Preservative effects of aqueous and ether extracts of *Aframomum melegueta* on West African soft cheese

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Abstract

The Total Bacterial Count, proximate composition, and sensory evaluation of West African Soft Cheese supplemented with aqueous and ether extracts of the spice *Aframomum melegueta* (at 0%, 1%, 2% and 3% each) were determined with a completely randomized design. Effects of the spice were monitored for 3 days. The results revealed that 3% concentration of the ether extract of the spice was the most inhibitory (p<0.05) of microbial growth for the duration of the experiment and it also significantly improved the fat, ash and protein contents of the cheese. The study recommended the addition of 3% ether extract of *A. melegueta* as an additive to fortify West African soft cheese.

Key words: cheese quality, microbial load, sensory evaluation, spice


Introduction

Cheese is one of the most important products of milk, known worldwide for its palatability, high nutritional content and versatility. It is highly desirable and can be taken as food or snack by people of all ages. Its high nutritional content is also an added advantage as it is a very rich source of protein, vitamins, and minerals which are essential for developing children and convalescing adults. Its ability to be used in almost any dish and also as a substitute for meat and fish adds to its popularity. Cheese is made in almost every country of the world with the existence of more than 2,000 varieties (O’Connor, 1993). It is today a major business worth billions of dollars in many industrialized countries. Indeed, cheese varieties are now unique products in their own right and cheesemaking has advanced beyond being merely a food preservation technique (Aworh, 2008). West African soft cheese *‘wara’* is it’s short shelf life of 2-3 days when immersed in whey (Belewu et al. 2005). A lot of research has gone into the improvement of its shelf life by using different preservatives. (Aworh & Egounlety, 1985; Anon, 1995). The use of 0.8% propionic acid and 0.8% sodium benzoate in the preservation of cheese for 8 days have been reported by Joseph and Akinyosoye (1997). Preservatives used in the food industry however are coming under increasing scrutiny and reappraisal. Synthetic antioxidants are effective oil stabilisers but concern about their possible adverse effects on consumption is increasing. Thus there is the need to consider the potency of natural spices and ingredients, as reported by Ashaye et al. (2006) and Belewu et al. (2005). This study aims at addressing the preservative capacity of alligator pepper (*Aframomum melegueta*) which has been found to have strong antimicrobial properties, and capable of improving nutritional components of soft cheese in storage. The seeds have pungent peppery taste due to aromatic ketones (Galal, 1996). According to Oladunmoye & Dada (2007), *Aframomum melegueta* was tested for antimicrobial effects on five pathogenic bacteria which include *Bacillus cereus, Staphylococcus*

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**Materials and Methods**

**Preparation of aqueous extract of *Aframomum melegueta***

The powder of the seeds was extracted as follows: The powdered seeds (250 g) were put in a round bottom flask. One litre of sterile distilled water was added into each flask, covered with aluminum foil and allowed to stand for about five days. The mixture was thoroughly shaken and filtered. The filtrate was then concentrated by heating over a water bath for evaporation of significant amount of moisture. The crude extract was then obtained in a beaker, and kept in the refrigerator at 4ºC (Bankole and Somorin, 2010).

**Preparation of ether extract of *A. melegueta***

The seeds were winnowed and milled into powder using mortar and pestle, 50 g of the powdered seeds was percolated with 250 ml of petroleum ether at room temperature (27ºC) for 24 hours and filtered. The extract obtained was concentrated at 50ºC (Afolabi et al., 2011).

**Preparation of West African soft cheese***

Some quantities of sodom apple stem (*Calotropis procera*) were collected, rinsed air dried and crushed with a sterilized enamel mortar and pestle. Ten grams (10 g) of the mash was weighed and sieved with 20 ml of distilled water (Badmos & Joseph, 2012), and labeled as sodom apple extract. Milk of 100ml was poured into each of seventy (70) heating vessels (7 treatments in 10 replicates), which were immersed in a heated water bath. The milk in the vessels were heated to approximately 50ºC for about 30 - 40 minutes, after which 3 ml of the fortified sodom apple extract (fortified with *A. melegueta* extract as described in the treatment plan below) were added to the warm milk in each vessel. Heating continued until coagulation started about 5 minutes later. It was boiled for a further 20 minutes to enable whey expulsion and inactivate the plant enzymes. The curd was then removed and put in a raffia basket to enable drainage of whey and give it shape and size.

**The treatment plan***

Seven (7) varying levels of *A. melegueta* (alligator pepper) fortifications/ inoculation represent 7 treatments, viz: Treatment 1, no extract added (Control); Treatment 2, 1 percent aqueous *A. melegueta* extract; Treatment 3, 2 percent aqueous *A. melegueta* extract; Treatment 4, 3 percent aqueous *A. melegueta* extract; Treatment 5, 1 percent ether *A. melegueta* extract; Treatment 6, 2 percent ether *A. melegueta* extract and Treatment 7, 3 percent ether *A. melegueta* extract.

