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Physico-chemical and sensory properties of unripened cheese fortified with food grade Vitamin C

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Abstract
Background*: Milk is a highly perishable product that is rich in nutrients, but deficient in vitamin C. In order to maintain itsnutrientsand physicochemical properties, it can be processed into products like cheese..Apart from the fact that vitamin C is not largely produced in milk it has been found that vitamin C content in milk is also reduced during heating process. Therefore, there is need to improve the vitamin C content in dairy products such as the soft cheese by its fortification with vitamin C.*
Materials and Methods*:Twentylitres of milk was obtained from lactating Bunaji cows and clarified and then divided into fifteen (15) parts of one litre. Each part (1L) was heated to a temperature of 50˚C with intermittent stirring. Thereafter, 20 g of coagulant (Calotropisprocera) leaves juice was added and immediately, vitamin C was added at varying levels of 0 mg (T1), 50 mg (T2), 100 mg (T3), 150 mg (T4) and 200 mg (T5) and allowed to form curd. The curd formed was drained to obtain the whey and both were stored for fourteen (14) days in the refrigerator.*
Results: *Storage effect revealed that vitamin C significantly ($p<0.05$) reduced in the whey (24.96-12.10mg/100) and in the cheese (32.10-18.55mg/100g) highest at day 1 period of storage and least at day 14. A decrease in pH (6.69– 6.45) was observed from day 1 to day 14. The highest free fatty acid was recorded as 8.10 mg/100 g at day 14 while 7.41 mg/100 g and 7.72 mg/100 g were recorded at 1 and 7 days storage periods respectively. The highest peroxide value (6.43 mg/100g) was at day 14 while days 1 and 7 recorded 5.23mg/100g and 5.79 mg/100g respectively. The 50g Vitamin C fortified cheese had the highest yield (23.30%) while cheese fortified with 150g Vitamin C had better acceptability response.*
Conclusion: ***T****he storage effect showed that Vitamin C concentration decreased with storage time in cheese, hence cheese fortified with vitamin should be consumed immediately inorder to get its full benefits.*
Vitamin C could improve cheese yield, however such cheese may decrease in quality with increased storage time.
Keywords*: Ascorbic acid, cheese yield, chemical, warakanshi, organoleptic, storage*

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I. Introduction

Unripened cheese is a fresh soft curd obtained after draining the whey and consumed fresh. Cheese is a product of milk and an excellent source of protein, fat, minerals, vitamins and essential amino acids, thus making it an important food in the diet of both old and young [1]. Cheese yield is a very important parameter, the higher the recovered percentage of solids, the greater is the amount of cheese obtained and therefore gains in economic terms [2]. Different factors influencing cheese yield as opined by [3] [4] are characteristics of the milk (contents of protein and fat, genetic variants of proteins, somatic cells), cheese making conditions (incorporation of whey proteins in the curd, homogenization of the fat, type of coagulant, use of different starters, curd firmness, type of vat, treatment of the curd, physiological factors, lactation stage, seasonal variations, storage of milk, standardization of milk, types of starter culture used, heat treatments of milk and curd handling systems).

However, milk is poor in Vitamin C which is a form of L-ascorbic acid [5] and it is lower than 4% of the US RDA per serving, and this may affect cheese nutritional quality as well as marketability. Ascorbic acid ($C_6H_8O_6$) also known as vitamin C is an organic compound belonging to the family of monosaccharide. It is highly soluble in water, and is often called one of the secrets of the Mediterranean diet [6]. Vitamin C is one of the essential vitamins that are required for healthy bodily function in humans but it cannot be synthesized in the human body, it is regarded as been essential because of its physiological health benefits. Vitamin C is not stored in the liver or fatty tissues like fat soluble vitamins. Milk is usually fortified with vitamins and minerals it lacks but attention is not directed to fortifying dairy products such as cheese, hence the aim of this study is to fortify unripened cheese with varied levels of Vitamin C and examine its chemical quality, yield, stability of vitamin C and sensory properties.

