JOBIMB, 2024, Vol 12, No 2, 74-80
https://doi.org/10.54987jobimb.v12i2.1069

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

JOBIMB VOL 12 NO 2 2024
Bacterial acrylamidase

Preservative Properties of Ginger (*Zingiber officinale*) and Alligator Pepper (*Aframomum danielli*) on Fried Soybean Cake (*Tofu/Wara*): Antioxidant, Microbial, and Sensory Evaluation

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HISTORY

Received: 19th Sep 2024
Received in revised form: 21st Nov 2024
Accepted: 25th Dec 2024

KEYWORDS

Antimicrobial resistance
Food spoilage
Preservation
Antioxidant
Apices

ABSTRACT

Spices have long been valued as flavor enhancers, coloring agents, and medicinal substances. This study investigated the preservative properties of ginger (*Zingiber officinale*), alligator pepper (*Aframomum danielli*), and their mixture on fried soybean cake (Tofu/wara). Three concentrations (0.25 g, 0.5 g, and 0.75 g) were tested, alongside untreated and chemically treated controls. Antioxidant and anti-inflammatory activities were evaluated using DPPH radical scavenging, reducing power, and membrane stabilization assays. Proximate composition, including moisture, ash, protein, lipid, fiber, and carbohydrate content, was assessed, along with microbiological quality using aerobic plate, coliform, and fungal counts. Storage stability tests and sensory evaluations were also conducted. Results demonstrated that the spices exhibited strong antioxidant and anti-inflammatory properties, which increased with concentration. Food samples treated with spices showed enhanced protein and fiber content, reduced moisture levels, and a significant decrease in bacterial and mold counts compared to untreated samples. Coliform levels were within acceptable limits, with no fecal coliform detected. Storage stability tests revealed reduced spoilage rates, with alligator pepper showing the greatest inhibitory effect, followed by ginger. However, the combination of the spices was less effective than individual applications. Sensory evaluation indicated high acceptance of treated samples in terms of appearance, taste, texture, and flavor. This study highlights the potential of ginger and alligator pepper as natural preservatives, providing a basis for their application in food processing and industrial uses. Further research should explore additional spices and combinations to enhance preservative efficacy and synergistic effects.

INTRODUCTION

Herbs and spices are of plant origin and are used for culinary purposes. The terms "herb" and "spice" are often used interchangeably, but they have specific definitions in botany. Herbs store flavor components in their leaves, while spices store them in the seeds, bark, and roots. A possible spice is the buds (cloves), bark (cinnamon), roots (ginger), aromatic seeds (fennel), and flower pistil (saffron) of the plant [1]. According to the Exploratorium, "Natural flavoring to foods is one of the most common uses of spices" Almost all spices are associated with a specific flavor, and they are the basis for culinary recommendations around the world. Depending on the region, different spices are used to flavor the dishes, bring their flavor to each style of food, and even create a culinary identity [2]. Spices are used as flavorings, preservatives, and coloring agents, as in medicine, religion, tradition, and magic in some parts of the world [3]. However, spices can also preserve and improve shelf

life apart from flavoring, as some findings prove that spices reduce food spoilage [4]. Some spices like Clove, Fenugreek, Garlic, and Ginger contain preservative properties that can be used in food processing without any adverse effects, and these spices have the power to preserve due to their high antimicrobial and antioxidant content. Therefore, a mixture of these spices can be a replacement for synthetic preservatives in the food industry [5].

Ginger, scientifically known as *Zingiber officinale* (Zingiberaceae), is classified as a perennial, edible rhizome. Its common name Zingiber is derived from the Greek word *zingibers* [6]. Ginger is famous worldwide and is primarily used in treating gastrointestinal disorders such as constipation, indigestion, and nausea. Ginger has been reported to have healing properties against digestive disorders, rheumatism, and diabetes [7]. The main pharmacological actions of ginger and compounds isolated therefrom include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic, and anti-emetic actions. Ginger may prevent the generation of free radicals due to its being a strong antioxidant substance and its being considered safe [8]. Kazeem *et al* [9] reported that the DPPH free radical scavenging ability of the extracts of ginger showed that it inhibits free radicals at certain concentrations.

