Impact of Physicochemical and Sensory Acceptability of Clove Oil Added Butter

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Abstract **-** Incorporation of clove oil to the butter is beneficial for human health. In this study, to developed the clove oil added butter and to evaluate the physical and chemical properties of of butter during storage. Butter was made using different concentration of clove oil (0.0%, 0.25%, 0.50% and 0.75% w w^{-1}) and kept in refrigerator at 4 ^{o}C for 8 weeks. The moisture, fat, pH, titratable acidity, free fatty acids contents and antioxidant activity as well as sensory characteristics of clove oil added butter samples were analysed during the storage period. At day 1, there is no any ($p>0.05$) changes observed among the treatment or between treatment during storage period but higher antioxidant activity was observed in butter with 0.75% clove oil. Highest and lowest values of moisture, titratable acidity and free fatty acids contents were recorded in without clove oil added butter and 0.75% clove oil added butter throughout the storage period, respectively. Fat contents and pH were higher in 0.75% clove oil added butter and lower values in without clove oil added butter during the storage period. In all butter sample, moisture, fat and pH were decreasing and titratable acidity and free fatty acids were decreasing until end of the storage period. While antioxidant activity was decreasing from day 1 to two weeks of storage in all butter samples. Sensory evaluation result revealed that majority of the panelist preferred butter with 0.50% clove oil in most of the sensory properties during the storage period.

Index Terms: *Butter, Clove oil, Physicochemical composition, Sensory properties, Storage*

I. INTRODUCTION

Butter plays an important role in supplying various health-enhancing components to the human diet. It aids in the prevention of various diseases, such as osteoporosis, atherosclerosis, and other degenerative disorders in humans. During the manufacture of sour butter, lactic acid bacteria, which can generate various metabolites during the fermentation process. Their metabolites have health enhancing functions, including antimicrobial activity on certain pathogens like Listeria monocytogenes and regulation of the immune system [1-2]. Butter undergoes oxidative degradation (rancidity) of fats during storage, resulting in an alteration of major quality parameters such as color, flavor, aroma, texture and nutritive value. During rancidity process, butter fat will hydrolysis and split into fatty acid chains. These free fatty acids can then undergo further auto oxidation. The development of rancidity reduce the shelf life of the butter [3]. Shelf life of the butter depends on the time taken for de-naturation of the fat or rancidity of the butter. Hydrolytic rancidity refers to the odor that develops when triglycerides are hydrolyzed and free fatty acids are released. The peroxide, when decomposed produce aldehyde, ketones and other volatile products. These products are responsible for rancid odor and flavor.

Clove oil is extracted by distillation of the flower, stems and leaves of the clove tree [4-5] and composed of eugenol (70-85%), eugenyl acetate (15%) and β-caryophyllene (5-12%). Other than that these volatile compounds, clove oil consists of some non-volatiles such as tannins, sterols, triterpenes and flavonoids. Clove oil has many essential ingredients beneficial for human health. It consists anti-bacterial properties and anti-cancer properties. It is a good medicine for arthritis, digestive problems, diarrhea, gastritis and vomiting. Other than that most important feature of the clove oil is anti-oxidant property. Clove and eugenol possess

strong antioxidant activity and considered to be better than synthetic antioxidants such as BHA (Butylated Hydroxyl Anisole) [6]. Adding clove oil to butter may increase the resistance of the fat to oxidation and consequent deterioration or rancidity. Adding of the clove oil to butter may also help to reduce the rancidity and increase the shelf life. In the previous study reported that clove oil used to increase the shelf life of Ghee [3]. Information is lacking using clove oil for butter production. Therefore, this study was designed to analyse the physico-chemical properties of clove oil added butter during the storage.

II. MATERIALS AND METHODS

A. Procedure of Butter Making

Milk was pasteurized at 68.5 °C for 30 minutes and cooled to 13 °C in an ice bath. Then cream was separated and churned until butter kernel was formed. The butter milk was separated from butter kernel by draining through the muslin cloth. The butter was pressed and washed with cold water. Salt was added at the rate of 2 $g100^{-1}$ g as indicated by Kaur *et al*.[7] . According to the method described by Shende *et al.* [3] clove oil was mixed at the rate of 0% (T1), 0.25% (T2), 0.50% (T3), 0.75% (T4) (w w^{-1}) during processing. Three replications were conducted for each sample. All samples were packed in plastic cups and kept under refrigerator condition (4 °C). The sample analysis was performed at day one, 2^{nd}, 4^{th}, 6^{th} and 8^{th} weeks of the storage period.