II. Materials And Methods

Study Location : The study was carried out at the Nutrition Laboratory in the Department of Animal Production and Health, Federal University of Technology Akure, Ondo State, Nigeria. Akure is located on longitude 4.944055^{o}E and 5.82864^{o}E, and latitude 7.491780 ^{o}N with annual rainfall ranging between 1300 mm and 1650 mm average maximum and minimum daily temperature of 38 ^{o}C and 27 ^{o}C respectively [7].

Study materials: Twenty litres of milk was harvested from lactating Bunaji cows, *Calotropisprocer*a leaves was plucked from a farm where it grew naturally while the food grade ascorbic acid was purchased at a reputable store.

Cheese production and Fortification: The raw milk (20L) obtained was clarified and divided into fifteen (15) parts of 1000 ml (1L) each. Each part was heated to a temperature of 50˚C inside an aluminum pot on a low intensity burner (regulated cooking gas) with intermittent stirring. Thereafter, 20 g of the *Calotropisprocera* leave was squeezed directly into the milk and allowed to coagulate and food grade Vitamin C was added at varying levels of 0, 50, 100, 150 and 200 mg/L of milk in three replicates. Heating of milk continued until total coagulation was achieved. The curd was obtained by pouring the coagulated milk into a 1mm sieve to drain the whey and draining of the whey lasted for 45 minutes. Thereafter, samples were kept in the refrigerator.

Chemical evaluation: The free fatty acid concentration was determined using the method [8],Vitamin C was estimated by the colorimetric method according to [9]. The pH was done using a portable pH meter. The total titratable acidity was determined by the method described by [10]. Peroxide value was determined according to Pearson D analyses [11].

Cheese and whey yield determination

The cheese yield was determined using the method described by [12]

$$\% \text{ cheese yield} = \frac{\text{grams of cheese produced}}{\text{grams of milk used}} \times 100$$

% whey yield was also calculated as the ratio of whey obtained to milk used multiplied by 100.

Sensory evaluation: The organoleptic properties of the cheese were evaluated by a 10-member panelist drawn from students and staff of the Department of Animal Production and Health, Federal University of Technology, Akure. They evaluated the cheese samples for colour, texture, mouth feel, flavour, taste and overall acceptability (scale 1-5). Samples were removed from the refrigerator about 1 h before evaluation and kept at room temperature. Approximately 100g of cheese was presented to each panel member. Enough water was provided to the panelists to rinse their mouths between samples. The panelists were requested to rank the samples in order of their acceptability.

Study Design and Statistical Analyses: The experimental design was completely randomized design in a 5×3 factorial arrangement (5 levels of vitamin C × 3 storage periods of 1, 7 and 14 days).Data obtained were subjected to two- way analysis of variance and significant means were separated using Duncan multiple range test using the [13] software, except for sensory evaluation which was analysed using one way ANOVA.

III. Result

Presented in Table 1 is the chemical concentration of soft cheese and whey fortified with synthetic vitamin C at 1, 7 and 14 days storage periods. Storage period had significant ($P<0.05$) effect on pH, vitamin C (in cheese and whey),free fatty acid (FFA) and peroxide value (PV).The storage effect which showed a decrease in pH (6.69– 6.45) observed from day 1 to day 14. The storage effect showed that vitamin C was highest (32.00 mg/100 g) in the cheese and whey (24.96 mg/100 g) at day 1 period of storage and least at day 14. Treatment effect showed significant ($P<0.05$) differences existed in all the parameters investigated. The results also showed that vitamin C content was highest (40.49 mg/100g) in cheese fortified with 150g Vitamin C (T4) at day 1 of storage and least (15.18 mg/100g) for unfortified cheese (T1) at day 14. The highest free fatty acid (FFA) was recorded as 8.10 mg/100 g at day 14 while 7.41 mg/100 g and 7.72 mg/100 g were recorded at 1 and 7 days storage period respectively.The peroxide value showed significant increase as storage period increased. The highest peroxide (6.43 mg/100g) value was observed at 14 days storage period while 5.23mg/100g and 5.79 mg/100g were recorded at 1 and 7 days period of storage respectively.Though not significantly ($p>0.05$) different, there was a steady increase in the Total Titratable Acidity (TTA) for the storage periods from 12.03-12.77 mg/1 00g.The interaction between storage periods and treatments showed significant ($P<0.05$) effects.

