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Effect of alligator pepper (*Aframommum melegueta*) and honey on proximate composition, microbial and sensory properties of West African soft cheese

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ABSTRACT: Cheese is a highly nutritious food but the supply is highly limited by its short shelf life in the tropics. Several attempts have been made through the use of natural preservatives to increase the shelf life of cheese. In this study, the effect of honey and ether extract of *Aframommum melegueta* (*A. melegueta*) on proximate, microbial and sensory properties of cheese was investigated using three levels (0, 3, and 6%) of the two additives in a 3×3 factorial experiment. The sensory value of the cheese samples was assessed by a 30-member panel, the proximate values determined and the bacterial status monitored over a 3-day period. The results showed at 3% level each of honey and ether extract of *A. melegueta* (3H;3A), there was a significant improvement in the protein and fat content of the soft cheese. The control cheese without the addition of the additives and the cheese fortified with 3% *A. melegueta* extract were the most acceptable to the sensory panelists. Lactic acid bacteria and non-lactic isolates were isolated from the cheese. The cheese treated with 6 % honey and 6 % *A. melegueta* extract (6H, 6A) had the lowest bacterial count. The following blends of honey and ether extract of *A. melegueta* - 6H, 6A; 3H;3A; 0H;6A and 6H;0A is recommended as preservatives and additives of West African Soft Cheese.

Keywords: Acceptability; Additives; Proximate composition; Bacterial assay.

Introduction

Cheese is a food derived from milk that is produced in a wide range of flavors, textures, and forms by coagulation of the milk protein casein. The West African soft cheese is an unripened soft cheese that is usually made by the addition of the extract from the Sodom apple plant (*Calotropis procera*) to the whole milk from cattle (Akinloye and Adewumi, 2014: Belewu, 2019).

The Nutritional importance of cheese has been overcome in West Africa by poor refrigerating facilities and poor preservative media (Oladipo and Jadesinmi, 2013). Many of the synthetic preservatives (expensive as they are) have major side effects and could be carcinogenic in nature (Alvarez-Suarez *et al*, 2010), hence the search of new, safer, and effective natural preservatives for the West African soft cheese. Natural preservatives earlier reported (Doherty *et al*., 2010) include Honey and Alligator pepper (*Aframomum melegueta*). The latter is a tropical herbaceous perennial plant of the genus Aframomum, which belongs to the family Zingiberaceae. The seeds have pungent peppery taste due to aromatic ketones (Iwu, 2014). It is a plant with both medicinal and nutritive values, found commonly in the rain forests.

The phytochemicals obtained from the seed of *A. melegueta* has been used for years in the treatment of infectious diseases. The seeds of *A. melegueta* possess active ingredients including phenolic compounds (saponins and tannins etc) that may be exploited for local development of antimicrobials (Sharma *et al*., 2016). Honey is a sweet and flavorful natural product, which is consumed for its high nutritive value and for its effects on human health, with antioxidant, bacteriostatic, anti-inflammatory, and antimicrobial properties. Honey contains alkaloids, flavonoids, glucosides, saponins and reducing sugars. (Alvarez-Suarez *et al*., 2010; Nwankwo *et al*. 2014). Badmos *et al*. (2017) reported preservative potency of *A. melegueta* extracts on soft cheese, while Belewu *et al*. (2011) also reported the use of honey and Moringa oil to reduce the microbial contamination of the West African soft cheese, and concluded that the synergistic use of different food additives has shown better quality and enhanced shelf-life of West African soft cheese compared to using a single additive. There is a need to study the combined preservative effect of honey and alligator pepper

The aim of this study is to determine the cheese preservative potency of (blends of) honey and ether extract of alligator pepper. The proximate, sensory, and microbial properties of fortified West African soft cheese in storage were studied.

Materials and Methods

Preparation of Honey solution: Pure honey purchased from University of Ilorin Apiary was diluted (at 10 percent) with distilled water (Badmos and Abdulsalam, 2012).

Preparation of Ether Extract of *A. melegueta*: The seeds of *A. melegueta* were winnowed and milled into powder using mortar and pestle and 50 g of the powdered seeds was percolated with 250 ml of petroleum ether at room temperature for 24 hours and filtered. The extracts obtained were concentrated at 50 oC to give crude extracts of the seeds (Afolabi *et al*., 2011)

Sodom Apple Juice Extraction: Some quantities of Sodom apple stem were collected, rinsed, weighed, and crushed with a sterilized enamel mortar and pestle. This mash (10 g of it) was filtered with 20 ml of distilled water and the juice was squeezed out with a muslin cloth (Badmos and Joseph, 2012).