B. Determination of Moisture and Fat

These butter samples were analyzed in triplicate for dry matter by oven drying at 105 °C to get constant weight according to Park *et al.* [8] method and percentage of dry matter was calculated. The fat content of the butter was determined by the Gerber method as described by AOAC [9].

C. Determination of pH and Titratable Acidity

Ten gram of butter was grated and the pH of butter sample was measured directly using a digital pH meter (model: Delta 320 pH meter) after calibration with fresh pH 4.0 and 7.0 stranded buffer. The titrable acidity was determined by titrating with 0.1 N NaOH according to AOAC [9].

D. Determination of Free Fatty Acids

Free fatty acids was determined according the method described by Kaur *et al.* [7] with little modification. Five gram of butter fat was dissolved with 50 ml of neutralized ethanol. Then 0.5 mL of phenolphthalein was added and titrated with 0.1 N NaOH to obtain the disappearance of pink colour. Results were expressed as percentage of free fatty Aaids (FFAs) with references of oleic acid.

E. Determination of Antioxidant Activity

The total antioxidant capacity was estimated by ferric reducing antioxidant power (FRAP), assay [10]. FRAP reagent was prepared by mixing 1 mL of (10 ml L^{-1}) TPTZ solution in 40 mmol L^{-1} HCl, 1 ml of $FeCl_3$ (20 mmol L^{-1}) and 10 ml of acetate buffer, (0.3 mol L^{-1}, pH=3.6). Twenty microliters of the extract was mixed with 1 mL FRAP reagent, incubated at room temperature for minutes and the absorbance measured at 593 nm exactly after 4 min. FRAP reagent was used as a control. The absorbance of 1000 micro-liters $FeSO_4$ standard was measured following the same procedure as for the samples. The ferric reducing antioxidant power was expressed in mM g^{-1} fresh weight (FW).

F. Sensory Evaluation

In sensory evaluation, the samples were subjected to nine-point hedonic scale test, and the acceptability of samples was judged by 30 untrained panel members to determine consumer preference as described by Abdalla *et al*. [11].. The sensory characteristics such as color, flavor, taste, texture and overall acceptability of the butter samples was judged by the panelists at day 1, 2nd , 4th , 6th and 8th of weeks of the storage.

G. Statistical Analysis

Samples were randomly collected and tabulated. Multivariate Analysis of Variance (MANOVA) was carried out to evaluate the effect of oil concentrations and storage time using SAS software. Duncan's Multiple Range Test (DMRT) was employed to compare significant differences (P<0.05) between means. The sensory analysis was carried out using Friedmans test for non-parametric data analysis.

III. RESULTS AND DISCUSSION

A. Changes in Physicochemical Composition of Butter at Day One

The chemical constitution of butter made from buffalo milk with different concentration of clove oil were no significant differences (p>0.05) observed in the nutritional properties among the treatments (Table I). It might be that butter samples did not show considerable changes in bio chemical reaction. Therefore, there was no any significant differences (p>0.05) among moisture, fat, free fatty acid, titratable acidity and pH content in different concentration of clove oil added butter at day one.

B. Changes in Physicochemical Composition of Butter During Storage Period

Butter having a pleasing and appetizing aroma. It has a considerably longer shelf life as compared to other indigenous dairy products. It undergoes oxidative degradation during storage, resulting in an alteration of major

Table I: Physicochemical properties of clove oil added butter at Day 1

Attributes	Treatment			
	T_1	**T_2**	**T_3**	**T_4**
Moisture %	31.96±0.82[a]	31.23±0.57[a]	31.56±0.38[a]	30.66±0.67[a]
Fat %	43.01±0.69[a]	44.24±0.38[a]	44.53±0.41[a]	44.91±0.02[a]
pH	6.44±0.09[a]	6.44±0.08[a]	6.43±0.06[a]	6.32±0.09[a]
Titratable acidity %	0.060±0.00[a]	0.066±0.01[a]	0.066±0.01[a]	0.066±0.01[a]
Free fatty acid %	0.71±0.05[a]	0.63±0.05[a]	0.60±0.13[a]	0.63±0.05[a]

T1-without clove oil added butter T2-0.25% clove oil added butter T3-0.50% clove oil added butter T4-0.75% clove oil added butter. Values are means ± standard deviations of replicate determination. Mean with the same letters are not significantly different at (p<0.05).

quality parameters such as color, flavor, aroma and nutritive value. Moisture and fat content are the most important parameters for the determination of butter quality and shelf life. In addition to that acid value also very important to determine shelf life of the butter [12].