Table 1.Chemical concentration(mg/100g) of unripened cheese and whey fortified with food grade vitamin C at different storage periods

Parameter	Titratable acidity	Free Fatty acid	pH	Vitamin C (Whey)	VitaminC(Cheese)	Peroxide value
Storage periods (days)						
1	12.03 ± 0.62	7.41 ± 0.10^{c}	6.69 ± 0.07^{b}	24.96 ± 1.04^{a}	32.10 ± 1.93^{a}	5.23 ± 0.16^{c}
7	12.16 ± 0.62	7.72 ± 0.10^{b}	6.52 ± 0.06^{a}	16.86 ± 0.87^{b}	25.17 ± 1.97^{b}	5.79 ± 0.17^{b}
14	12.77 ± 0.76	8.10 ± 0.10^{a}	6.45 ± 0.05^{c}	12.10 ± 1.03^{c}	18.55 ± 2.02^{c}	6.43 ± 0.19^{a}
P value	0.610	0.001	0.001	0.001	0.001	0.001
Treatment						
T1	9.87 ± 0.50^{c}	7.14 ± 0.21^{b}	6.92 ± 0.08^{a}	14.88 ± 2.48^{c}	24.35 ± 2.58^{b}	5.28 ± 0.36^{b}
T2	12.53 ± 2.37^{b}	7.53 ± 0.12^{b}	6.76 ± 0.10^{b}	15.59 ± 1.96^{c}	25.26 ± 1.99^{b}	5.39 ± 0.21^{b}
T3	12.32 ± 0.33^{b}	7.77 ± 0.14^{ab}	6.60 ± 0.07^{c}	17.64 ± 2.16^{bc}	25.86 ± 2.05^{b}	5.85 ± 0.25^{ab}
T4	10.57 ± 0.31^{bc}	8.05 ± 0.13^{a}	6.53± 0.05^{cd}	21.51 ± 1.91^{a}	33.63 ± 4.73^{a}	6.40 ± 0.22^{a}
T5	16.31 ± 0.16^{a}	7.96 ± 0.12^{a}	6.42 ± 0.06^{d}	20.25 ± 1.90^{ab}	22.08 ± 1.80^{b}	6.16 ± 0.19^{a}
P value	0.001	0.001	0.001	0.001	0.001	0.001
Treatment* Storage period						
T1*1	9.60 ± 0.89^{d}	7.11 ± 0.37^{d}	6.93± 0.11^{ab}	21.84 ± 3.67^{b}	32.22 ± 0.99^{b}	4.68 ± 0.65^{d}
T1*7	9.87 ± 0.93^{d}	7.34 ± 0.35^{c}	7.10 ± 0.15^{a}	13.50 ± 2.14^{d}	25.66 ± 0.14^{d}	5.29 ± 0.58^{c}
T1*14	10.14 ± 0.93^{c}	7.19 ± 0.37^{c}	6.73 ± 0.07^{b}	9.29 ± 3.70^{e}	15.18 ± 2.26^{f}	5.88 ± 0.65^{bc}
T2*1	11.70 ± 2.16^{bc}	7.18 ± 0.13^{cd}	6.77 ± 0.19^{b}	22.50 ± 1.35^{b}	31.22 ± 1.77^{b}	4.80 ± 0.24^{d}
T2*7	12.07 ± 2.16^{b}	7.54 ± 0.17^{b}	7.60 ± 0.06^{a}	14.65 ± 1.21^{d}	25.55 ± 1.95^{d}	5.39 ± 0.23^{c}
T2*14	13.81 ± 3.07^{b}	7.85 ± 0.13^{ab}	6.50 ± 0.10^{b}	9.62 ± 0.81^{e}	19.01 ± 1.77^{f}	6.00 ± 0.24^{bc}
T3*1	12.16 ± 0.53^{b}	7.44 ± 0.19	6.73 ± 0.03^{b}	25.11 ± 1.95^{a}	27.40 ± 2.26^{c}	5.26 ± 0.35^{c}
T3*7	12.28 ± 0.69^{b}	7.76 ± 0.19^{b}	6.73 ± 0.07^{b}	16.81 ± 0.85^{c}	20.01 ± 1.53^{e}	5.83 ± 0.37^{bc}
T3*14	12.53 ± 0.64^{b}	8.11 ± 0.19^{a}	6.33± 0.03^{bc}	11.02 ± 1.10^{e}	15.18 ± 2.26^{f}	6.46 ± 035^{b}
T4*1	10.48 ± 0.50^{c}	7.75 ± 0.16^{b}	6.50 ± 0.00^{b}	28.26 ± 1.59^{a}	40.49 ± 8.27^{a}	5.82 ± 0.28^{bc}
T4*7	10.46 ± 0.58^{c}	7.98 ± 0.14^{ab}	6.67 ± 0.03^{b}	20.22 ± 0.43^{b}	33.46 ± 8.55^{b}	6.37 ± 0.15^{b}
T4*14	10.78 ± 0.62^{c}	8.43 ± 0.16^{a}	6.40 ± 0.06^{b}	16.04 ± 1.59^{c}	26.95 ± 8.97^{c}	7.02 ± 0.28^{a}
T5*1	16.19 ± 0.21^{a}	7.57 ± 0.15^{b}	6.37± 0.07^{bc}	27.08 ± 0.08^{a}	28.65 ± 0.70^{c}	5.61 ± 0.14^{bc}
T5*7	16.14 ± 0.27^{a}	8.00 ± 0.03^{ab}	6.60 ± 0.06^{b}	19.14 ± 1.39^{bc}	21.16 ± 0.41^{e}	6.05 ± 0.07^{b}
T5*14	16.59 ± 0.36^{a}	8.31 ± 0.08^{a}	6.30 ± 0.10^{c}	14.53 ± 0.63^{d}	16.44 ± 0.70^{f}	6.81 ± 0.15^{a}
P value	0.050	0.020	0.030	0.050	0.040	0.040