Cheese Production: Cattle milk (100 ml) was poured separately into ninety heating vessels (9 treatments x 10 replicates) which were immersed in a heated water bath. The milk in the vessels was heated to approximately 50 0C for about 35 minutes. Three ml of the extracted Sodom apple juice were then added to the warm milk in each vessel. Gentle heating continued until coagulation started about 15 minutes later and specified volumes of alligator pepper extract and/or honey solution were added to the coagulating cheese as in the treatment plan below. The curds were heated in whey for a further 20 minutes to enable

whey expulsion and inactivate the plant enzyme. This is similar to the methods reported by Belewu (2019) and Badmos *et al*. (2017). The cheese curds were afterward removed and taken for analysis.

Table 1: Experimental treatment plan

Treatments	Honey Juice (ml)	*A. melegueta* (ml)
1	0	0
2	3	0
3	6	0
4	0	3
5	3	3
6	6	3
7	0	6
8	3	6
9	6	6

Parameters evaluated

These include proximate composition, (Crude protein, moisture content, ether extract, and ash), sensory evaluation (flavour, texture, taste, and overall acceptability), and Total Bacterial Count.

Proximate Analysis: The proximate composition of the Cheese samples was determined using the method of A.O.A.C. (2010).

Sensory Analysis: A thirty (30) member trained panelists familiar with the taste of Cheese examined (each of the cheese) samples independently (Badmos *et al*., 2017), as the curds were removed from the raffia baskets. The evaluation was based on colour, texture, aroma, taste, overall acceptability. The score was based on a hedonic scale of 1-9 (1 = like extremely and 9 = dislike extremely).

Total Bacterial Count: Cheese Samples were aseptically taken daily for the next 48 hours after cheese production (cheese samples kept on the shelf) and the bacterial counts were determined according to Fawole and Osho (2007). For bacterial analysis, 10 g of each (Day 1) cheese sample was homogenized with 90 mL of sterile distilled water. Serial dilutions were prepared in 9ml sterile 0.1% peptone water until a dilution of 105 was achieved, 1ml was then plated on nutrient agar in triplicate. The plates were incubated at 37oC for 18-24 hours. The total number of viable bacterial cell colonies were counted for the three replications and the values expressed as Log10Cfu/g. The Days 2 and 3 cheese samples were similarly assessed and the colony counts similarly taken. The bacterial isolates were identified on day 2 as described by Fawole and Osho (2007).

Statistical Analysis: The data collected from the quality and quantity assessment were subjected to the analysis of variance using a 3×3 factorial experiment. The difference between the means was separated using Duncan Multiple range test using the SPSS version 16 tool (Steel and Torrie, 1980).

Results and Discussion

Nutritive Value: The dry matter, fat, crude protein and ash content of soft cheese (Table 2) were highest ($p \leq 0.5$) with the inclusion of 6 % honey as well as 6 % *A. melegueta* extract. This result corroborates the earlier report of Belewu and Morakinyo (2008) where the highest crude protein content was observed for the 15 % honey-treated cheese sample. Details of the interaction in this study (Figures 2 and 3) showed that Treatment 9, the blend of 6 % ether extract of *A. melegueta* plus 6 % honey (6H, 6A) gave the highest

cheese protein and fat content. The high cheese fat may be due to the fact that the ether was able to extract more fat from the spice at higher inclusion levels (Dari, 2009). According to the report of Belewu *et al*. (2005), natural preservatives (garlic and ginger) prevent the degradation of cheese protein and fat, by reducing lipid oxidation and the activities of proteolytic organisms. This is similar to the reports of improvement in protein and fat content with natural preservatives by Kikuzaki *et al*. (1994) and Badmos and Abdulsalam (2012).

Table 2: Effect Of Ether Extract of *A. melegueta* and Honey on the Nutritive Properties of West African Soft Cheese

		Parameters			
Cheese preservatives	Levels (%)	Dry Matter (%)	Fat (%)	Protein (%)	Ash (%)
Honey	0	38.94^{c}	15.77^{c}	14.27^{c}	1.63^{c}
	3	41.45^{b}	16.25^{b}	14.41^{b}	2.00^{b}
	6	42.70^{a}	16.80^{a}	15.22^{a}	2.11^{a}
	SEM	1.20	0.51	0.21	0.09
	P-value	0.012	0.038	0.001	0.044
A.melegeuta	0	41.83^{r}	15.72^{s}	14.62^{s}	1.77^{t}
	3	41.89^{r}	16.25^{rs}	14.56^{s}	1.90^{s}
	6	39.49^{s}	16.85^{r}	14.72^{r}	2.07^{r}
	SEM	1.01	0.31	0.87	0.04
	P-value	0.043	0.001	0.023	0.048
Honey*A.*melegeuta*		*	*	*	*

($^{abc}/^{rst}$): means followed by different superscripts along the column are significantly different; * indicates the interaction is significant.

(*): indicates the interaction between honey and *A. melegueta* is significant.