Aframomum danielli also known as alligator pepper is a large robust perennial plant of the family *Zingiberaceae* that grows in Central and West African countries [10]. Some physical and mechanical properties of alligator pepper pods and seeds have been documented by [11]. Given the importance of alligator pepper in herbal medicine and its socio-cultural applications in Nigeria and other neighboring African countries, this data bears relevance in developing or adapting technologies for its processing into value-added products, thereby boosting obtainable income. Kazeem *et al* [9] reported that the DPPH free radical scavenging ability of alligator pepper extract showed that the spice was able to inhibit free radicals. The effect of natural preservatives (*Aframomum danielli*) on the chemical, microbiological, and sensory properties of fried bean cake was investigated by [12] and the result showed reduced moisture content with an increase in the concentration of *Aframomum danielli* powder. Other proximate content increased significantly ($p<0.05$) as the concentration increased. Bacterial and fungal count decreased significantly ($p<0.05$) as the concentration increased.

The food industry is highly concerned with microbial food spoilage as a result of a 25% total loss of food production each year after harvest. The growth of spoilage microorganisms in food affects the shelf life and overall quality of the finished product; though it may not be harmful to human health, it affects consumer choice, leading to substantial loss in business. Therefore, preventing or inhibiting their growth in food is of prime importance for today's globalized food production [13]. Food spoilage and poisoning remain major problems despite the varieties of preservatives available; due to consumer insistence on fresh and natural foods, food manufacturers use these synthetic preservatives instead of simple preservation techniques like refrigeration, which only intensifies the problem [2]. While some synthetic preservative substances are harmless when used in small amounts, the use of others is not without risk to human health. Among the many side effects, one may see a rash, itching, difficulty breathing, sneezing, or digestive upset [14]. The growing consumer demand for healthy and long-lasting foods has spurred the search for food preservatives with less potential for health risks. Plants and their derivatives are viable alternatives in the preservation of foods, ensuring the stability of the organoleptic and nutritional properties of these products, as well as the quality and consistency of these products [15]. Therefore, there is always a need for new treatments, either alone or in combination with existing ones, capable of reducing or eliminating pathogens and spoilage bacteria in food.

MATERIALS AND METHOD

Sources of samples

All the spices samples: *Aframomum danielli* (Alligator Pepper), and *Zingiber officinale* (Ginger), and the food samples: *Glycine max* (Soybeans), groundnuts oil, and salt were purchased from Aliero local market in Kebbi State, Nigeria in July 2024, and stored at room temperature before use.

Preparation of spice-powdered samples

The method described by Adedeji and Ade-Omowaye [12] and that of Bello *et al.* [10] was adopted. The spices (alligator pepper and ginger) were sorted from any debris, washed with water and oven-dried at 37°C for 72 hours. The spices were dry-milled and sieved using a blender. The powdery substance obtained was packaged in an airtight container and labeled accordingly.
The three (3) different spice samples were prepared as follows:

- Powdered Ginger sample (G)
- Powdered Alligator pepper sample (AP)
- Equal mixture of ginger and alligator pepper sample (MIX)

Each of the above samples was divided into 3 different concentrations (0.25 g, 0.5 g, and 0.75 g which is equivalent to 250 mg, 500 mg, and 750 mg) making one (21) sample, plus two controls (positive and negative) making a total of twenty-three (23) samples.

Preparation of the spice extracts

The method described by Han *et al.* [16] was used with some modifications. Dried spice powdered samples (2g) each were extracted with 50 mL of 80% methanol at room temperature (23 °C) for 24 h in a shaking water bath. The shaking water bath was set to a heating temperature of 50 °C with a rotation speed of 50 OPM. Freeze-drying was carried out under the following conditions: a temperature of 4 °C and a pressure of 100 mT. A Millipore filter filtered the extract with a 0.45-µm nylon membrane under vacuum at 23 °C. The filtrate was then freeze-dried, ground into a fine powder and passed through a sieve, the extracts were divided into three (3) concentrations as mentioned above. The samples were then stored in an airtight container before use.