**Parameters evaluated***

These include proximate composition- crude protein, moisture content, ether extract, and ash (AOAC, 1995); and Total Bacterial Count (TBC); sensory evaluation (flavour, texture, taste and overall acceptability), which was done after 48 hours of incubation at 37 ºC by counting individual colony in the petri dish (Fawole & Osho, 2007). Sensory analysis (flavour, texture, taste and overall acceptability) of the cheese samples was also made by a panel of judges. A thirty member trained panelists familiar with the taste of Cheese examined the samples independently (Lammond, 1970; Ashaye et al., 2006). Evaluation was based on colour, taste, texture, aroma, and the overall acceptability. Score was based on hedonic scale of 1-9 (1 = like extremely and 9 = dislike extremely).

**Statistical analysis***

The data collected from the above were subjected to the analysis of variance (ANOVA) by the Completely Randomized Design. The difference between the means was separated using Duncan Multiple Range Test (Steel & Torrie, 1980). In total 10 replications were performed.

**Results***

Table 1 shows that soft Cheese fat content increased (p<0.05) with increasing level of...
extract. The highest cheese fat was obtained by the 3 % ether extract, while the control cheese had the lowest fat content. The cheese protein content increased steadily (p<0.05) with increasing levels of the extract of *A. melegueta*. The trend was not consistent for the aqueous extract of the spice. The highest cheese protein was obtained by the 3% ether extract, while the 1% aqueous extract cheese was lowest in protein. The cheese ash content was higher (p<0.05) with increasing levels of the extracts, and the 3% ether extract gave the highest cheese ash. The cheese moisture content was similar in all the treatments, but was significantly lowest for the 3% ether extract. Cheeses made from higher levels of extract were consistently lower (p<0.05) in TBC for the three days of microbiological examination (Table 2). The counts increased from day 1 to day 2, and onwards to day 3. The rise was however smaller with higher levels of the extract. The 3% ether extract appeared to be most inhibitory on bacteria growth, and actually decreased the bacteria population in day 3.

The sensory study (Table 3) showed that the control cheese was the most preferred (p<0.05) by the panelists while the cheese containing ether extract of the spice was not so preferred by the panelists. The ratings followed this same trend for cheese colour, taste, aroma, texture, and general acceptability.

**Table 1.** Proximate analysis of West African soft cheese treated with aqueous and ether extracts of *A. melegueta*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Eth1%</th>
<th>Eth2%</th>
<th>Eth3%</th>
<th>Aq1%</th>
<th>Aq2%</th>
<th>Aq3%</th>
<th>±SEM</th>
<th>F&lt;sub&gt;0.05&lt;/sub&gt;</th>
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<td>20.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.84&lt;sup*e&lt;/sup&gt;</td>
<td>20.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.56&lt;sup&gt;e&lt;/sup&gt;</td>
<td>20.89&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.06</td>
<td>522.74&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>Protein</td>
<td>12.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.99&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12.10&lt;sup&gt;f&lt;/sup&gt;</td>
<td>12.00&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.01</td>
<td>23.102&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>1.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.60&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>0.07</td>
<td>50.15&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture</td>
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<td>55.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>53.47&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>173.78&lt;sup&gt;*&lt;/sup&gt;</td>
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<sup>abc</sup>: means followed by different superscripts in the same row differ significantly. Eth/ Aq means percentage of ether and aqueous extracts

**Table 2.** Bacterial load of West African soft cheese treated with aqueous and ether extracts of *A. melegueta* (10<sup>6</sup>cfu/ml)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Eth1%</th>
<th>Eth2%</th>
<th>Eth3%</th>
<th>Aq1%</th>
<th>Aq2%</th>
<th>Aq3%</th>
<th>±SEM</th>
<th>F&lt;sub&gt;0.05&lt;/sub&gt;</th>
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<tr>
<td>0</td>
<td>1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.80&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>1.14&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>14.73E2&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
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<td>7.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.53&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.24&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.38&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.00</td>
<td>36.96E4&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>7.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.24&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.41&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.99&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.00</td>
<td>88.61E2&lt;sup&gt;*&lt;/sup&gt;</td>
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</table>

<sup>abc</sup>: means followed by different superscripts in the same row differ significantly. Eth/ Aq means percentage of ether and aqueous extracts

**Table 3.** Effect of aqueous and ether extracts of *A. Melegueta* on the sensory quality of West African soft cheese

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Eth1%</th>
<th>Eth2%</th>
<th>Eth3%</th>
<th>Aq1%</th>
<th>Aq2%</th>
<th>Aq3%</th>
<th>±SEM</th>
<th>F&lt;sub&gt;0.05&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>Colour</td>
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<td>2.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.43&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>0.04</td>
<td>60.34E4&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Taste</td>
<td>4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.93&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.13&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.00</td>
<td>217.55&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Aroma</td>
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<td>2.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.93&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2.59&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>0.08</td>
<td>2.22&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>Texture</td>
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<td>2.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.81&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>0.01</td>
<td>421.09&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>Acceptability</td>
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<td>2.59&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2.96&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>3.21&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.07</td>
<td>56.87&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abc</sup>: means followed by different superscripts in the same row differ significantly. Eth/ Aq means percentage of ether and aqueous extracts
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Discussion