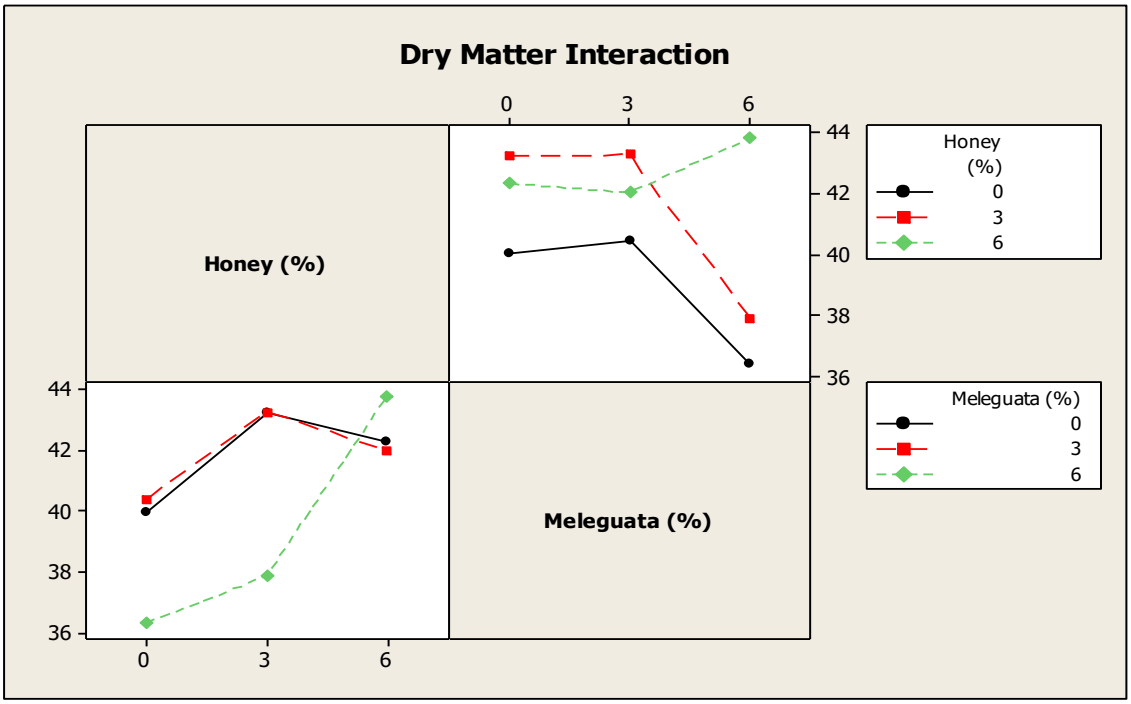

Figure 1: Interaction of the Effect of Honey and *A. melegueta* on Cheese Dry Matter