Soya bean cake (Wara/Tofu) preparation

After purchase, soya beans were sorted and then washed to remove any dirt and soaked after which the soaked soya beans were wet milled and sieved, the resultant juice was boiled and 50 mL of vinegar/calcium sulphate / 250 mL of whey of akamu was added as a coagulant that brings Tofu to gather, the resultant Tofu was then collected into a clean muslin cloth, pressed out to remove whey and divided into 3 portions of 30 g each, the spice samples were then added accordingly, pressed to bring it to gather and cut into pieces and fried accordingly for 15 minutes.

Determination of antioxidants and anti-inflammatory activity of the spices

Antioxidant Activity Assay

The DPPH free radical scavenging activity was accessed according to the established procedure of Govindappa *et al*. [17].

The reducing power was determined according to the established procedure of Cuendet *et al.* [18].

Membrane stabilization test
The method described by Shinde *et al.* [19] and modified by Juverkar *et al.*[20] was employed.

Preparation of Red Blood cells (RBCs) suspension
The blood was collected from a healthy human volunteer who had not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with an equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline.

Heat-induced hemolysis
The reaction mixture (2 mL) consisted of 1 mL test sample of different concentrations (100 - 500 µg/mL) and 1 mL of 10% RBCs suspension, instead of the test sample only saline was added to the control test tube. Diclofenac sodium was used as a standard drug. All the centrifuge tubes containing the reaction mixture were incubated in a water bath at 56 ºC for 30 minutes. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of hemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Hypotonicity induced hemolysis
Different concentrations of extract (100-500 µg/mL), reference sample, and control were separately mixed with 1 mL of phosphate buffer, 2 mL of hypo-saline, and 0.5 mL of HRBC suspension. Diclofenac sodium was used as a standard drug. All the assay mixtures were incubated at 37 ºC for 30 minutes and centrifuged at 3000rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated by a spectrophotometer to be 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection = 100- (OD sample/OD control) x 100.

Proximate composition of the food samples treated with the spices
Moisture, ash, fat, crude fiber, and crude protein and carbohydrate content of the soya beans cake with the extract and non-extracted spice samples with the control were determined according to the method of AOAC [21].

Microbiological analysis of the spiced Tofu
Total viable bacterial and Mold counts of the freshly prepared food sample treated with the spices and spoiled samples after storage were examined using the method adopted by Bello *et al.* [10]. The total coliform count was examined according to the procedures of Chikezie [22].

Storage stability test
The experiment was carried out in the preparation room of the general biology laboratory of Kebbi State University of Science and Technology Aliero.

The subjective evaluation (storage stability/ sensory test) of Tofu treated with different spice concentrations was done by adopting the procedure of Wakoli *et al.* [23].

Consumer acceptability test
The consumer acceptability test was carried out in the preparation room of the general biology laboratory at Kebbi State University of Science and Technology. This was done according to the method described by Adedeji [24] with some modifications.

A group of people were recruited to assess the acceptability Tofu treated with different spice concentrations. An invitation to participate in the acceptability group was sent via direct SMS and WhatsApp messages to some Kebbi State University of Science and Technology Aliero staff and students. 60 healthy adults were selected to take part in the study. Tofu was prepared on the day of testing, and one piece containing each sample's different concentrations was given differently to each participant on white disposable plates.

Each sample was coded (care was taken to ensure that each participant received samples in a different order). A score sheet accompanied each sample, and the participants were asked to score the acceptability of the food samples using a 9-point hedonic scale, with nine representing like extremely and one representing dislike extremely. Bottle water was provided for participants to rinse their mouths between samples. Good hygienic practice was followed throughout the process.

Statistical analysis
The data obtained from proximate composition and antioxidant activity were subjected to analysis of variance and means were separated using Tukey tests. The results are expressed as mean ± SD. We used a one-way analysis of variance (ANOVA) to find significant differences between the means. Turkey test was used to group the means. Results were considered to be significant when p-values were less than 0.05 ($p < 0.05$).