The ash content of the cheese was higher with increasing level of extracts, and particularly so for the ether extract treatments. This may be due to the minerals present in the spice and those produced by microbial activities (Adegoke and Gopalakrishna, 1998; Ashaye et al., 2006). Odebunmi et al. reported that A. melegueta has considerably high quantities of calcium (388 mg/Kg dry matter), magnesium (960 mg/Kg), iron (37.8 mg/Kg), zinc (32.93 mg/Kg) and manganese (68.53 mg/Kg). The extract of the spice is thus expected to be rich in minerals.

The increase of cheese fat content with increasing level of ether extract inclusion could be due to the fact that the ether was able to extract most of the fat in the spice (Dari, 2009) and at an inclusion level of 3%, the fat content extracted was higher when compared to the other inclusion levels. Odebunmi et al.(2009) reported a crude fat content of 2.55% for A. melegueta. There was no significant difference in the protein content between the treatments, but the 3% ether extract cheese had the numerically highest protein content. It appeared that many factors affected the cheese protein content, and the interaction of such factors did not allow for a simple trend. The relationship between cheese protein and fat has been earlier established. Chen et. al. (1996) reported that casein forms the structural matrix that traps moisture and fat. The presence of some microorganisms and/or their enzymes in the control (which had the highest microbial count) is another factor. These microbes aid in the synthesis of nitrogenous substance (Ashaye et al., 2006), thereby increasing the protein content. The moisture content of the cheese were statistically similar, except for the 3% ether extract cheese which was the lowest. This might be due to the high fat content as reported above. Higher content of extract might have enhanced higher activities of microorganisms and enzymes produced by them, as was reported by Ashaye et al., (2006). This differences in volume would definitely have allowed for higher fat content with increasing level of extract.

The sensory study showed that the control cheese was the most preferred by the panelists while the cheese from the ether extract of the spice was the least preferred by the panelists. This may be attributed to the fact the panelists of bacteria growth, on the other hand, the aqueous extract of the spice had the least effect on the bacteria isolates and microbial load of the cheese produced. The control was observed to have the highest microbial load among all the treatments. The difference in the antibacterial properties of the plant extracts might be attributable to age of the plant, extracting solvent, method of extraction and time of harvesting of plant materials (Amadioha et al, 1999). The result of this work showed that the seed extract of A. melegueta inhibited the growth of bacteria. The antibacterial effect of A. melegueta is apparently due to the phytochemical constituents present in the extract. A. melegueta seeds have been reported to be a rich source of phytonutrients such as flavonoids, phenolic compound tannins, sapolin, terpenoids, cardiac glycosides and alkaloids (Doherty et al, 2010; Chiejina & Ukeh, 2012).

The biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Okwu, 2004). This may be the reason for the use of the extracts of this plant in the treatment of intestinal troubles in herbal medicine (Okwu, 2004). The presence of phenolic compounds in the seed of A. melegueta is also an indication of the plant being an antibacterial agent. This is because phenols and phenolic compounds have been extensively used in disinfection and remains the standard with which other bactericides are compared. Phenolic compounds as electron donors are readily oxidized to form phenolate ions or quinine, an electron acceptor (Doherty et al, 2010). Extracts from A. melegueta therefore have potent antiseptic or bactericidal properties (Okwu, 2004). Ilondu et al. (2001) reported that some plants contain phenolic substances and essential oils, which are inhibitory to microorganisms. The presence of these compounds in these extracts has also been reported to be responsible for their antifungal properties (Ahmed & Stoll, 1996).

The sensory study showed that the control cheese was the most preferred by the panelists while the cheese from the ether extract of the spice was the least preferred by the panelists.
were more familiar with the sensory qualities of the cheese produced without the extract and it could also be attributed to the quantity of saponin content in the extracts. Some of the general characteristics of saponin include: formation of foam in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). The high tannin content could be partly responsible for the hot, bitter and pungent taste of *A. melegueta* seed (Doherty et al., 2010).

**Conclusion**

Ether extract of the spice generally had the highest inhibitory effect on the isolates, the microbial load and nutritional value of cheese made from it, with 3% of the extract being the most effective. It was also effective in significantly improving the percentage cheese yield, although its sensory attributes were not good. From the results above, it is recommended that 3% ether extract of the spice *A. melegueta* should be added as a supplement in cheese preparation as it can significantly reduce the bacterial load of the cheese and also significantly improve the nutritional value of West African Soft Cheese made from it. Further work could be done to blend the *A. melegueta* fortification with other natural additives like honey as this might further improve the sensory and microbial status of the cheese.

**References**


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