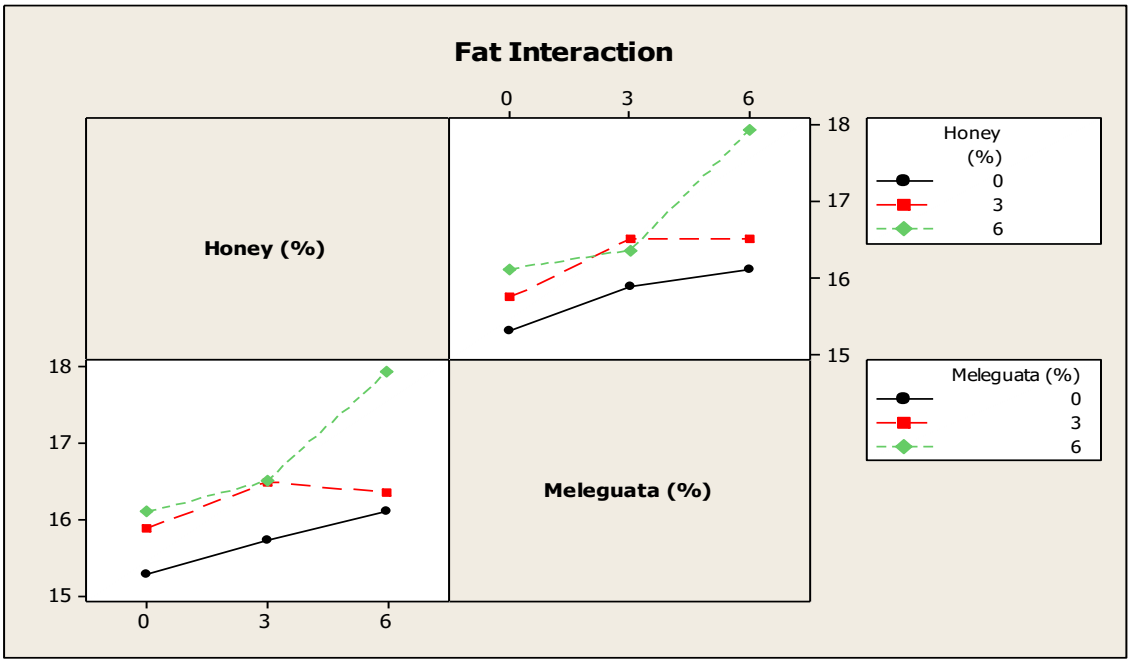

Figure 2: Interaction of the Effect of Honey and *A. melegueta* on Cheese Fat

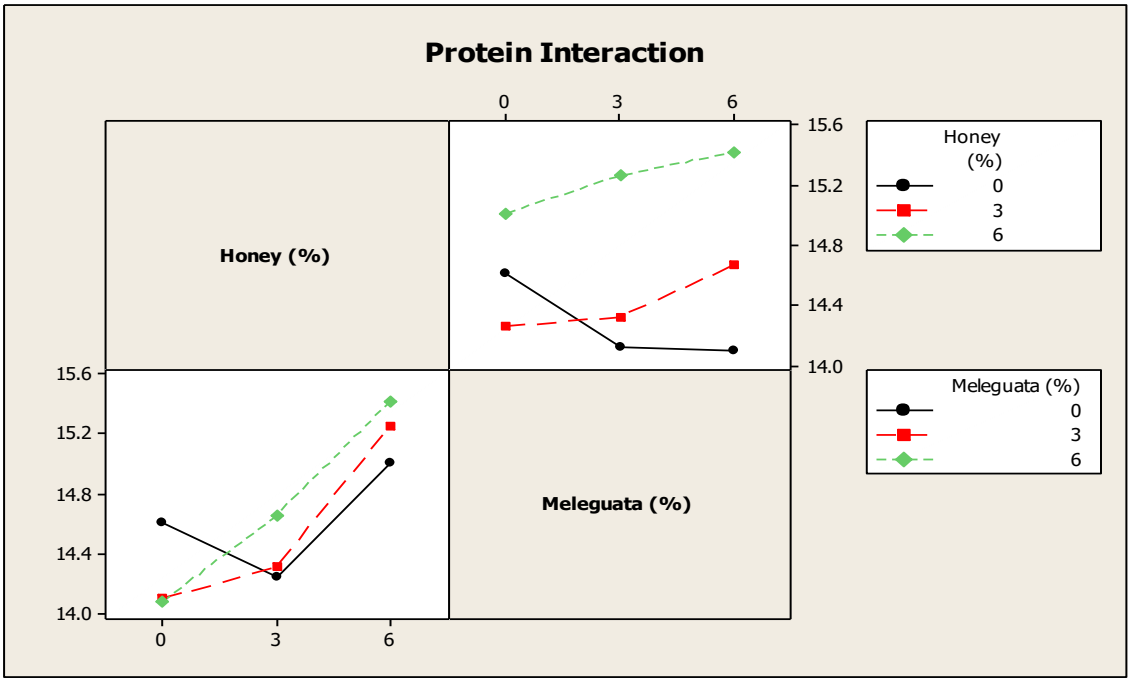

Figure 3: Interaction of the Effect of Honey and *A. melegueta* on Cheese Protein

Table 3: Effect of Ether Extract of *A. melegueta* and Honey on the Sensory Qualities of West African Soft Cheese

Cheese preservatives	Levels (%)	Sensory Parameter				
		Colour	Aroma	Taste	Texture	OA
Honey	0	6.33^{a}	6.00^{a}	5.50^{a}	5.67^{a}	6.00^{a}
	3	6.33^{a}	5.17^{b}	6.00^{b}	5.67^{a}	5.67^{b}
	6	5.00^{b}	4.33^{c}	4.33^{c}	4.67^{b}	4.67^{c}
	SEM	0.44	0.57	0.32	0.54	0.85
	P-value	0.03	0.04	0.01	0.00	0.01
A.melegeuta	0	7.00^{r}	5.46^{r}	6.00^{r}	6.00^{r}	6.33^{r}
	3	5.67^{s}	5.17^{s}	5.67^{s}	5.00^{s}	5.33^{s}
	6	5.00^{t}	4.67^{t}	4.17^{t}	5.00^{s}	4.67^{t}
	SEM	0.24	0.32	1.07	0.89	1.33
	P-value	0.00	0.04	0.04	0.001	0.03
Honey*A.*melegeuta*		*	*	*	*	*

$^{abc}/^{rst}$: means followed by different superscripts along the column are significantly different;
OA means overall acceptability.
*: indicates the interaction between honey and *A. melegueta* is significant.