Ethical Approval
Ethical approval for this study was obtained from the Institutional Ethical Committee of Kebbi State University of Science and Technology Aliero. All procedures were conducted in accordance with institutional and international ethical guidelines. Informed consent was obtained from all participants prior to their involvement in the study.

RESULTS

The antioxidant activity of the spice samples was assessed by testing DPPH radical scavenging activity (**Table 1**) and reducing power ability (**Table 2**) whereas anti-inflammatory activity was assessed using the membrane stabilization (heat-induced and hypotonicity-induced) ability of the spices (**Table 3**). Values are means of triplicates. Values with the same letter along the column are the same while those with different letters along the same column are significantly different at p-value = ≤0.05.

Table 1. DPPH Radical scavenging activity of the spices.

S/N	S/Conc.	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL
1	Ginger	65.10±0.10^{a}	43.50±1.04^{a}	35.67±0.00^{a}	30.10±0.73^{a}
3	AP	54.00±0.89^{c}	45.50±0.00^{c}	32.00±0.95^{c}	22.00±0.95^{c}
2	MIX	70.10±0.10^{b}	67.50±0.50^{b}	53.50±0.50^{b}	42.00±0.40^{b}
4	Control	83.00±0.00^{d}	82.00±0.00^{d}	81.00±0.00^{d}	78.00±1.00^{d}

KEY: AP= Alligator pepper, MIX=mixed spices, S=sample, Conc.= concentrations

Table 2. Reducing power activity of the spices.

S/N	S/Conc.	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL
1	Ginger	60.00±0.00^{a}	60.00±5.00^{a}	53.00±2.00^{a}	52.00±2.00^{a}	45.00±1.00^{a}
3	AP	80.00±0.00^{c}	78.00±0.00^{c}	76.00±2.00^{c}	70.00±1.00^{c}	68.00±0.00^{c}
2	MIX	81.00±0.00^{c}	75.00±1.00^{b}	65.00±1.00^{b}	63.00±1.00^{b}	53.00±0.40^{b}
4	Control	96.00±1.00^{d}	95.00±1.00^{d}	90.00±0.00^{d}	90.00±1.00^{d}	88.00±0.00^{d}

KEY: AP= Alligator pepper, MIX=mixed spices, S=sample, Conc.= concentrations

Table 3. Membrane stabilization activity of the spices.

Method	Heat induced Hemolysis				Hypotonicity Induced hemolysis			
S/Conc.	Ginger	AP	MIX	Control	Ginger	AP	MIX	Control
500µg/mL	66.0±0.6^{b}	60.4±0.6^{d}	51.4±0.9^{c}	100.0±0.0^{e}	98.3±1.5^{a}	99.3±0.6^{a}	99.0±0.1^{a}	98.0±1.0^{a}
400µg/mL	45.3±1.5^{b}	49.7±5.8^{a}	50.5±0.5^{a}	100.0±0.0^{c}	96.0±0.0^{a}	96.3±0.0^{a}	96.6±0.9^{a}	95.0±0.0^{a}
300µg/mL	43.1±0.1^{b}	33.3±0.5^{a}	42.2±1.0^{a}	87.0±0.0^{c}	95.0±0.0^{a}	96.3±1.0^{a}	96.0±0.1^{a}	95.0±0.0^{a}
200µg/mL	28.1±0.1^{b}	12.3±0.4^{d}	41.0±0.2^{c}	85.0±1.0^{e}	92.0±2.0^{a}	95.0±0.9^{a}	95.0±0.5^{a}	94.0±0.0^{a}
100µg/mL	24.2±0.2^{b}	6.5±0.3^{d}	39.0±0.3^{c}	84.0±0.0^{e}	71.0±1.0^{b}	94.0±0.0^{a}	93.0±0.2^{a}	90.0±0.0^{a}

KEY: AP= Alligator pepper, MIX=mixed spices, S=sample, Conc.= concentrations

Table 4. Proximate composition of fried soybeans cake (war/Tofu) treated with spices.