C. Moisture, pH and Titratable Acidity Content of the Butter During Storage Period

The moisture content of the butter among treatment (p<0.05) declined during the storage (Table II). At 2nd week of storage, higher and lower of moisture contents was recorded for without clove oil added butter (31.56±0.96%) and 0.75% clove oil added butter (28.83±0.38), respectively. Similarly at 8th week of storage, higher and lower of moisture contents was recorded without clove oil added butter (25.16±0.20%) and 0.75% clove oil added butter 24.83±0.41%), respectively. On the other hand, moisture content was gradually decreasing when increasing of clove oil in the butter. It was also observed earlier by Park *et al*. [8]. The

gradual decrease in moisture content of butter during storage was mostly due to expulsion of moisture from the butter and this may lead to the salting that took place during this period (Hofi *et al*. [13].

pH content of butter ($p<0.05$) decreased during the storage period. pH was increased with the increase of clove oil concentration whereas titatable acidity in the butter ($P< 0.05$) increased during storage period (Table III). The titratable acidity of the butter also normally decreased with increasing concentration of clove oil due to clove oil minimize the fermentation process in the butter. These results coincide with previous research findings of Mallia [14] (2008) and Mallia *et al*. [15] .

D. Fat and Free Fatty Acid of Butter During Storage Period

The value of the fat content decreased during the storage period (Table IV). At 2nd week of storage, higher and lower of fat contents was recorded for 0.75% clove oil added butter (44.15±0.04%) and without clove oil added butter (42.05±0.58%), respectively. Similar pattern was observed at 8th week of storage for higher and lower of fat contents were 41.99±0.09% for 0.75% clove oil added butter and 39.24±0.46% for without clove oil added, respectively. The decreasing of fat during storage due to formation of free fatty acid by hydrolyzing the fat. This result was supported with finding of Shende *et al*. [3]. They said that, butterfat was hydrolysis during the storage conditions. There was highly ($P<0.05$) increased in free fatty acid in all the samples during the storage period. The FFA level of the butter with clove oil also increased, but rate of increase was slower than butter

Table II: Changes of moisture content (%) of butter during storage period

Treatment	Week 2	Week 4	Week 6	Week 8
T 1	31.56 ± 0.96^{a}	30.60 ± 0.80^{ab}	29.26 ± 0.97^{cd}	25.16 ± 0.20^{g}
T 2	29.80 ± 0.21^{bc}	29.06 ± 0.30^{cd}	28.06 ± 0.49^{de}	24.96 ± 0.81^{g}
T 3	28.76 ± 0.28^{cde}	27.66 ± 0.49^{ef}	26.55 ± 0.80^{f}	24.76 ± 0.47^{g}
T 4	28.83 ± 0.38^{cde}	28.36 ± 0.46^{de}	27.63 ± 0.40^{ef}	24.83 ± 0.41^{g}

T1-withiut clove oil added butterl T2-0.25% clove oil added butter T3-0.50% clove oil added butter T4-0.75% clove oil added butter. Values are means ± standard deviations of replicate determination. Mean with the same letters are not significantly different at ($p<0.05$).

