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Full Length Research Paper

Comparative studies of ginger (*Zingiber officinale*) and black pepper (*Piper guinenses*) extracts at different concentrations on the microbial quality of soymilk and kunuzaki

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Extracts of two spices namely ginger (*Zingiber officinale*) and black pepper (*Piper guinenses*) were prepared in 0.4, 1.2, 2.4 and 3.6% concentrations. Soymilk and kunuzaki, were treated, respectively with the different concentrations and stored at ambient temperature for 5 days. The microbial load and identification were determined every day of storage until samples were adjudged spoiled. On the first day, 0.4% ginger extract in soymilk and kunuzaki had a microbial load of 7.77 × 10^{6b} and 5.17 × 10^{6b}, respectively. 3.6% ginger extract in soymilk and kunzakki recorded 3.73 × 10^{6b} and 3.30 × 10^{6} each. 0.4% black pepper extract in soymilk had 6.273 × 10^{6b} and recorded 4.63 × 10^{6b} in kunuzaki. 3.6% black pepper extract in soymilk and kunuzaki had a microbial load of 3.20 × 10^{6d} and 2.90 × 10^{6c}, respectively. On the 3rd day, the microbial load increased for both ginger and black pepper extract. Ginger extract recorded 9.13 × 10^{6b} in soymilk and 5.60 × 10^{6b} in kunuzaki at 0.4% concentration. Black pepper extracts recorded 7.43 × 10^{6b} in soymilk and 3.27 × 10^{6b} in kunuzaki also at 0.4% extract. 3.6% black pepper extract recorded 4.10 × 10^{6a} in soymilk and 2.20 × 10^{6c} in kunuzaki. There was linear reduction of microbial load as spice concentration increases. Black pepper recorded lower microbial load, thus has more anti microbial activity and may be preferred to be used as national anti microbial preservatives to extend the shelf-life of food.

Key words: *Zingiber officinale*, *Piper guinenses*, soymilk, kunzakki.

INTRODUCTION

The use of soybean for various food products has become popular and has acquired a confirmed health food status by the United States food and drug Administration (USFDA) of U.S.A (Enwere, 1998; Ohr, 2004). One main product of soybean is soymilk, which contributes to the provision for the much needed cheap protein source that would alleviate the problems of declining protein availability (Nsofor and Osuji, 1997). It is a non-alcoholic and non-fermented beverage. However, soymilk is susceptible to spoilage, it has poor shelf stability during ambient temperature (Nsofor and Anyanwu, 1992) and pasteurization treatment is not sufficient to inactivate the bacterial spores in it. Sterilization by heat causes denaturation of soy protein, thus resulting in coagulation (Nsofor and Osuji, 1997). Kunuzaki is a traditionally fermented non-alcoholic beverage mostly consumed in Northern Nigeria. It is usually produced from millet (*Pennisetum typhoidum*), sorghum (*Sorghum bicolor*) or maize (*Zea mays*). Graffa et al. (2002) reported that 73% of the population in Nigeria consumes Kunuzaki daily and 26% occasionally. Kunuzaki is low in viscosity and has a sweet sour taste, with creamy appearance.

According to Odunta (1985), numerous different fermented products have the advantage of prolonged shelf life due to the organic acid produced during

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fermentation, which is fatal to diverse spoilage micro organisms. This could be the reason why kunuzaki preserved longer than soymilk under the same condition as observed in this work. However, kunuzaki does not store long at ambient temperature, though it is a fermented product. It has a shelf life of 24 h at ambient temperature (Adeyemi and Umar, 1994). Efforts are being made in order to maintain the nutritional components of food, using various means such as reduction in temperature or the use of local spices as a preservative. The use of local spices to control the activities of microorganisms in food has been reported (Akpomedaye and Ejechi, 1998; Nwafor and Ogiehor, 2003).The anti microbial activity varies depending on the type of spice or herb. The need to which a spice material is used as a spice is dictated primarily by its essential oils or oleoresins (Dziezak, 1989). The volatile oils are responsible for the aroma and taste of most spices. These volatile oils contain terpenes, sesquiterpenes, alcohols, esters, aldehydes, ketones, phenols etc. (Juliani et al., 2004). The application of extracts of spice could possibly control the microbial activities associated with food samples while retaining the nutritive and economic quality. Hence in this work, two spices, ginger (*Zingiber officinale*) and black pepper (*Piper guinenses*) were used to enhance the shelf life of soymilk and kunuzaki at ambient temperature.

MATERIALS AND METHODS

Soybeans, millet, ginger and black pepper were all obtained from a local market (Ahaiohuru) in Abia state.

Production of soymilk

Soymilk was processed by the method described by Iwe (2003). Soybeans were sorted, 250 g of sample and steeped in water for 12 h. The soaked soybean were hand dehulled by rubbing and hulls removed by floatation. The steeped beans were ground to fine paste using locally fabricated attrition mill and 2.4 L of water was added to the paste. The resulting paste was sieved using muslin cloth and the filtrate boiled for 1 h.

