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Effect of Cholesterol Reduction on Goat Milk Yoghurt Components by Cyclodextrin from Different Carbon Sources and *Bacillus spp*

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Authors' contributions

This work was carried out in collaboration between all authors. Author ACA designed the study, Author NTU performed the statistical analysis. Author ECF wrote the protocol and wrote the first draft of the manuscript. Authors ACA and ECF managed the analyses of the study. Author NTU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To produce Cyclodextrin from three CGTase producing *Bacillus* species (BI, B2, B3) and different starch sources, cassava varieties (30572, 419, and umu 37), sweet potato root (*x-igbariam*), cocoyam (*edeuhie* and NXS003). To treat Goat milk yoghurt with two different level of cyclodextrine (0.3% and 0.5%). To determine its cholesterol reducing effect during 6days refrigerated storage and to ascertain the effect on other yoghurt components.
Study Design: Multifactor randomized complete block design (RCBD) was used for this study. Each experiment was repeated in duplicate. Data obtained were subjected to analysis of variance (ANOVA) at the significant level of 5% *(p≤ .05)* using SPSS 20.
Place and Duration of Study: Department of Food Science and Technology, Michael Okpara

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University of Agriculture, Umudike, between May 2015 and october 2016.
Methodology: A Total of 37 yoghurt samples were formulated from 6 samples of starch, 3 samples of CGTase source and two (2) levels of cyclodextrin. total cholesterol, Fat, Protein, reducing sugar and total soluble solid content of cyclodextrin treated goat milk yoghurt were determined,
Results: During 6 days refrigerated storage, the cholesterol content of goat milk yoghurt decreased *(p≤ .05)* as a result of cyclodextrin treatment from 3.9 mg/ml (control) to 1.67 ml/ml (NXS 205) in cocoyam, 1.10 mg/ml (419-205) in cassava starch source and 1.52 mg/ml (pot 105) for sweet potato starch. Cyclodextrin from *B. licheniformis* and cassava starch (419) at 0.3 level was best in terms of cholesterol reduction. Cyclodetrin treatment also led to significant *(p≤ .05)* decrease in fat, protein, reducing sugar and total soluble solid content of goat milk yoghurt. Cholesterol reductions were not significantly affected at *p≤ .05* by percentage of cyclodextrin.
Conclusion: The results showed that all cyclodextrins was effective in reduction of cholesterol in goat milk yoghurt. Cyclodextrin from cassava starch 419 and *B. licheniformis* at 0.3% level (419-303 = 1.10 mg) resulted in a goat milk yoghurt with lowest level of cholesterol among all the samples analysed. As the storage period progressed, the cholesterol levels also reduced as a result of cyclodextrin treatment. There was a direct relationship between increase in cyclodextrin and decreasing level of fat, protein, reducing sugar and total soluble solid of cyclodextrin treated goat milk yoghurt.

Keywords: Bacillus spp; cholesterol; cyclodextrin; goat milk; yoghurt.

1. INTRODUCTION

Cholesterol is a waxy substance made by animal liver and also supplied in diet through animal products such as meats, poultry, fish and dairy products. Cholesterol is needed in the body to insulate nerves, make cell membranes and produce certain hormones, and it is an important lipid in some membranes. However, the body makes enough cholesterol, so any dietary cholesterol is not needed [1].

Cholesterol plays a major role in human heart health. Cholesterol can be both good and bad. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. Excess cholesterol in the bloodstream can form plaque (a thick, hard deposit) in artery walls. The cholesterol or plaque build-up causes arteries to become thicker, harder and less flexible, slowing down and sometimes blocking blood flow to the heart. When blood flow is restricted, angina (chest pain) can result When there is too much LDL cholesterol in the blood, it is deposited inside the blood vessels, where it can build up to hard deposits and causes atherosclerosis, the disease process that underlies heart attacks [1].

Since a strong positive correlation exists between increased serum cholesterol concentrations and risk of coronary heart disease, most consumers are concerned about excessive intake of cholesterol (Grundy et al. [2]; Gurr [3]). Therefore, physical, chemical, and biological methods to reduce cholesterol have been studied in foods, including dairy products (Szjetli, [4]; Ahn and Kwak, [5]; Lee et al. [6]; Kwak et al. [7]).

Cholesterol from food is hard to get away from the body, even though one may be watching his/her diet. All foods of animal origin contain cholesterol, including eggs, milk, red meat, and shrimp. Generally, foods that are high in saturated fats or Trans fats should also be limited. These include foods you may not even think of, such as grilled-cheese sandwich, margarine, potato with butter and chicken pot pie, etc. As we eat, cholesterol from food is absorbed by our digestive tract. It then makes its way into our liver and can circulate through our body in the bloodstream. Milk and dairy products contain relatively high level cholesterol that can elevate the blood cholesterol [8].