The inclusion of honey and *A. melegueta* improved all the evaluated nutritive properties (Figure 1). Inclusion of 6 ml each of both honey and *A. melegueta* led to significantly higher cheese dry matter (4.4 %) compared to other preservative combinations. This shows a better synergistic effect of *A. melegueta* towards dry matter increase of the cheese. The cheese preserved with 6% *A. melegueta* and 6 % honey also exhibited a significantly higher fat content (1.8 %) than other preservative combinations which were not different from one another. Honey level of 6 % with additional levels of *A. melegueta* yielded a significantly higher cheese protein, and at an increasing trend. The highest ash (2.45 %) in the cheese was obtained when honey and *A. melegueta* were added at 6% levels and was different ($p \leq 0.5$) from other levels of the combinations.

Sensory Properties: The control was observed to be the most preferred by the panelists (Table 3). There was a decrease in cheese sensory values with an increase in cheese fortification levels. Details of the interaction (Figure 4) showed that the sensory values do not fit into a particular trend, but the control cheese (Treatment 1) had the highest overall acceptability, while the blend of 6 % ether extract of *A. melegueta* plus 6 % honey (6H, 6A- Treatment 9) was least acceptable. This may however be attributed to the fact the sensory panelists were more familiar with the cheese produced without the preservatives. It could also be attributed to the saponin content of *A. melegueta* which leads to the formation of foam in aqueous solution, hemolytic activity, cholesterol-binding properties, and bitterness (Okwu 2004). The high tannin content of *A. melegueta* could be partly responsible for the hot, bitter, and pungency taste of the treated cheese samples (Doherty *et al*., 2010).

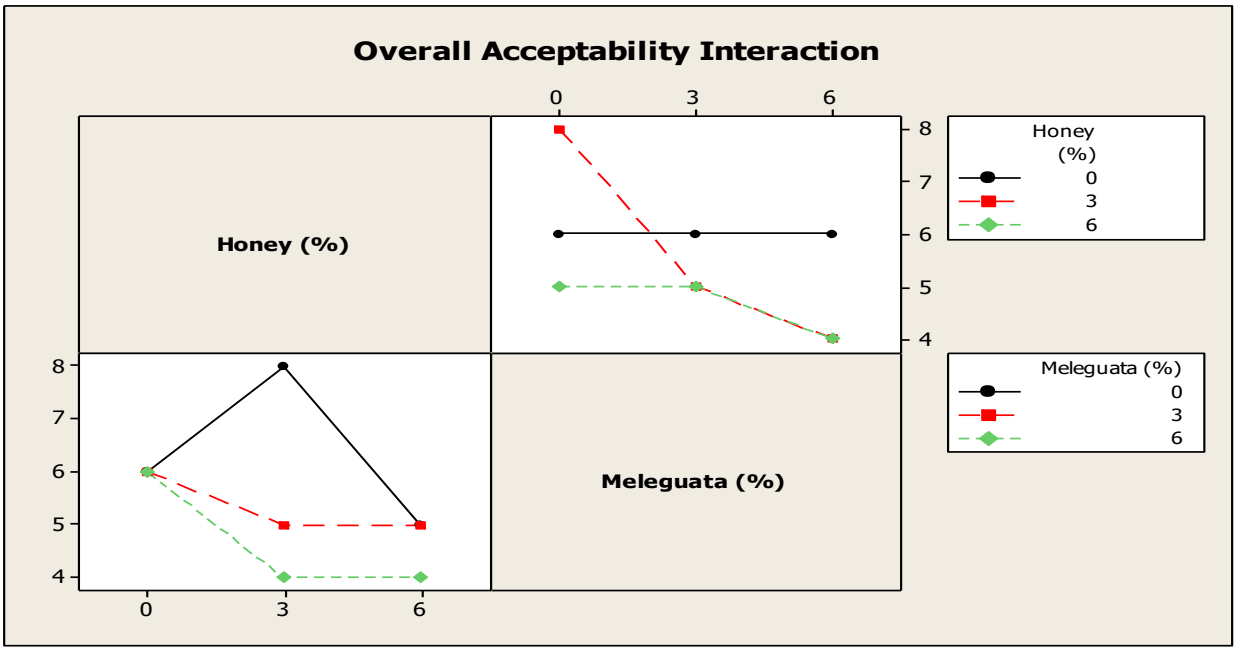

Figure 4: Interaction of the Effect of Honey and *A. melegueta* on Sensory Acceptability