S/N	S.	Conc.	Moisture	Ash	Fibre	Lipid	Protein	Carbohydrate
1	G	0.75 g	2.00±0.00^{cde}	3.30±0.17^{de}	3.10±0.10^{f}	13.00±1.00^{de}	65.00±1.00^{bcde}	16.00±0.10^{cde}
2		0.5 g	1.90±0.10^{bcd}	3.30±0.10^{de}	2.90±0.00^{ef}	2.00±0.00^{cde}	64.00±0.10^{abcd}	15.00±0.00^{abc}
3		0.25 g	1.60±0.17^{ab}	3.10±0.17^{cde}	2.80±0.10^{de}	10.67±1.15^{bc}	64.00±0.50^{abcd}	13.00±1.73^{ab}
7	AP	0.75 g	1.90±0.00^{bcd}	2.80±0.10^{abcd}	3.10±0.10^{f}	8.600±0.17^{a}	67.00±1.00^{de}	13.00±0.50^{ab}
8		0.5 g	1.80±0.10^{abc}	2.67±0.67^{ab}	3.00±0.00^{ef}	8.500±0.10^{a}	65.00±1.00^{bcde}	12.50±0.00^{a}
9		0.25 g	1.50±0.00^{a}	2.40±0.00^{abc}	2.80±0.10^{de}	8.200±0.10^{ab}	64.33±1.61^{abcd}	11.67±0.76^{a}
13	MIX	0.75 g	1.90±0.00^{cde}	2.67±0.67^{abcd}	3.00±0.10^{ef}	12.00±0.00^{cde}	64.00±1.00^{abcd}	13.00±0.00^{ab}
14		0.5 g	1.60±0.17^{ab}	2.90±0.10^{bcde}	2.90±0.10^{ef}	11.00±1.00^{bc}	62.00±2.00^{ab}	12.00±0.00^{a}
15		0.25 g	1.60±0.10^{ab}	2.80±0.10^{abcd}	2.60±0.10^{c}	10.00±0.00^{ab}	62.00±0.00^{bc}	12.00±0.00^{a}
22	Control		2.30±0.27^{ef}	2.63±0.15^{abcd}	2.80±0.10^{de}	11.10±0.10^{bc}	62.00±0.50^{bc}	14.22±0.00^{ab}

Key: S.= samples, G= Ginger, AP= Alligator pepper, MIX = mixed spices

The proximate composition of Tofu treated with extracts of ginger, Alligator pepper, and Mixed spices at 0.25 g, 0.5 g, and 0.75 g is presented in **Table 4**. The result shows the percentage of these samples' moisture, ash, crude fiber, crude lipid, protein, and carbohydrate content at varying concentrations. All values are means of triplicates. Values with the same letter along the column are the same, while those with different letters along the same column are significantly different at p-value = ≤0.05. The microbiological quality of food samples treated with different concentrations of the spice extracts and un-extracted spices, before and after spoilage was assessed using Aerobic plate count (**Table 5**), Total Coliform Count (TCC) (**Table 6**) and Total fungal count (TFC) (**Table 7**). All values are means of triplicates.

Table 5. Total Bacterial count.

s/n	Sample code	No. of colonies (cfu/g)
1.	A1	$7.0x10^{3}$
2.	B1	$1.5x10^{4}$
3.	C1	ng
4.	A2	$4.0x10^{9}$
5.	B2	$8.0x10^{9}$
6.	C2	$3.0x10^{10}$
7.	D	$1.0x10^{10}$

KEY: ng= no growth, cfu/g = colony forming unit per gram, A1 – C1 = Tofu sample containing ginger, alligator pepper and mixed spices respectively. However, 1 represents the samples before spoilage and 2 after spoilage while D is the control.

Table 6. Mean Total Fungal Count on the food samples treated with the spices.

S/N	sample code	No of colonies (cfu/g)
2	A1	----
3	B1	----
7	C1	----
8	D1	----
10	A2	$1.0x10^{2}$
11	B2	----
15	C2	----
16	D2	$3.0x10^{2}$

KEY: cfu/g = colony forming unit per gram, A1 – D1 = Tofu sample containing ginger, alligator pepper, mixed spices and control respectively. 1 = represent the samples before spoilage while 2 represent samples after spoilage.