Table III: Changes of pH and Titratable Acidity of butter during storage period

	Attributes	Week 2	Week 4	Week 6	Week 8
T 1	pH	6.00 ± 0.10^{def}	5.68 ± 0.09^{g}	5.36 ± 0.09^{h}	5.04 ± 0.10^{i}
	Titratable Acidity %	0.13 ± 0.17^{e}	0.21 ± 0.14^{c}	0.28 ± 0.14^{b}	0.36 ± 0.14^{a}
T 2	pH	6.16 ± 0.05^{abcd}	5.89 ± 0.05^{ef}	5.64 ± 0.04^{g}	5.29 ± 0.16^{h}
	Titratable Acidity %	0.11 ± 0.07^{f}	0.16 ± 0.05^{d}	0.21 ± 0.05^{c}	0.27 ± 0.04^{b}
T 3	pH	6.30 ± 0.07^{ab}	6.15 ± 0.07^{abcd}	6.02 ± 0.07^{cdef}	5.87 ± 0.07^{f}
	Titratable Acidity %	0.10 ± 0.16^{g}	0.14 ± 0.14^{e}	0.17 ± 0.12^{d}	0.21 ± 0.06^{c}
T 4	pH	6.33 ± 0.09^{a}	6.22 ± 0.08^{abc}	6.10 ± 0.08^{bcde}	5.98 ± 0.09^{def}
	Titratable Acidity %	0.08 ± 0.007^{g}	0.10 ± 0.007^{fg}	0.12 ± 0.006^{ef}	0.14 ± 0.006^{e}

T1-withiut clove oil added butterl T2-0.25% clove oil added butter T3-0.50% clove oil added butter T4-0.75% clove oil added butter. Values are means ± standard deviations of replicate determination. Mean with the same letters are not significantly different at ($p<0.05$).

Table IV: Changes of Fat content and Free Fatty Acid in butter during storage period

Attributes		Week 2	Week 4	Week 6	Week 8
T 1	Fat (%)	42.05 ± 0.58^{fg}	41.09 ± 0.49^{hi}	40.46 ± 0.17^{i}	39.24 ± 0.46^{j}
	Free fatty acid	0.79 ± 0.53^{abd}	0.86 ± 0.52^{abc}	0.93 ± 0.51^{ab}	1.01 ± 0.51^{a}

T 2	Fat (%)	43.41 ± 0.43^{abcd}	42.73 ± 0.54^{def}	41.93 ± 0.53^{fg}	41.08 ± 0.51^{hi}
	Free fatty acid	0.69 ± 0.53^{cd}	0.74 ± 0.54^{bcd}	0.80 ± 0.53^{bcd}	0.85 ± 0.51^{abc}
T 3	Fat (%)	43.79 ± 0.52^{ab}	42.99 ± 0.40^{bcde}	42.25 ± 0.37^{efg}	41.38 ± 0.20^{gh}
	Free fatty acid	0.63 ± 0.39^{d}	0.67 ± 0.18^{cd}	0.71 ± 0.38^{cd}	0.74 ± 0.38^{bcd}
T 4	Fat (%)	44.15 ± 0.04^{a}	43.64 ± 0.04^{abc}	42.84 ± 0.05^{cdef}	41.99 ± 0.09^{fg}
	Free fatty acid	0.65 ± 0.052^{d}	0.67 ± 0.24^{cd}	0.70 ± 0.05^{cd}	0.71 ± 0.22^{cd}

T1-withiut clove oil added butterl T2-0.25% clove oil added butter T3-0.50% clove oil added butter T4-0.75% clove oil added butter. Values are means ± standard deviations of replicate determination. Mean with the same letters are not significantly different at ($p<0.05$).

without clove oil. The increase in free fatty acid content was mostly due to lipolysis of butter fat. The increase of free fatty acid of butter samples was similar to previously reported value of Shende *et al*. [3] and Sindhu *et al*. [16] . Who said that free fatty acid level increased due to hydrolysis as well as by oxidation of fat.

E. Antioxidant Activity of Clove Oil Added Butter

Table V shown that the antioxidant activity for day 1 and week 2 was recorded and the antioxidant activity was decreasing at the second week of storage. It might be reaction with lipid radicals and convert them into more stable products. Therefore clove oil acts as antioxidant, which maintains the self-life of the butter during storage.