A significant interaction was also observed between honey and *A. melegueta* for all the sensory parameters of the cheese. Cheese preserved using only 3% honey (0% *A. melegueta*) was the most preferred in terms of colour. Preservation using *A. melegueta* at 0% and 3% without honey was not significantly different from each other but were more preferred than the cheese with 6% *A. melegueta* and 0% honey. Cheese with 6% honey and 6% *A. melegueta* were of the least preferred colour. The highest taste scoring was obtained from the cheese preserved with 3% honey and 3% *A. melegueta*. This was followed by the control cheese (no preservative) which was not significantly different from the cheese preserved with 3% *A. melegueta* alone (without honey). The use of 6% of either *A. melegueta* or honey in combination with 0%, 3%, and 6% of honey or A. meleguata respectively led to the cheese of the least taste score. The cheese rated to be of the best aroma was the one without any preservative (0% honey and 0% *A. melegueta*). Cheese with 3% of *A. melegueta* without honey (0% honey) had the best aroma among the cheese with preservatives. Cheese containing 6% honey and 3% *A. melegueta* had a comparable aroma with the cheese with 3% honey added with 0%, 3% or 6% *A. melegueta*). The most preferred texture of the cheese by the panelist was the one without preservatives. Honey at 3% yielded a significantly better texture than 6% regardless of the *A. melegueta* level. The overall acceptability of the cheese preserved with 3% honey without *A. melegueta* was rated best while preservation by using only *A. melegueta* (3% and 6%) without honey yielded cheese of comparable acceptability with control cheese (0% honey; 0% cheese) which was ranked second. The cheese conditioned with 6% honey preservative with or without *A. melegueta* was rated similar but were of the least acceptability by the panelists.

Bacterial Count: Cheese preservation using both honey and *A. melegueta* at 3% each reduced the bacterial count to the lowest level (Table 4) which was not significantly different from the cheese preserved with both 6% honey with or without *A. melegueta* and 6% *A. melegueta* with or without honey for the considered number of days. The interaction between *A. melegueta* and Honey as cheese preservatives on the total bacteria count between day 1and 3 is presented in Figures 5, 6, and 7. The bacteria growth of the control cheese (0% honey and 0% cheese) was significantly higher than the cheese with preservatives regardless of storage day.