^{abcdef}Means along the same column with different superscripts are significantly ($P<0.05$) different. T1=0g vitamin C, T2=50g vitamin C, T3= 100g vitamin C, T4= 150g vitamin C, T5= 200g vitamin C

The cheese and whey yield shown in Fig1 reveals that the fortification of cheese with vitamin C influenced the percentage cheese yield. It was observed that cheese yield was inversely proportional to vitamin C fortification. Cheese fortified with 50 g vitamin C (T2) had the highest cheese yield of 23.30 % while T1 (0g vitamin C), T3 (100 g vitamin C), T4 (150 g vitamin C), and T5 (200 g vitamin C) recorded 21.45, 19.69, 18.84 and 16.79 % respectively. However, the highest percentage of whey yield was 53.66 % for T1 while T2, T3, T4 and T5 had 50.73, 45.37, 35.66 and 52.51 % respectively.

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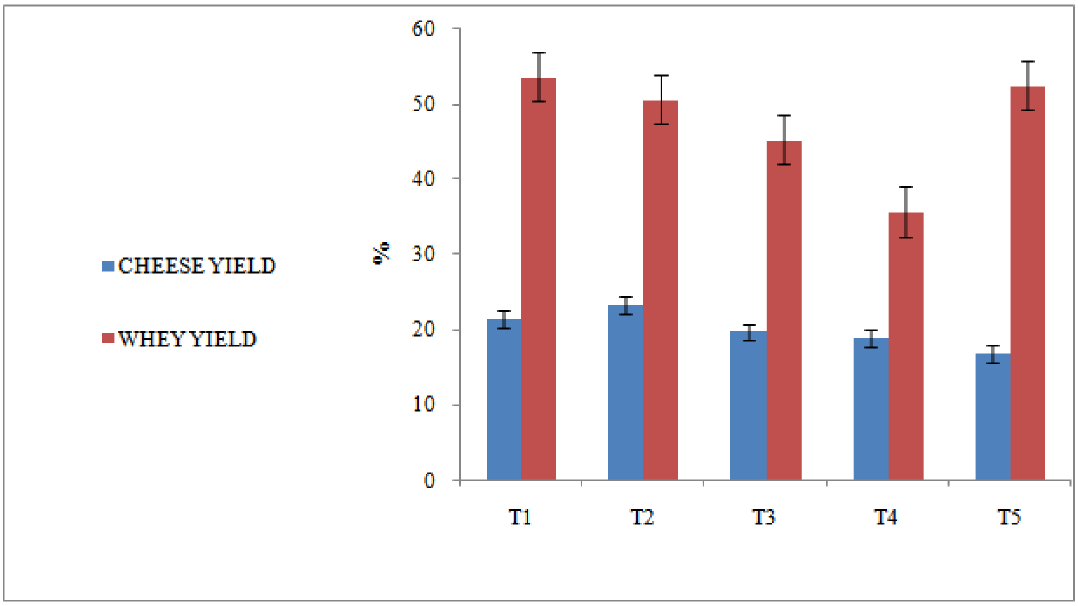

Figure 1. Percentage cheese and whey yield.

T1=0 g vitamin C, T2=50 g vitamin C, T3= 100 g vitamin C, T4= 150 g vitamin C, T5= 200 g vitamin CPresented in Table 2 is the sensory evaluation of the fresh cheese fortified with vitamin C.The fortification of the cheese with vitamin C had significant (P<0.05) effect on the taste and mouth feel. In terms of colour, the T5 (200 g vitamin C) had the highest score of 2.14 while the unfortified cheese (T1) had the least score (1.71). The highest flavour score of 3.86 was recorded in T4 (100 g Vitamin C). Though not significantly (p>0.05) different, the highest overall acceptability score of 4.00 was recorded in T4.

Table 2. Sensory evaluation of unripened cheese fortified with food grade vitamin C

Treatment	Colour	Texture	Taste	Flavour	Mouth feel	Overall acceptability
T1	1.71±0.18	2.86±0.14	$3.29±0.18^{b}$	3.57±0.20	$4.00±0.22^{ab}$	3.43±0.20
T2	2.00±0.01	3.21±0.47	$3.71±0.29^{ab}$	3.29±0.18	$3.43±0.20^{b}$	3.71±0.36