Large number of physicochemical methods was recommended to reduce cholesterol in food as well as blood cholesterol (Gurr) [3]. cholesterol could be removed in an efficient manner from milk fat up to 90% using supercritical co_2 technology [9].

In spite of effectiveness of the methods, some complications exist because of the organic solvents and saponin residues in food and safety problems for human body. The worries about solvent residue and harmful saponin

consumption for human health motivated the investigated of find non-toxic and effective substances instead of unsafe materials.

Cyclodextrins are cyclic oligosaccharides consisting of α-(1-4)- linked D-glycopyranose sugars. The crown structures are water soluble and their use in lipid exchange studies resides in possession of a hydrophobic cavity that binds lipids. The inward-facing non-polar surfaces of the cyclodextrin monosaccharide subunits form a hydrophobic cavity whose size depends on the number of monosaccharide units. The size of the pocket confers selectivity for the type of lipid that can be accommodated and β-cyclodextrins have a particular affinity for sterols like cholesterol. The stacking of two cyclodextrin molecules is required to form a large enough hydrophobic pocket to solubilize one cholesterol molecule and solubility and specificity of the complex in water can be improved by using derivatives of β-cyclodextrin. The use of cyclodextrin to determine physical parameters associated with the interaction of cholesterol with phospholipid membranes, however, needs to be regarded with circumspection (Zidovetzki and Levitan) [10]. Also cyclodextrins bind phospholipids [11] and trans-locate them between membranes [12].

It has been proved that the β-CD molecule can be used as non-toxic and non-digestible molecule to remove cholesterol effectively from milk and animal products for improving their nutritional characteristics (Astray, et al., 2009) [13]. A number of studies have indicated that cholesterol removal from dairy products was most effectively achieved by the powder β-cyclodextrin (β-CD) (Oakenfull and Sidhu, 1991 [14]; Makoto et al. [15]; Ahn and Kwak, [5]; Lee et al. [5]; Kwak et al.) [7].

Ha et al. [16] measured the amount of nutrients (lactose, short-chain FFA, FAA, and water-soluble vitamins) that were entrapped during cholesterol removal from cream by cross-linked β-CD. They concluded that the amount of entrapped nutrients was negligible during cholesterol removal from cream by cross-linked β-CD. Dias et al. [17] investigated butter cholesterol removal using different combinatorial methods with β-CD. Although many investigations were carried out to demonstrate the feasibility of β-CD as excellent substance for removing cholesterol from food including milk, few investigations have been reported on the effect of cyclodextrin on cholesterol during storage of dairy product and its effect on major component milk products.

The aims of this study are: To evaluate the feasibility of cyclodextrin from different carbon sources and *Bacillus spp* on cholesterol removal during storage of goat milk yoghurt and its effect on main components of goat milk yoghurt.

2. MATERIALS AND METHODS

2.1 Source of Materials

Cyclodextrin was previously produced using different starch sources (cassava varieties; 30572, 419, and umu 37, sweet potato root; *x-igbariam* and cocoyam; *edeuhie* and NXS003) and different *Bacillus* spp (B1- *B. cereus,* B2- *B. thuringences,* and B3- *B. licheniformis*).

Goat milk from West African Dwarf Goat (*Capra hircus*) at mid-lactation period was obtained from the Goatry Section of Micheal Okpara University of Agriculture Umudike Farm. Starter culture (*Lactobacillus brevis and Streptococcus thermophillus*) was obtained from Mr Vee Yoghurt Company Ikwuano, Chemicals and equipment used for the analysis were obtained from Crop Utilization Laboratory, Bio Science Laboratory of International Institute for Tropical Agriculture (IITA), Ibadan, Central service laboratory of National Root Crops Research Institute Umudike and Department of Plant Science/Biotechnology (PSB) Post Graduate laboratory of Michael Okpara University of Agriculture, Umudike Abia State.

2.2 Cholesterol Reduction Process

Exactly 100 ml of raw goat milk were separately placed in 500 mL beakers and different percentages of cyclodextrin (0%, 0.3%, and 0.5%) from different starch source, cassava (419 um37 and 30572) sweet potato (*X-igbariam)* and cocoyam (*edeuhie* and NX003) and Bacillus species (B1: *B. cereus,* B2: *B. thuringences*, and B3**:** *B. licheniformis*) were used for reduction of cholesterol in goat milk yoghurt. Sample without cyclodextrin was used as control. Skimmed milk powder was added to increase solids. The mixture was stirred vigorously by VELP stirrer 700 rpm at temperature 20°C for 10 min.