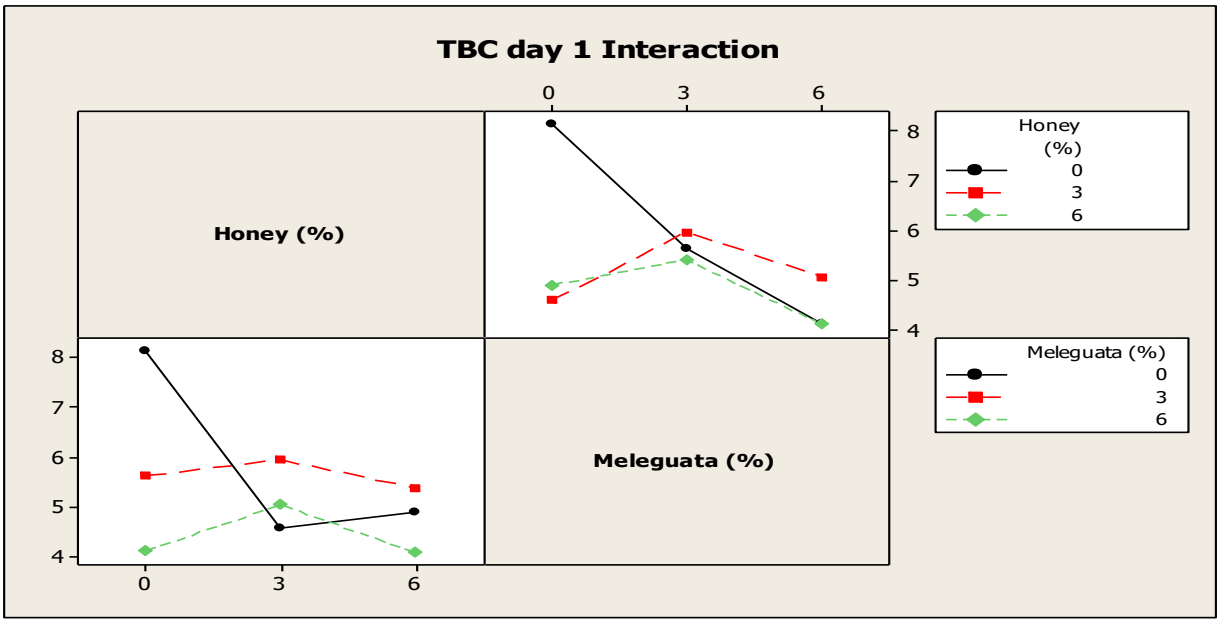

Figure 5: Interaction of the Effect of Honey and *A. melegueta* on Day 1 TBC

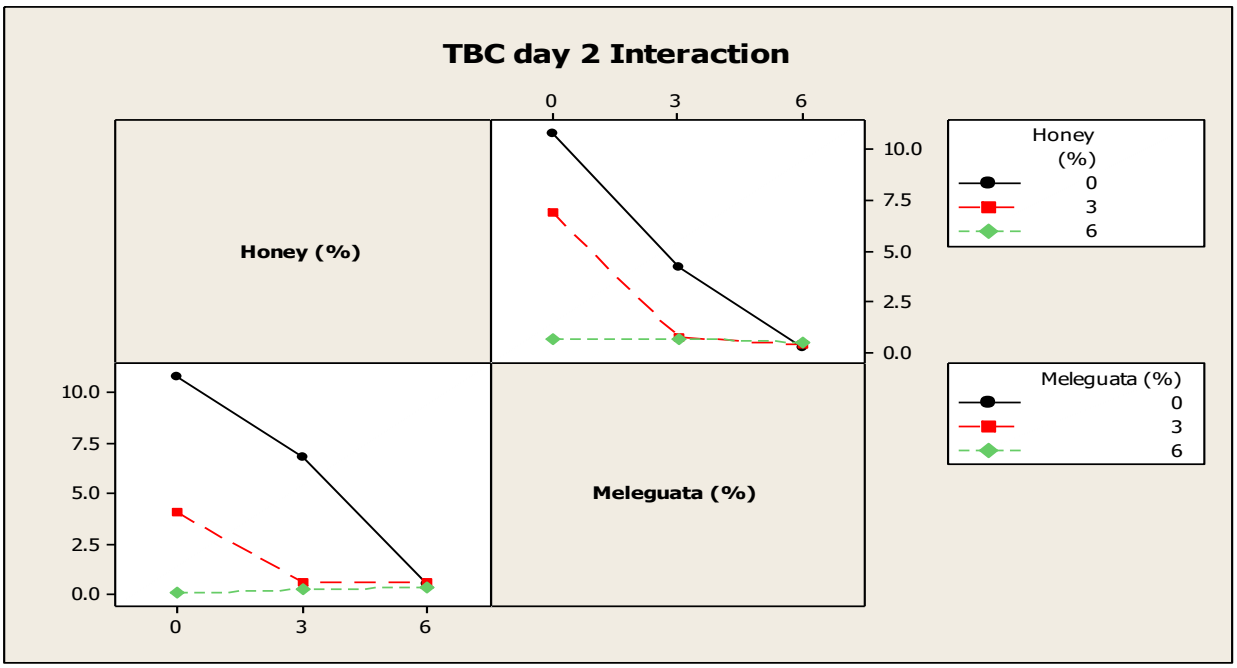

Figure 6: Interaction of the Effect of Honey and *A. melegueta* on Day 2 TBC

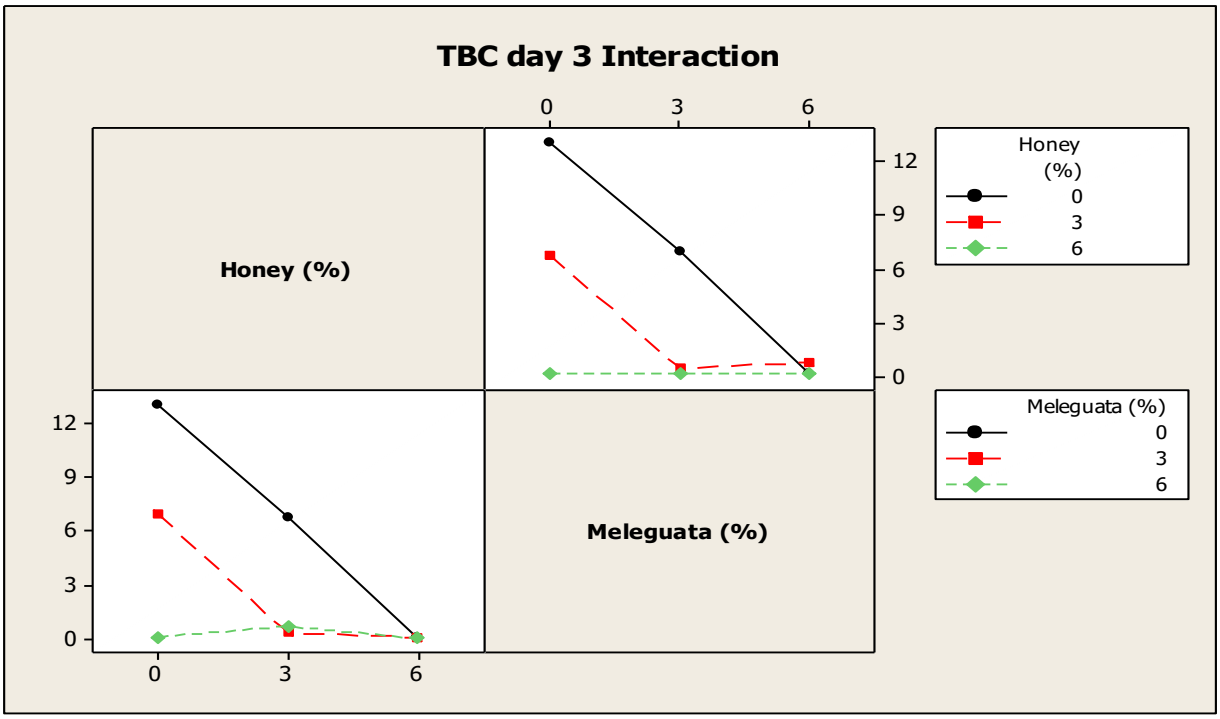

Figure 7: Interaction of the Effect of Honey and *A. melegueta* on Day 3 TBC

Bacterial Assay: Seven bacteria species were isolated from soft cheese in this study (Table 5). The control cheese (Treatment 1) contained all the bacteria isolates examined. Bacterial isolation studies show that Treatments 3, 5, 7 and 9 i.e. 0 % ether extract combined with 6 % Honey solution, 3 % ether extract combined with 3 % honey solution, 6 % ether extract combined with 0 % Honey solution, as well 6 % ether extract combined with 6 % honey are equally potent against the growth of various species, except Pseudomonas sps, which was observed on all treatment cheeses. Earlier work (Badmos *et al*., 2017) revealed that 3% concentration of the ether extract of *A. melegueta* was the most inhibitory ($p<0.05$) of microbial growth on cheese. The inhibitory potency of the honey solution against the growth of Streptococcus species and Escherichia coli has been reported by Mahendra and Kumarasamy (2015) and is in line with the findings in this study. The result of this work showed that the ether extract of the spice inhibited the growth of most isolates, and this shows that the extract is broad-spectrum inactivity. The treatment 9 (6 % ether extract combined with 6 % honey) has the most significantly reduced bacterial growth in cheese. The antibacterial potency of *A. melegueta* appears superior to that of honey solution. This might be due to the 10 % dilution of the honey, which reduced its potency. Afromomum melegueta seeds are reported to be rich in phytonutrients such as flavonoids, phenolic compounds, tannins, saponin, terpenoids, cardiac glucosides, and alkaloids (Doherty *et al*. 2010; Chiejina and Ukeh, 2012).

Table 4: Effect of Ether Extract of *A. melegueta* and Honey on Bacterial Load (TBC) of West African Soft Cheese (log_{10} Cfu/g)

