

**Effect of Ethanol Extract of Clove on the Nutritive and Storage
Attributes of West African Soft Cheese**

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Abstract

The microbial spoilage of cheese results in multi-billion naira loss and global food insecurity. The need to preserve West African soft cheese using clove ethanolic extract and determine its preservative potency is the theme of this study. Five treatments studied were varied levels of inclusion of Clove Ethanolic Extract (CEE 0, 0.5, 1, 1.5 and 2ml) variously into 200 ml milk in a Completely Randomized Design. Sodom apple was the coagulant used, but was fortified with different inclusion levels of CEE into 200ml of coagulating milk. Parameters evaluated include bacterial count, nutritional composition and sensory analysis. It was observed that increasing the inclusion level of clove extract improved ($p < 0.5$) the protein, fat and sensory properties, while reducing bacterial growth. The study recommended that clove ethanolic extract is a potent additive and its addition at 1 % of coagulating milk will enhance the nutritional, sensory and storage attributes of West African soft cheese.

Keywords: *Nutritive value, Sensory values, Bacterial attributes*

Introduction

Cheese is universally desired food item, a highly nutritious food product made by the coagulation of milk protein known as casein (Badmos *et al*, 2018). Cheese is one of the major products obtained from processing the milk of cows, goats, sheep, buffalos, camels and yaks (Akinloye and Adewumi, 2014). It is rich in proteins, lipids, sugars, vitamins and minerals and it serves as an excellent medium of milk preservation (Hidreth, 1977; Belewu, 2019). Cheese is presently a highly commercialized food item (Aworh, 2008; Badmos *et al.*, 2017). The shelf life of Cheese however varies with its moisture

content and soft cheese, with moisture content above 60% has a shelf life of 3 days when in whey (Adegoke *et al.*, 1992; Belewu *et al.*, 2005). This is due to the high moisture content and almost neutral pH making cheese, particularly soft cheese an ideal growth medium for bacteria and spoilage micro-organisms (Adesokan *et al.*, 2008). There is a continuous need to inhibit bacterial growth and extend the shelf life of soft cheese, beyond the use of synthetic preservatives which are implicated in health problems (Alvarez-Suarez *et al.*, 2010). Natural preservatives are a more preferred option. Such natural spices and herbal products include Alligator pepper (*Aframomum melegueta*), Locust bean husk (*Parkia biglobosa*) eucalyptus oil, Ginger (*Allium sativum*), lemon grass oil and Sorghum stalk extract [Joseph and Akinyosoye (1997); Belewu *et al.* (2005); Belewu *et al.* (2012); Ashaye *et al.* (2006) and Badmos *et al.*, 2017)]. Clove is another additive with strong antimicrobial properties (Cock and Cheeseman, 2018). It is one of the most valuable spices that have been used traditionally as food preservatives and for many other purposes. This plant represents one of the richest sources of phenolic constituents as eugenol, and eugenol acetate possessing great potential for pharmaceutical, cosmetics, food and agricultural applications (Suárez-Vega *et al.*, 2024). Clove contains appreciable amounts of volatile oil used for flavouring foods and pharmaceuticals. The antioxidant and antimicrobial activities of clove are higher than for many fruits and spices (Burst and Reinders, 2003) and it has remarkable antibacterial, antiviral, anti-inflammatory, anti-mutagenic and antiparasitic attributes (Cai and Wu, 1996; Mittal *et al.*, 2023). The thrust of this study is thus to evaluate the potential of clove extract as a preservative and determine its efficacy as an additive to improve the nutritive and sensory properties of West African soft cheese in storage.

Materials and Methods

Preparation of clove ethanolic ethanol extracts (CEE): Fresh dried clove obtained from Ilorin Market was destoned, cleaned and blended using Black & Decker smartgrind and cuisinart spice nut grinder to a powdery form in the Animal production laboratory of the University of Ilorin. The clove powder was immersed in absolute unadulterated ethanol at ratio of 1g of the clove powder to 4ml of the ethanol which was stirred thoroughly, placed in an electric shaker and allowed for 48hrs to become a homogenous mixture. The clove- ethanol mixture was then transferred, filtered (bottle top filter) and evaporated in a water bath which was set at 50°C until a thick extract was obtained. (Zakaria *et al.*, 2011) This process took approximately 7 days and reconstitution followed with a Dimethyl Sulfoxide (DMSO) at concentration of 1024mg/ml (Zakariyah *et al.*, 2017).

Milk: Fresh cow milk was obtained from the cattle herd in Kara, Okeose, adjacent to the Teaching hospital of University of Ilorin Teaching Hospital, under strict hygienic guidelines. The milk was transported in an ice jacket within 25 minutes to the department of Animal Production (latitude 08030'N and longitude 04035'E) laboratory.

Sodom Apple juice extraction: Some quantities of Sodom apple (*Calotropis procera*) leaves were collected, rinsed and weighed. Portions were weighed to 10g,

crushed with a sterilized enamel mortar and pestle with addition of 20 ml water. The resulting mash was squeezed out with a muslin cloth (Badmos and Joseph, 2012).