T3	2.00±0.22	3.26±0.36	$3.14±0.26^{b}$	3.57±0.20	$4.29±0.18^{a}$	3.80±0.22
T4	2.00±0.22	3.29±0.18	$4.14±0.26^{a}$	3.86±0.14	$4.00±0.31^{ab}$	4.00±0.31
T5	2.14±0.26	3.25±0.36	$3.29±0.29^{b}$	3.57±0.20	$3.67±0.30^{ab}$	3.57±0.20
P-value	0.650	0.150	0.040	0.350	0.020	0.460

Value represent mean of triplicate reading.^{ab}Means along the same column with different superscripts are significantly (P<0.05) different.

IV. Discussion

The storage effect which showed a decrease in pH (6.69– 6.45) observed from day 1 to day 14 could be due to increase in fermentative activity in the cheese. The low pH observed is in line with the findings of [14], who reported decrease in pH from 5.04 – 4.55 in a study on the effect of short term storage on chemical composition of cheese also with the reports of [15] who examined the effect of local preservative (*Aframomumdanielli*) on the chemical and sensory properties of stored warakanshi. The storage effect showed that vitamin C was highest (32.00 mg/100 g) in the cheese and whey (24.96 mg/100 g) at day 1 period of storage and least at day 14. This shows that Vitamin C concentration in dairy food can decrease with storage.It was observed that the cheese retained more of the vitamin C while much of vitamin C was lost in the whey. This could be attributed to the cheese matrix which may have the ability to trap nutrients. Vitamin C loss during the cooking process is due partly to oxidative destruction and partly to leaching of the vitamin into the water used for cooking [16]. [17] also opined that Vitamin C is highly sensitive to losses during processing and storage. The results also showed that vitamin C content was highest (40.49 mg/100g) in cheese fortified with 150g Vitamin.C (T4) at day 1 of storage and least (15.18 mg/100g) for unfortified cheese (T1) at day 14. [18] reported that degradation may occur during storage even in vitamin C-fortified milk.