Table 7. Mean Total Coliform Count on the food samples.

S/N	Sample	Number of positive tubes			MPN index per 100mL
		Double strength 10mL	(1mL) single strength	(0.1mL) single strength	
2	A1	0	0	0	0
3	B1	0	0	0	0
4	C1	0	0	0	0
5	D1	1	1	0	7.3
6	A2	0	1	0	3
7	B2	1	0	0	3.6
8	C2	0	0	0	0
15	D2	2	1	1	20

KEY: MPN= Most Probable Number, CFU/g = colony forming unit per gram, A1 – D1 = Tofu samples containing ginger, alligator pepper, mixed spices, and control respectively. However, 1 represents the samples before spoilage while 2 represents samples after spoilage

The absence of the indicator organism *Escherichia coli* isolates for fecal contamination was confirmed by the absence of a greenish metallic sheen on Eosin methylene blue agar (EMB). **Table 8** below shows the storage stability test of Tofu treated with different concentrations of different spice samples in question based on a number of days taken to spoil after an open-air inoculation with two controls (positive and negative).

Table 8. Storage stability test of Tofu treated with spices.

S/N	Samples (g)	Week 1	Week 2	Week 3	Week 4	Week 5
	G = 0.75	_	_	_	+	+
	G = 0.5	_	+	+	+	+
	G = 0.25	_	+	+	+	+
	AP = 0.75	_	_	_	+	+
	AP =0.5	_	_	+	+	+
	AP = 0.25	_	+	+	+	+
	MIX = 0.75	_	_	_	+	+
	MIX = 0.5	_	_	+	+	+
	MIX = 0.25	_	+	+	+	+
	+ve Control	_	_	_	_	+
	-ve Control	+	+	+	+	+

Key: AP= Alligator pepper, MIX= Mixed spices

Table 9. Consumer acceptability test.

Samples (g)	Level of Preferance (%) L.E	L.V	L.M	L.S	NLND	D.S	DM	D.V	D.E
G = 0.75	25.00	25.00	10.00	00.00	1.67	3.33	05.00	00.00	00.00
G = 0.5	25.00	25.00	50.00	00.00	00.00	00.00	00.00	00.00	00.00
G = 0.25	25.00	16.67	50.00	05.00	0.00	01.66	00.00	01.67	00.00
AP = 0.75	10.00	33.33	41.70	10.00	01.67	01.67	01.673	00.00	0.00
AP = 0.5	05.00	16.67	1.67	66.60	05.00	03.00	00.00	01.67	00.39
AP = 0.25	33.33	05.00	26.67	26.67	08.33	00.00	00.00	00.00	00.00
MIX = 0.75	50.00	25.00	10.00	15.00	00.00	00.00	00.00	00.00	00.00
MIX = 0.5	66.67	08.30	10.00	11.67	00.00	01.67	01.67	00.00	00.00
MIX = 0.25	50.00	16.67	33.33	00.00	00.00	00.00	00.00	00.00	00.00
Control	50.00	16.67	33.33	00.00	00.00	00.00	00.00	00.00	00.00

DISCUSSION

One of the most accepted methods of screening the antioxidant activities of plant extracts is DPPH free radical scavenging, which is very important in preventing autoxidation in the food system and the deleterious role of free radicals in different diseases [25]. The percentage inhibition of DPPH radical scavenging activity of the three spice samples (Ginger, Alligator pepper, and mixed spices) was assessed at 400,300,200 and 100µg/mL. There is a significant difference between the spices and the control (83.0 -78.00) at various concentrations of the spices used. Interestingly, mixed spices (70.10 – 42.00%) have higher scavenging percentage inhibition than the remaining spices, followed by Ginger (65.10 – 30.10%) and Alligator pepper (54.00 -22.00) at the same concentrations, which shows strong scavenging of these spices and synergistic effect when they are mixed.