Production of kunuzaki

The millet grains were sorted to remove extraneous materials. It was weighed, steeped in water for 18 h at ambient temperature, washed, wet milled with attrition mill and sieved. The filtrate was mixed with water and divided into two unequal portions in the ratio 1:2. The greater portion was boiled until it gelatinized and was allowed to cool. The cooked and uncooked portions were mixed together and allowed to ferment for 12 h to give kunuzaki.

Extraction of spices

Ginger and black pepper extract were prepared by the method described by Zia-ur-Rehman and Habib (2003). Samples were grounded into powder form and extracted with hot sterile water (80°C at 5 g/100 ml for 72 h). Extracts were filtered using filter papers. The spice extracts were concentrated by exposure to 100°C for 20 min in a water bath. Different concentration of the spices were made prepared according to the method developed by Akpomedaaye (1988) and Ogiehor et al. (1998) (10.4, 1.2, 2.4 and 3.6% w/v), respectively. Fifty millliter (50 ml) each of soymilk and kunuzaki were placed in five separate sterile containers, respectively and treated with the different concentration of the spices.

Group A: Ginger extract (0.4, 1.2, 2.4, 3.6% w/v); Group B: Black pepper extract (0.4, 1.2, 2.4, 3.6% w/v) and Group C: Control (No treatment). After the various treatments, the samples were stored at ambient temperature until they were adjudged spoilt.

Microbiological analysis

One millliter (1 ml) from each of the samples was separately homogenized in 9.0 ml of sterile peptone water. The dilution was serially made until 10^{-5} level of dilution was obtained. Isolation and identification was done according to the method of Ogbulie et al. (2005) and ICMMSF (1978). For bacterial isolation, nutrient agar, macConkey agar was used, while sabouraud dextrose agar was used for fungi isolation. Total viable counts of bacteria were determined by enumerating the colony forming units (cfu) by pour plating 1.0 ml of 10^{-5} diluent incubated at 37°C for 48 h. Total fungi counts were determined by pour plating also and incubated at 37°C for 3 days. The experiments were carried out in triplicates. Pure cultures of bacterial and fungal isolates were obtained on the nutrient agar and sabouraud dextrose agar, respectively.

Characterization and identification of isolates

Bacteria isolates were characterized and identified by initially examining colonies macroscopically on their cultural properties followed by physiological and biochemical tests (Motility test, citrate test, coagulase test, indole test, starch fermentation test, gram stain, spore stain catalase test and oxidase test etc.). The fungal isolates were characterized by their cultural properties stained with cotton-blue lactophenol solution and observed under low power objective lens (Chessbrough, 2002; Kovac, 1956; ICMMSF, 1978; Ogbulie et al., 2005)

RESULTS AND DISCUSSION

Results of the effect of spice concentration on storage time of 3 to 4 days on the microbial quality of soymilk and kunuzaki is presented in Tables 1 and 2. The microbial count on the day of production was generally low, 1.30×10^6 and 9.7×10^5 in both samples, respectively until the second day of storage when there was a spontaneous increase in the bacterial load in both soymilk and kunuzaki, ranging from 7.77×10^{6b} at a concentration of 0.4% and 3.73×10^{6d} at a concentration of 3.6% for ginger extract, and 6.27×10^{6c} at a concentration of 0.4% and 3.20×10^{6d} at a concentration of 3.6%, using black pepper extract. 5.17×10^{6b} at 0.4% and 3.30 at 3.6% ginger extract. 4.63×10^{6b} at 0.4% and 2.90×10^{6d} at 3.6% black pepper extract for kunuzaki. It was observed that there was a linear reduction in the bacteria load with increase in concentration of spice. This indicates the anti microbial activity of ginger and black pepper extract. In the ginger treated samples, there was a moderate reduction in microbial load when compared with those of

Table 1. Bacteria load of spice treated soymilk during storage at ambient temperature.

Spice extract	Spice concentration (%)	Storage time (days)			
		1	2	3	4
Ginger extract	0.4	7.77×10^{6b}	10.01×10^{6b}	9.13×10^{6b}	9.00×10^{6b}
	1.2	5.63×10^{6c}	8.80×10^{6c}	7.80×10^{6b}	7.20×10^{6b}
	2.4	4.40×10^{6d}	6.57×10^{6d}	5.80×10^{6b}	5.20×10^{6b}
	3.6	3.73×10^{6d}	6.13×10^{6d}	4.70×10^{6b}	4.10×10^{6b}
Black pepper extract	0.4	6.27×10^{6c}	8.67×10^{6d}	7.43×10^{6b}	7.10×10^{6b}
	1.2	5.03×10^{6d}	6.43×10^{6d}	5.53×10^{6b}	5.25×10^{6b}
	2.4	3.93×10^{6d}	5.37×10^{6d}	4.47×10^{6b}	4.23×10^{6b}
	3.6	3.20×10^{6d}	5.27×10^{6d}	4.10×10^{6a}	3.90×10^{6b}
	Control	10.90×10^{6a}	12.40×6^{a}	12.20×10^{6a}	11.90×10^{6a}

Means with the same superscript on the same row are not significantly different (P < 0.05).