Table 1: The Treatment Plan

Treatments	Eth Ext (ml)	Percent Inclusion	Sodom Apple (ml)
CEE 0	0	0	3.5
CEE1	0.5	0.25	3.5
CEE 2	1.0	0.5	3.5
CEE 3	1.5	0.75	3.5
CEE 4	2.0	1	3.5

Note: CEE means Clove Ethanol extract and Eth Ext means Ethanol Extract

Treatment Plan: Milk coagulation for all cheese samples was effected by the addition of Sodom apple leave as the coagulant after which different inclusion levels of the ethanol extract of clove (0.5ml, 1.0ml, 1.5ml, 2.0ml) were also measured into 200ml of milk. The control sample had no (sodom apple coagulant only) inclusion of ethanol extract of clove.

Cheese Preparation: Five stainless cups were rinsed and 200ml of milk was measured and poured into each cup i.e. each cup contained 200ml of milk. The cups were placed into a water bath. The milk was heated to approximately 45 °C and 3.5ml of Sodom apple extract was strained into each cup. Coagulation started after some few minutes and fortification was done at this stage with the clove extract being added to the coagulating curd at different levels (0.5, 1.0, 1.5 and 2.0ml as described in the treatment plan). The control sample cheese had no extract added (0 ml).The curd was heated further for 10minutes to inactivate the plant enzyme and facilitate whey expulsion (Belewu 2005; Badmos and Joseph 2012). The cheese curds were removed from the cups and ladled individually into raffia baskets to enable drainage of whey and to give it desired shape. The cheese samples were labelled and prepared for proximate, microbial and sensory analyses.This procedure was repeated 3 times for precision.

Proximate Analysis: Proximate content of cheese were conducted, including the crude protein, moisture, fat and ash contents according to AOAC (2000) methods.

Total Bacterial Count (TBC): The methods of Fawole and Osho (2007) and Badmos *et al*, (2018) was followed, where the cheese samples of Days 1, 3 and 5 were taken aseptically and subjected to serial dilution of cheese samples, such that 1g of cheese was diluted in 9ml distilled water. The samples were smeared on Agar plates and incubated for 48 hours at 37 °C. Counting of viable bacterial colonies for the respective cheese samples followed and the values were expressed as colony forming units per gram. Characterization and identification of the isolates in cheese samples used standards microbiological procedures (Barrow, 2004 and Garrity *et al*, 2005).

Sensory Analysis: A Thirty-member trained panellist, consisting of University of Ilorin students were employed for the test. Cheese samples in white plastic cups were labelled and placed on benches in a way that there was no interference between the panellists (Lammond, 1970; Badmos and Abdulsalam, 2012) and water was provided for the panelists to rinse their mouth after each taste to avoid interference or overlap between samples. Evaluation was based on colour, taste, flavour, texture and overall acceptability. Scores were based on hedonic scale of 1-9 (1=dislike extremely and 9=like extremely).

Statistical Analysis: The results obtained were subjected to analysis of variance (ANOVA) using the completely randomized design and the means were compared by Duncan Multiple Range Test (Steel *et al.*, 1997)

Result and Discussion

Phytochemical screening of ethanol clove extract: The screening of the ethanol extract of clove showed that saponin, steroid, triterpenoid and glycoside are present in the extract (Table 2). Other phytochemicals were not observed. Lone and Jain (2022) earlier reported that clove; a native of Indonesia contains alkaloids, flavonoids, tannins, sterols and triterpenes in its dry bud. Dahiru and Paliwal (2021) reported saponins, alkaloids, phytosterols, flavonoid pigments, tannins, terpenoids in clove. Bharadwaj (2020) reported phytochemicals in clove to include tannins, saponins, terpenoids and cardiac glycosides. These observations confirm the phytochemical screening in the present study.

Table 2: Phytochemicals present in ethanol extracts of cloves

Phytochemicals	Present
Saponin	+
Steroid	+
Triterpenoid	+
Glycoside	+

NOTE: *Phytochemical precense is +*

Bacterial count: The bacterial study shows that the control (CEE 0) had the highest microbial count (113.50×10^{-6} cfu/g) of colony forming units of bacteria throughout the five storage days. It can be observed from Table 3 that the Total bacterial count (TBC) increased as the days of storage increased for all the cheese samples. The effect of increasing the inclusion level of clove is general decrease in bacterial count for the cheese samples and for the days of storage. The bacterial population increased daily as expected with increasing spoilage but the spoilage rate was inhibited with clove extract addition. This observation on bacterial growth is consistent with the previous studies reporting that clove oil reduced total bacteria content in cake (Ibrahim *et al.*, 2013) and buffalo meat (Naveena *et al.*, 2017).Clove contains eugenol and other phytochemicals with intense antioxidant activity that is as potent as BHA, pyrogallol and other synthetic

anti-oxidants (Dorman *et al.* 2000) as linolenic acid peroxidation was inhibited above 95 % at concentration of 15 µg/ml. The phytochemicals in clove in addition to denaturing proteins of microbes, changes cell membrane permeability by reacting with phospholipids. Burst and Reinders (2003) reported that clove oil inhibited the growth of some *E. coli* strains. Gupta *et al.* (2014) similarly observed that clove oil inhibited the growth of gram-positive bacteria in food including *Listeria monocytogenes*, *Streptococcus aureus* and *Escheria faecalis*. These reports can explain the lower bacterial content of the cheese samples with clove extract in the present study.

