.2 (0.8)	1.0 (2.1)
4	0.2 (0.8)	1.4 (3.1)	0.6 (1.5)	0.4 (1.0)	1.2 (2.5)

Figures in parenthesis represent data for June, 2006

Table 3: Distribution of microbial isolates in different soy oil cultivars

	Soya oil cultivars				
Isolates	Refined	1681-3F	1740-2F	1448-2F	1440-1E
Staphylococcus aureus	+	-	-	+	+
Bacillus subtilis	-	+	+	-	+
Aspergillus flavus	+	-	-	-	-
Aspergillus fumigatus	-	+	+	-	-
Aspergillus niger	+	+	+	-	-
Fusarium poae	-	+	-	+	+
Rhizopus oryzae	-	+	-	+	+

Key: + = Present; - = Absent

Table 4: The mean value of physico-chemical properties of stored soy oil samples in January and June 2006

	Stored soya oil samples				
Physico-chemical properties	Refined	1681-3F	1740-2F	1448-2F	1440-1E
Moisture content	0.47 (0.53)	0.59 (0.67)	0.53 (0.59)	0.51 (0.56)	0.57 (0.63)
Free fatty acid (%)	0.28 (0.38)	0.56 (0.82)	0.43 (0.57)	0.34 (0.42)	0.59 (1.04)
Iodine value	126.8 (122.27)	117.89 (115.79)	120.94 (119.25)	122.32 (119.36)	119.57 (118.58)
Peroxide value	4.48 (1.68)	7.18 (4.23)	5.72 (2.97)	5.20 (2.39)	6.55 (3.44)

Figures in parenthesis represent data for June 2006

(Erickson, 1995). The P.V decrease as storage period increases because the higher the degree of unstauration the higher the degree of auto-oxidation as stated by Dorko (1995). The oil showed non positive reaction to aflatoxin screening despite the isolation of A. flavus, this might be that the level of toxin in the oil is so minimal that it cannot be detectable and also the strain of A. flavus as revealed by Orum *et al*. (1998) and Oboh *et al*. (2000) that not all strains of A. flavus produce aflatoxin. S. strain produces aflatoxin while L strain produces no aflatoxin. Despite the slight deterioration of the oils from the soyabean cultivars for this study, the results showed that the soyabean cultivars are good and can be used to produce oil for domestic and industrial use but the oils are most susceptible to deterioration in June than in January thus the oils are best stored during the dry season.

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