Reducing power activity results showed a decrease significantly with a decrease in the spice concentrations. There is also a significant difference between all the spice samples and the control. With control (96.00 -88.00%), mixed spices (81.00 53.00%), Alligator pepper (80.00 – 68.00%), and Ginger respectively (60.00 – 45.00%), respectively. This shows that the spice samples used in this study have a reduced power ability and can serve as antioxidants in food since the addition of antioxidants is required in the food system to serve as a defense mechanism against reactive oxygen species [25].

Stabilization of the RBC membrane was studied further to establish the mechanism of anti-inflammatory actions of the spices. All the extracts were effectively inhibiting the heat-induced hemolysis. The result showed a significant difference at ($p<0.05$) between the spices at varying concentrations, with ginger having the range of (66.0 -24.2 %) followed by Alligator pepper (60.4 – 39.5%) and mixed spices (51.4 – 39.0%). However, the percentage of hemolysis in the spices is less significantly compared to the control (100 – 84%). When compared with hypotonicity induced, there is no significant difference between the spices and the control but the percentage is higher, showing the ability of these spices to hemolyse cell membranes in hypotonic environment.

The percentage hemolysis of 99% for alligator pepper and mixed spices and 98% for ginger and control at the highest concentrations was observed. This work is in line with the work of Yesmin *et al*. [26] with a similar work on membrane stabilization as a mechanism of anti-inflammatory activity of the methanolic extract of Choi (*Piper chaba*). It reveals that the extracts possess anti-inflammatory ability at induced hypotonicity and heat. This can serve as evidence for their anti-inflammatory effect. Proximate compositions showed reduced moisture content with an increase in the spice concentrations which is of higher advantage in terms of shelf life, as moisture is one of the most important factors that affect the growth of microorganisms in food. The control sample had a moisture content of 2.50% while a range of 2.00 - 1.6% was recorded for the remaining samples at varying concentrations. This conforms with the work of [27] and that of Adedeji and Ade-Omowaye [12] who reported low moisture content in foods treated with spices from their research. Ash content is an indication of mineral content in food (Adelakun *et al*., 2009). Samples containing Ginger (3.30 -3.10%) and Mixed spices (3.00 -2.80) had higher ash content than Tofu spiced with Alligator pepper (2.80 -2.40), and the control sample had an ash content of 2.50% at varying concentrations.

The addition of ginger, Alligator pepper, and mixed spices to the Tofu sample resulted in a significant ($p<0.05$) increase in their ash content. Observations from this study suggest that the Tofu snack if treated with spices, is an excellent and nutritious food commodity. The fiber content of the Tofu snack was found to be relatively higher in spiced samples than the control this is also of great advantage as fiber is reported to lower serum cholesterol, control blood sugar, prevent constipation by increasing bulk stool, prevent pil and colon cancer among others [12]. Mixed spices (13.00 – 10.00%) had higher lipid content followed by Ginger (12.00 – 10.67%) and Alligator pepper (11.60 -9.20%), respectively and control (8.10%) had the least content. This is in line with the work of Adedeji and Ade-Omowaye [12] who reported that ginger has higher lipid content, although he worked on only ginger and alligator pepper, so it can be justified that the addition of ginger and alligator pepper or their mixture to the soybean cake samples to gather with groundnut oil used resulted in significant ($p<0.05$) increase in their fat content.

The result shows a significant difference in the fat content with an increase in the spice concentrations in the samples except with the samples at 0.75g and 0.5g. Soya bean cake spiced with Alligator pepper (67.00 -64.33%) had higher protein content than samples spiced with Ginger (65.00 – 64.00%) and mixed spices (64.00 – 62.00%) respectively. The protein content of Alligator pepper to that of ginger has contributed significantly at ($p<0.05$) to the increase in protein content of the food samples. This is in line with the work of Adedeji and Ade-Omowaye [12], who reported similar findings when ginger and Alligator pepper were used to spice fried bean cake. The control sample had a protein content of 62.00%. This indicates that despite the food sample being a rich source of protein, ginger, alligator pepper, or their combinations with fried soybeans cake snacks resulted in a significant ($p<0.05$) increase in the protein level of the sample. The observation also shows a significant decrease in the protein content of all the samples with decreasing their concentrations. This can serve as proof that these spices increase the protein content of food.