Table 2. Bacteria load of spiced treated kunuzaki during storage at ambient temperature.

Spice extract	Spice concentration (%)	Storage time (days)			
		1	2	3	4
Ginger extract	0.4	5.17×10^{6b}	4.33×10^{6b}	5.60×10^{6b}	2.83×10^{6b}
	1.2	4.90×10^{6b}	$3.7.3\times10^{6b}$	3.33×10^{6b}	2.60×10^{6b}
	2.4	3.83×10^{6c}	3.33×10^{6bc}	3.07×10^{6bc}	2.47×10^{6b}
	3.6	3.30×10^{6c}	3.03×10^{6bc}	2.53×10^{6c}	2.17×10^{6b}
Black pepper extract	0.4	4.63×10^{6b}	4.10×10^{6b}	3.27×10^{6b}	2.87×10^{6b}
	1.2	3.83×10^{6c}	3.43×10^{6bc}	3.03×10^{6bc}	2.60×10^{6b}
	2.4	3.37×10^{6c}	2.63×10^{6cd}	2.40×10^{6c}	2.20×10^{6b}
	3.6	2.90×10^{6c}	2.40×10^{6cd}	2.20×10^{6c}	2.00×10^{6b}
	Control	8.44×10^{6a}	10.31×10^{6a}	9.23×10^{6}	8.0×10^{6b}

Means with the same superscript on the same row are not significantly different (P < 0.05).

black pepper treated samples; showing that black pepper spice has more anti microbial activity than ginger. The active component of spices at low concentration might have interacted synergistically with other factors to increase preservative effect. The preservative effect of these spices is due to their chemical composition, especially the essential oil fractions which are inhibitory to microbial growth (Powers et al., 1995). These chemical compositions vary in spices, hence black pepper kept the shelf life of the soymilk and kunuzaki longer than ginger as found in this test.

However the microbial load started to decline by the third day. This was attributed to the possibility of increase in acidity resulting from microbial fermentation of the samples and depletion of nutrient available and the release of toxins by the micro-organisms (Oyeagbo, 2003). Tables 3 and 4 presents a summary of the micro-organism identified. At lower concentration of the spice extract, five species of bacteria were isolated including, *Staphylococcus aureus, Bacillus cereus*, *Pseudomonas, Proteus and Lactobacillus* species. At higher concentration of spices, two species were identified. This indicates that at higher concentration of these spices, some types of bacteria are killed. Comparing Tables 1 and 2, it was observed that the microbial load in soymilk is generally higher than kunuzaki sample, also kunuzaki was able to preserve longer than soymilk. Kunuzaki is a fermented product. According to Odunta (1985) different fermented products have the advantage of prolonged shelf life because of the organic acid produced during fermentation, which is fatal to certain micro-organisms. This also agrees with the work done by Achi (1991) who observed that fermentation helps to increase the shelf life and digestibility of foods.

Conclusion

It is evident from this research work that ginger and black pepper extract possess antimicrobial properties, since they can extend the shelf life of soymilk and kunuzaki from 12 and 24 h, respectively to a maximum of 4 days at ambient temperature. The microbial activity of the spices varies especially at concentration from 0.4% and above.

Table 3. Bacteria flora of the spice treated soymilk at ambient temperature**.**

Spice extract	Spice concentration (%)	Organisms				
		Staphylococcus	***Bacillus***	***Pseudomonas***	***Proteous***	***Lactobacillus***
Ginger extract	0.4	+	+	+	+	+
	1.2	+	+	+	+	+
	2.4	+	+	-	+	+
	3.6	+	+	-	-	+
Black pepper extract	0.4	+	+	+	+	+
	1.2	+	+	-	+	+
	2.4	+	+	-	-	+
	3.6	-	+	-	-	+

Table 4. Bacteria flora of the spice treated kunuzaki at ambient temperature.

Spice extract	Spice concentration (%)	Organisms				
		Staphylococcus	***Bacillus***	***Pseudomonas***	***Proteous***	***Lactobacillus***
Ginger extract	0.4	+	+	+	+	+
	1.2	+	+	+	+	+
	2.4	+	+	-	-	+
	3.6	-	+	-	-	+
Black pepper extract	0.4	+	+	+	+	+
	1.2	+	+	+	-	+
	2.4	+	+	-	-	+
	3.6	-	+	-	-	+

+ = Present; - = absent.

Black pepper spice possess more anti microbial properties than ginger and may be preferred and used as natural anti microbial preservatives to extend the shelf life of food.

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