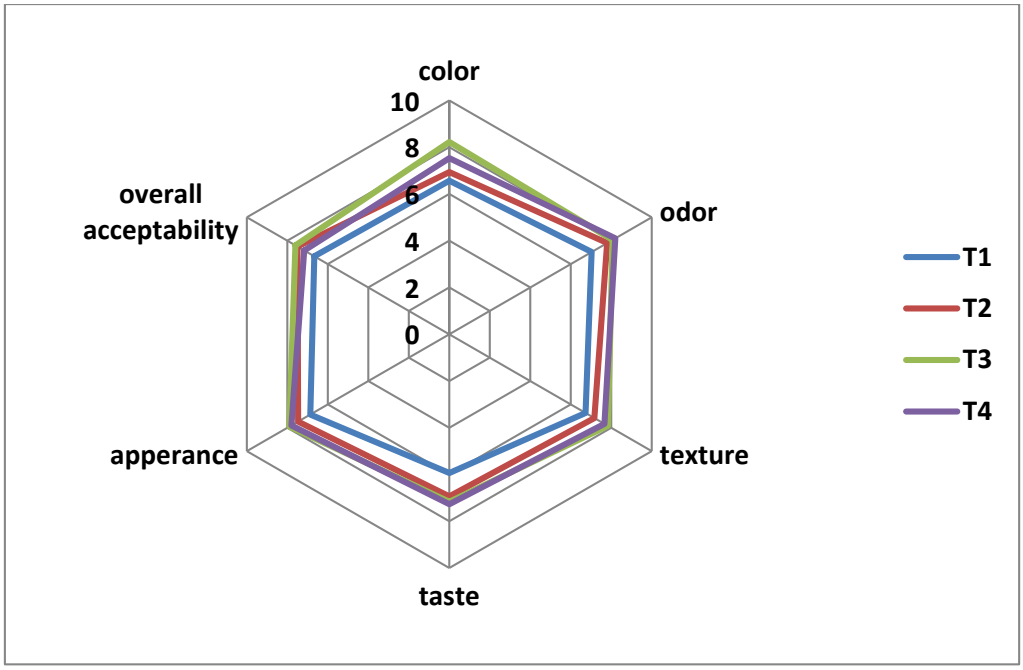
Table V. Antioxidant activity at 593 nm absorbance

Treatment	Day 1	Week 2
T_1	0.173	0.167
T_2	0.380	0.371
T_3	0.734	0.720
T_4	1.056	1.032

T1-without clove oil added butter T2-0.25% clove oil added butter T3-0.50% clove oil added butter T4-0.75% clove oil added butter added butter

F. Sensory Properties of Clove Oil Added Butter During Storage Period

Results of the sensory evaluation of samples on a scale from 1 (poor) to 9 (excellent) are shown in Figure 1. The sensory properties of clove oil added butter samples were evaluated on the attributes of colour, appearance, taste, flavor, texture and overall acceptability at day 1, week 2, week, week 4, week 6 and week 8. Sensory properties of the butter samples were found to be affected significantly by the addition of clove oil. Butter with 0.50% clove oil had higher score for odor, taste of butter at day one but no changes observed in color, texture and appearance in the butter samples. At the second week, highest score for the odor, taste and overall acceptability were given for the butter with 0.50% clove oil while lowest score for the taste, odor and overall acceptability is given for butter with 0.75% clove oil. There was a slight changes in texture, color and appearance of stored butter samples with the increase in storage period. However, no considerable changes occur in texture, color and appearance within the treatments. At fourth week of storage butter with 0.50% clove oil was received highest score for texture, color, appearance and overall acceptability among the panelist. At the sixth week of storage, higher score for texture, color, appearance and overall acceptability were recorded for butter made with 0.50% clove oil and butter without clove oil and butter with 0.25% clove oil showed lower score for all attributes. At 8^{th} week of storage, most of the panelists preferred for texture, color, appearance and overall acceptability for 0.50% clove oil added butter. However, organoleptic properties was showing a decreasing trends with storage period In overall quality, butted with 0.50% clove oil had more preference for sensory properties among the panelist during the storage period.



T1-without clove oil added butter T2-0.25% clove oil added butter T3-0.50% clove oil added butter T4-0.75% clove oil added butter added butter

Figure I: Overall quality of the butter during storage period

IV. CONCLUSION

In this study at day one, quality attributes like moisture and free fatty acid of with and without added butter did not ($p>0.05$) show the changes but titratable acidity, fat content, pH and antioxidant were varying within treatments. Free fatty acid level and ttratable acidity of clove oil added butter samples increased during the storage period while moisture, fat content, pH and antioxidant value was decreasing with storage period. Finally, butter with 0.50% clove oil was taken the highest score for the overall quality among the panelist throughout the storage period.

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