Ginger spiced samples had higher carbohydrate content than Tofu spiced with alligator pepper and their combinations. The carbohydrate content of ginger (16.0 – 13.0%) has contributed significantly at $p<0.05$ to the increase in carbohydrate content of the cake. This conforms with the work of Adedeji, [24], who reported a similar result, except that in this work, alligator pepper does not contribute significantly to the increase in carbohydrate content as stated here. The control sample had a carbohydrate content of 14.0%, while a range of 13.00-12.00% was recorded for the remaining samples of alligator pepper and the combinations of the extracts at varying concentrations. The microbiological quality of the samples was assessed through Aerobic plate count (APC) for the total bacterial, mold count and coliform count. The bacterial count shows reduced microbial

load with increasing concentration of the spices on the freshly prepared samples compared to the spoiled ones, as the spoiled samples may contact the organisms during storage. And an indication that hygienic practices were followed while preparing the food samples. At the same time, the freshly prepared samples treated with the spices have lower microbial load than the untreated ones, indicating the inhibitory effect of those spices. This work is in line with the work of Adedeji and Ade-Omowaye [12], who reported similar findings. For the mold count, only one out of the spoilt samples with the control shows mold growth, which is also within the stated limits of food microbiological standards and specifications.

The absence of growth on the remaining samples can indicate hygienic practices used during the food preparation. It is of great interest that the total coliform count revealed the absence of the fecal coliform (E. coli) indicator in the food samples throughout the time of preparation and storage. The MPN is also within the stated limit. The results are in line with the work of Adedeji and Ade-Omowaye [12] with a similar analysis on fried bean cake using ginger and Alligator pepper. The shelf life increased with an increase in the concentration of the spice extracts. The result showed a reduced spoilage rate on the treated samples compared to the untreated ones at ambient temperature. Similar findings have been documented elsewhere [28]. The result also shows an increased shelf-life in the extracted samples compared to the un-extracted. This may be attributed to the inhibitory effect of methanol used in the extraction. However, all three samples used are able to delay spoilage for three weeks at the highest concentration (0.75) and 1 – 2 weeks for the remaining concentrations. This disagrees with the work of Wakoli *et al*. [23], who reported that the food sample with mixed spices spoiled faster than those with the rest of the selected spices. This can be due to the combined spices' synergistic effect, which could increase the antimicrobial activity.

The increased shelf life of ginger agrees with the work of [29], who reported the effect of ginger in increasing the shelf life of zobo drinks at specific concentrations. Unfortunately, the control sample containing a synthetic preservative was able to stop spoilage for up to four weeks, after which rancid spoilage was observed. For the consumer acceptability tests, the food samples treated with different spice concentrations were well accepted by the panelists in terms of appearance, taste, texture, flavor, and overall acceptability. Interestingly, the sensory evaluation result shows a significant difference ($p<0.5$) between the samples with the highest concentration and other samples. The food samples treated with 0.25 g and 0.5 g of all the spices had higher ratings than the ones spiced with 0.75 G, which is the least appreciated result of the higher concentration of the extracts. The unspiced (control) samples had the lowest ratings compared to the spiced ones in all the sensory attributes, indicating the potential of the spices to produce acceptable food products. This is in line with the work of Adedeji and Ade-Omowaye [12] with similar results.

CONCLUSION

Spices such as Ginger and alligator pepper and their mixture have higher antioxidants and anti-inflammatory content and can increase the shelf life of food at specific concentrations, especially mixed spices, which indicate the synergistic effect of these spices when combined. The spices should, therefore, replace synthetic preservatives in the food industry as they can preserve and increase some nutritional contents of food. However, the spices should be treated with care as higher concentrations may affect the organoleptic properties of the food. Further research is needed to test other spices' efficacy for their preservative properties. Other extraction methods also need to be explored to obtain the best extract that can beat the synthetic preservation.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENT

The authors would like to acknowledge financial support from TETFund through the Institution-Based Research (IBR).

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