2.3 Yoghurt Production

A total of 37 different samples of goat milk yoghurt were made from goat milk, different percentage of cyclodextrin (0%, 0.3%, 0.5%). The goat milk was heated to 60°C and homogenized and pasteurized at 62.5°C for 30 min then rapidly cooled to 45°C. *Lactobacillus brevis* and *Streptococcus thermophillus* (1%) served as starter culture which was also introduced into goat milk. The inoculated goat milk was incubated at 45°C until pH reaches 4.5. The yoghurt samples were cooled to 25°C by resting in a temperature controlled room and then stored at 3.5°C for a period of 12 h. The yoghurt was stirred to smoothen and to obtain thick consistency. The product was packaged in sterile containers and stored in refrigerator for further analyses.

2.4 Total Cholesterol Determination

Total cholesterol in goat milk yoghurt was determined using the Product Manual for Total Cholesterol Assay Kit (Colorimetric) of Cell Biolab Company Catalog number STA-384 192 assays.

2.4.1 Optimization of saponification conditions of clarified milk fat

Rapid saponification of milk fat was essentially based upon the method of Fletouris et al. [18]. The flow diagram (Fig. 1) of saponification is discussed below:

Milk fat (0.1-0.15 g) in test tube with teflon lined screw cap

↓

Add 5 ml of (2.5%, 5%, 7.5%) methanolic KOH and mix thoroughly

↓

Incubate the capped tubes in water bath for (80°C, 85°C, and 90°C/ 20 min) with intermittent shaking after every 5 min

↓

Cool the contents under tap water

Fig. 1. Protocol for optimization of saponification conditions

2.4.2 Extraction of unsaponifiable matter

After saponification, 1 ml of distilled water was added to the contents followed by the addition of 5 ml of hexane. The contents were then thoroughly mixed using vortex mixer for 1 min followed by centrifugation at 2000 rpm for 2 min. After centrifugation, the upper hexane layer was pipetted out and passed through anhydrous sodium sulphate, to remove traces of moisture. Exactly 0.5 ml of this extract was transferred in a dry test tube and evaporated at 50-60°C under nitrogen. The dried unsaponifiable matter obtained was used for colour development.

2.4.3 Colour development

3 ml of cholesterol reagent given in the kit was added to the dried unsaponifiable matter (2.4.2) and mixed well using vortex mixer. After mixing, the tubes were incubated in boiling water bath for 90 sec, with intermittent shaking. The same protocol was followed for standard in which 15 µl of standard cholesterol solution, 200 mg% (given in the kit) was utilized instead of unsaponifiable matter. The colour intensity was measured at 560 nm using spectrophotometer and cholesterol content was calculated using the following formula

Cholesterol (mg/100 g) =

{0.03 × absorbance of sample × ml of hexane (5 ml) × 100} / {absorbance of std. × ml of hexane aliquot (0.5 ml) × wt of sample (g)}

0.03 is the concentration (mg) of cholesterol in 15 µl of standard solution

2.5 Crude Protein Determination

The method for protein determination used was the kjeldahl method describe in James [19]. Exactly 2 g of sample was weighed into a kjeldahl flask and tablets of kjeldahl catalysts added. Also 25 ml conc suphluric acid and 5 glass beads (to prevent bumping during heating) were added. The sample was heated in a fume cupboard, heated very gently at first and then increased heat with occasional shaking until the solution assumes a green color (temperature of digestion was above 420°C for 30 min).

At this point, the sample was cooled and washed down any black particles showing at the tip and mid of the flask with distilled water. It was reheated gently at first until the green colour disappeared then allowed to cool.

The digest was transferred into 100 ml volumetric flask and made up to mark with distilled water.

Protein Distillation was done with Markham distillation apparatus the Markham distillation apparatus was steamed for 15 min. Exactly 100 ml of boric acid and two (2) drops of screened methyl red indicator was placed under the condenser such that the condenser tip was dipped below the boric acid solution. Exactly 5ml of the digest was pipetted into the apparatus via the small funnel aperture and washed down with distilled water followed by 5 ml of 60% NaOH solution. This was allowed to steam for about 5-7 min to collect enough NH_3SO_4 after which the conical flask was removed and titrated against 0.0IN hydrochloric acid and the Nitrogen content calculated hence the protein content of the food.