Table 3: Total Bacterial Counts (TBC) of cheese samples (10⁻⁶cfu/g)

Treatment	Day 1	Day 3	Day 5
CEE0	58.00 ^c	80.00 ^d	113.50 ^e
CEE 0.5	35.00 ^b	69.50 ^c	87.00 ^d
CEE 1	33.00 ^b	57.00 ^{bc}	67.00 ^c
CEE 1.5	31.00 ^{ab}	54.50 ^b	56.50 ^{ab}
CEE 2	28.00 ^a	48.00 ^a	52.00 ^a
SEM	1.14	1.22	1.09

^{a-e}Means sharing the same lowercase letters in the same column differ significantly ($p < 0.05$). CEE means Clove Ethanol Extract

Table 4: Proximate Analysis of West African soft cheese samples

Treatment	Moisture (%)	Fat (%)	CP (%)	Ash (%)
CEE0	65.46 ^a	12.57 ^d	10.97 ^a	2.22 ^c
CEE0.5	65.17 ^b	13.17 ^b	11.33 ^a	2.42 ^b
CEE 1	65.12 ^b	12.84 ^c	11.37 ^a	2.39 ^b
CEE 1.5	65.09 ^{bc}	13.43 ^a	11.05 ^a	2.40 ^b
CEE 2	64.82 ^c	13.09 ^b	11.28 ^b	3.41 ^a
SEM	2.19	4.72	3.04	0.04

^{a-e}Means sharing the same lowercase letters in the same row differ significantly ($p < 0.05$). CEE means Clove Ethanol Extract
CP means Crude Protein

Cheese Nutritive Content: Table 4 shows the proximate composition of clove extract treated cheese samples. The control cheese (0% clove) was highest in moisture and the clove inclusion generally reduced the moisture content of cheese. The opposite was the case for the protein and fat content of cheese samples, two nutrients which

increased generally but inconsistently with increasing inclusion of clove extract. The ash content of the CEE 2 cheese was highest. The high protein and fat content observed with increasing clove extract content is apparently due to inhibition of proteolysis and lipolysis that is associated with bacterial growth. Belewu *et al.* (2013) earlier reported higher crude protein, ether extract, ash and dry matter in cheese with inclusion of ether extract of garlic. A similar nutrient content improvement was observed by Badmos *et al.* (2020) with treatment of soft cheese with honey and *Aframomum melegueta* (alligator pepper). Earlier studies (Kikuzaki *et al.*, 1994; Belewu and Morakinyo, 2008) had similar conclusion. The extraction of fat during the ether extract preparation might also be responsible for higher fat content (Dari, 2009) of clove extract treated cheese.

Sensory Properties: Table 5 shows the Appearance, Flavour, Taste, Texture and General Acceptability of soft cheese with inclusion of clove ethanolic extract. CEE 0.5 was rated best in Taste, while CEE 2 was rated best in Appearance, Flavour, Texture and General acceptability. The stringent taste of clove, particularly in the absence of sugar apparently reduced the taste preference with increasing clove extract inclusion in cheese samples. Badmos *et al.* (2018) reported similar observation on the use of *Parkia biglobosa* husk extract additive for soft cheese. The high fat content of CEE 2 might have been responsible for the high cheese flavour rating. The effect of cheese fat on flavour and consumer preference was earlier reported (Childs and Drake, 2009; Johnson *et al.*, 2001).

Table 5: Sensory values of West African soft cheese samples

Treatment	Appearance	Flavour	Taste	Texture	G. Acceptability
CEE0	7.4 ^a	6.5 ^b	6.45 ^b	4.75 ^c	5.25 ^d
CEE0.5	6.95 ^b	6.6 ^b	6.85 ^a	6.45 ^b	6.1 ^b
CEE 1	7.35 ^a	6.9 ^a	6.4 ^b	6.55 ^b	6.85 ^{ab}
CEE 1.5	6.35 ^b	6.9 ^a	6.2 ^b	6.6 ^b	5.85 ^{bc}
CEE 2	7.45 ^a	7.02 ^a	6.2 ^b	6.95 ^a	7.2 ^a
SEM	2.32	3.02	3.04	2.81	2.55

^{a-h}Means sharing the same lowercase letters in the same row differ significantly ($p < 0.05$). CEE means Clove Ethanol Extract

Conclusion

The conclusion of this study is that ethanolic extract of clove improved the dry matter, fat and protein of cheese, reduced bacterial growth and enhanced sensory properties of soft cheese. Clove extract is thus a potent additive and preservative of cheese and is hereby recommended to be included into coagulating cheese curd at the rate of 2ml extract per 200ml milk.

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