This result is in line with the report of [19] who reported 20.09 mg/100g for buffalo's milk fortified with non-encapsulated ascorbic acid. The range of Vitamin C values obtained in this study is within the recommended amount of [20]. The highest free fatty acid (FFA) was recorded as 8.10 mg/100 g at day 14 while 7.41 mg/100 g and 7.72 mg/100 g were recorded at 1 and 7 days storage period respectively. These values could be comparable with 4.91-7.80mg/100g reported by [21] in Bafut cheese. Free fatty acids in cheese are important in defining the specific flavourof cheese and high values of FFA is desirable in cheese [21]. They are derived from two majors sources; breakdown of fat by lipolysis and metabolism of carbohydrates and animo acids by bacteria [21]. The peroxide value showed significant increase as storage period increased. The highest peroxide (6.43 mg/100g) value was observed at 14 days storage period while 5.23mg/100g and 5.79 mg/100g were recorded at 1 and 7 days period of storage respectively. Peroxide value measures hydroperoxides that are produced in the early stages of the oxidation process, this increase may be attributed to oxidative activity during storage. The increase in TTA contents with decreasing pH values would probably extend its shelf life by impeding the growth of spoilage and pathogenic microorganisms, consequently making it safe for human consumption. The result obtained corroborates the report of [22] who reported an increase (0.39 – 1.55 %) in TTA as storage period increased in Sudanese white cheese but were lower than 2.46-2.49% in cheese made from raw mik and 1.51-2.21 in cheese made from pasteurized milk with starter culture as reported by [21].

The cheese yield obtained from this study is lower than that of [23] who reported cheese yield range of 26 - 28% when sheep milk was used. This variation might be due to the source of the milk as bovine milk was used in this study. Also, the values obtained were lower than the range of 5.52-14.79 % reported by [24] in cheese made from four plant sources as coagulants, but higher than 15-16 % cheese yield reported by [25], 11.42-19.61 % reported by [26] in the use of some indigenous plants in cheese production in Nigeria. The differences in cheese yield could be due to the types of coagulant and vitamin C inclusion in this study. For optimal cheese yield and cheese quality, good quality milk from healthy animals with good flavour, cold stored for a limited amount of time and has a high protein content with the BB genotypes of s-lactoglobulin and κ-casein (bovine milk) should be used and also, the milk should be low in somatic cell count, as proteases from somatic cells attacks αS2- and s caseins and reduce the cheese yield [25]. Also pH will have an influence on the yield [27]. This can be explained with the effect pH has on the casein matrix. At pH values above 5.1 the matrix is more or less intact whereas at pH below 4.8, the casein sub-micelles have to a greater extent been disintegrated. A disintegrated or looser matrix has the possibility to influence the yield as well as the texture if the water content is high, this is because it has a higher ability to retain water [27]. The content of free fatty acids should be low, as free fatty acids bind Ca2+ and thereby reduce the coagulation properties of the milk, in addition free fatty acids contribute to the development of rancid flavour in cheese [25]. Moreso, [28] [29] opined that cheese yield potential of milk is largely dependent on milk composition, particularly fat and protein.The acceptability and satisfaction derived in food products by nutrition-conscious consumers is influenced by the nutritional and sensory qualities of the product. Cheese fortification with Vitamin C improved its overall acceptability. However, consumers preferred 150 g/l Vitamin C fortification in cheese production. Vitamin C being a reducing agent could have influenced oxidative changes in the milk thereby impacting theflavour which had effect on the overall acceptability.

V. Conclusion

The fortification of cheese with vitamin C influenced the percentage yield. Cheese with 50 g/L Vitamin C produced the highest yield. Storage effect of vitamin C revealed that Vitamin C fortified cheese should not be stored up to seven days as the quality reduced with increased storage time. Also cheese fortified with 150 g of vitamin C was preferred by the consumers.

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