Calculation

% Nitrogen = (V_S-V_B x N acid X 0.01401 x 100) / Weight of sample

Where,

V_S volume (ml) of acid required in titrating sample
V_B = Volume (ml) of acid required in titrating the blank
N acid =Normalityof acid (0.IN)

%crude protein =N × conversion factor = 6.25

0.01 ml of the 0.1 mol/l sodium hydroxide standard solution corresponds to 0.0140 mg of nitrogen.

2.6 Fat Content Determination

The Soxhlex fat extraction method was used as described by Pearson [20] and James [19].

Exactly 250 ml clean boiling flask was dried in the oven at 110°C for 30 min and transferred into desiccators and allowed to dry. Exactly 2 g of sample was weighed into labeled thimble. Conical flasks were weighed, filled with 200 ml of petroleum ether (boiling point 40-60°C).

The extraction thimble was plugged lightly with cotton wool and the Soxhlex apparatus assembled and allowed refluxing for about 6 h. The thimble was removed and petroleum ether collected in the top container of the set-up and drained into a container for reuse when the flask is almost free of petroleum ether, it was removed dried at 110°C for 1 hr cooled in the dedicator and then weighed.

The fat content calculated using the formula below:

% fat = (Weight of fat x 100) / Weight of sample

2.7 Total Soluble Solid (TSS) Determination

The method of AOAC [21] was used for determination of Total soluble solid (TSS) determination.

Clean dish was dried at 105°C in an oven until constant weight was achieved. It was cooled to room temperature in a desiccator and the weight was noted. After mixing thoroughly, 100 ml of deodorized goat milk yoghurt samples and control were accurately pippetted into the dish and evaporated to dryness in a steam bath. The residue was dried in the oven for about 1hr at 105°C. The dish was quickly transferred into the desiccator for cooling to room temperature after which it was weighed. The dish was returned in to the oven for further drying for 20 min range and re-weighed after cooling to room temperature. This process was repeated until the weight of the dish plus residue was constant, the weight of the dish was subtracted to obtain the weight of total solids.

Total solids (mg/ml) = mg total solid x1000

2.8 Reducing Sugar (Lactose)

The method of AOAC [21] was used for determination of reducing sugar in the yoghurt samples. Exactly 0.1 g of sample was weighed and extracted with 5 ml hot 0% ethanol (twice). The supernatant was collected extracted and evaporated in a water bath at 80°C for 30 min. exactly 10 ml of distilled water was added and 0.5, 1, 1.5…3 ml of the extract was pipette in six test tubes and 4ml of distilled water was added in all the tubes. Three milliliter (3 ml) of DNS reagent was added, heated in water bath for 5min and 1 ml of 40% Rochelle salt added. The intensity of dark red color formed was read at absorbance of 510 nm.

2.9 Preparation of Standard for Reducing Sugar

Exactly 100, 200, 300, 400, and 500 µg of glucose was collected and 3 ml of distilled water added to each, the 3 ml of DNS reagent was added and boiled for 5 mins. When content of the test tube was warm, about 1 ml of 40% Rochelle salt solution was added and allowed to cool. The absorbance was read at 510 nm. The amount of reducing sugar was calculated using this equation:

$$Y = mx + c$$

Where,

Y =absorbance, c = concentration, m = slop of the standard graph

2.10 Experimental Design and Statistical Analysis

Multifactor randomized complete block design (RCBD) was used for this study. A Total of 37 yoghurt samples were formulated from 6 samples of starch, 3 samples of CG Tase source and two (2) levels of cyclodextrin. Each experiment was repeated in duplicate. Data obtained were subjected to analysis of variance (ANOVA) at the significant level of 5% *(p≤ .05)* using SPSS 20 (analytical software). Statistical differences were found using least significant difference (LSD) at the 5% significant levels (Landau and Everitt, 2004).

3. RESULTS AND DISCUSSION

3.1 Effect of Cyclodextrin Level, Carbon Source and *Bacillus spp* on Cholesterol Content of Goat Milk Yoghurt for 7 Days at 4°C

Table 1, 2 and 3: shows the effect of different levels of cyclodextrin levels (0.3 and 0.5%), cyclodextrin and carbon source on the cholesterol content of goat milk yoghurt as storage period progressed. At day 2, cholesterol was highest (4.08 mg/g) in control while there where slight reduction range of 0.05-0.08 mg/g difference between the control and cyclodextrin treated goat milk yoghurt *(p≤ .05)*.