Cheese preservatives	Levels (%)	TBC (log_{10} Cfu/g)		
		DAY 1	DAY 2	DAY 3
Honey	0	7.78^{a}	7.72^{a}	7.83^{a}
	3	7.72^{b}	7.41^{b}	7.42^{b}
	6	7.68^{c}	6.74^{c}	6.08^{c}
	SEM	0.04	0.11	0.34
	P-value	0.00	0.00	0.03
A.melegeuta	0	7.70^{s}	7.78^{r}	7.82^{r}
	3	7.75^{r}	7.26^{s}	7.40^{s}
	6	7.65^{t}	6.45^{t}	6.54^{t}
	SEM	0.12	0.25	0.12
	P-value	0.04	0.00	0.01
Honey*A.melegeuta		*	*	*

$^{abc}/^{rst}$: means followed by different superscripts along the column are significantly different;
* indicates the interaction is significant; Cfu/g; colony forming unit per gram;
TBC: Total Bacteria Count

Table 5: Inhibitory Effect of Ether Extract of *A. melegueta* and Honey on the growth of Bacteria Isolates of West African Soft Cheese

	Bacteria Isolates					
Preservative Treatments	*Pseudomonas Sp*	*Bacillus subtilis*	*Streptococcus spp*	*Lactobacillus casei*	*Proteus vulgaris*	*Micrococcus luteus*
Control (0%H, 0%AM)	+	+	+	+	+	+
0%H, 3%AM	+	-	-	-	-	+
0%H, 6%AM	+	-	-	-	-	-
3%H, 0%AM	+	-	+	-	-	-
3%H, 3%AM	+	-	-	-	-	-
3%H, 6%AM	+	+	-	-	-	-
6%H, 0%AM	+	-	-	-	-	-
6%H, 3%AM	+	+	-	-	-	+
6%H, 6%AM	+	-	-	-	-	-

- : Inhibition, + : No inhibition;
% H and % AM - percentage Honey and percentage *A. melegueta* respectively.

Conclusion and Recommendation

Inclusion of honey and *A. melegueta* ether extract blends in cheese had significant effects on the proximate, sensory, and bacterial parameters evaluated. The blend of 6 % ether extract of *A. melegueta* plus 6 % honey (6H, 6A) is recommended as an additive as well as other combinations like 3H;3A: 0H;6A and 6H;0A, as they can significantly improve microbial stability of West African soft cheese. The

inhibition of microbial growth is expected to improve shelf life and enhance the nutritional value of stored cheese. Further work needs to be done on improving the sensory acceptability of preserved cheese and determining the number of days and storage conditions related to the shelf life of preserved cheese.

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