Table 1. Effect of different levels of cyclodextrin (0.3%, 0.5%) isolated from cocoyam starch source and *Bacillus Spp* on cholesterol content of goat milk yoghurt at day 2, 4 and 6 at 4°C storage (mg/100 g)

Sample	Storage time (day)		
	2	4	6
Control	4.08^{a}	4.05^{a}	3.9^{a}
Ede-103	4.02^{a}	3.38^{b}	1.74^{ef}
Ede-105	4.02^{a}	2.94^{e}	2.04^{c}
Ede203	4.00^{a}	3.21c	1.82^{de}
Ede205	4.00^{a}	3.00^{e}	1.80^{de}
Ede303	4.01^{a}	3.12^{d}	2.82^{b}
Ede305	4.00^{a}	2.98^{e}	2.87^{b}
Nxs-103	4.01^{a}	2.82^{f}	1.82^{de}
Nxs-105	4.00^{a}	2.74^{g}	1.74^{ef}
Nxs-203	4.02^{a}	3.00^{e}	1.88^{d}
Nxs-205	4.00^{a}	2.74^{g}	1.67^{f}
Nxs-303	4.02^{a}	2.99^{e}	184^{de}
Nxs-305	4.00^{a}	3.42^{b}	2.11^{c}
LSD	0.133	0.85	0.60

ede (edeuhie) and Nxs(xanthosoma) are cocoyam starch, 1,2,3 denotes CGTase from B.cereus, B.thuringences,and B.licheniformis respectively while 03 and 05 are the levels of cyclodextrin in %. Means with different superscripts are significantly different at P≤.05

At day 4: cholesterol content of the experimental samples greatly reduced as a result of addition of cyclodextrin. The decrease in cholesterol content of samples when compared with the control ranged from 0.67-2.13 mg/g. The cholesterol content of the control in day 2 was slightly higher when compared to that of day 4. This could be as a result of probiotic organism used during fermentation.

Hongbao Ma [22] reported that *Bifidobacterium* and *Lactobacillus acidophilus* may play an important role in cholesterol metabolism of their host. Several other mechanisms have been hypothesized, which include enzymatic assimilation of cholesterol by probiotics [23], cholesterol binding to cell walls of probiotics [24], incorporation of cholesterol into the cellular membranes of probiotics during growth [25], conversion of cholesterol into coprostanol [26] and production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics [25].

Table 2. Effect of different levels of cyclodextrin (0.3%, 0.5%) isolated from cassava starch source and *Bacillus* Spp on cholesterol content of goat milk yoghurt at days 2, 4 and 6 4°c storage (mg/100 g)

Sample	Storage time (day)		
	2	4	6
Control	4.08[a]	4.05[a]	3.9[a]
Um37-103	4.04[a]	2.11[j]	1.62[h]
Um37105	4.00[a]	2.90[d]	1.70[fg]
Um37-203	4.01[a]	2.18[j]	1.78[e]
Um37-205	4.02[a]	2.52[g]	2.00[c]
Um37-303	4.03[a]	2.40[h]	2.00[c]
Um37-305	4.00[a]	2.28[i]	2.15[b]
419-103	4.01[a]	2.51[g]	1.92[d]
419-105	4.02[a]	2.10[j]	1.74[e]
419-203	4.02[a]	2.00[g]	1.57[h]
419-205	4.02[a]	1.92[j]	1.10[i]
419-303	4.00[a]	3.10[k]	1.92[d]
419-305	4.03[a]	3.02[k]	1.68[g]
30572-103	4.03[a]	2.72[c]	1.77[ef]
30572-105	4.02[a]	3.30[c]	1.98[c]
30572-203	4.02[a]	2.62[e]	1.70[fg]
30572-205	4.03[a]	2.50[b]	1.91[d]
30572-303	4.00[a]	2.82[f]	1.77[e]
30572-30	4.00[a]	2.74[g]	1.62[h]
LSD	0.051	1.00	0.08

419, um 37 and 30572 are cassava starch, 1,2,3 denotes CG Tase from B. cereus, B. thuringences, and B. licheniformis respectively while 03 and 05 are the levels of cyclodextrin in %.Means with different superscripts are significantly different at P≤.05

Day 6 also showed the same form of decrease for both samples and control. Control reduced from 4.08-3.9 mg/g while cyclodextrin treated goat milk yoghurts reduced from 4.04-1.1 mg/g. cholesterol content also reduced with increase in level of cyclodextrin (0.5%). The cholesterol content of goat milk yoghurt decreased from 3.9 mg/ml (control) to 1.67 ml/ml (NXS 205) in cocoyam, 1.10 mg/ml (419-205) in cassava and 1.52 mg/ml (pot 105). Cyclodextrin from *B. thurengiences,* cocoyam starch (NXS003) at *p≤ .05* level was best in terms of cholesterol reduction.

Different starch sources and *Bacillus spp* used in production of cyclodextrin showed different activities during reduction of cholesterol. For cocoyam starch, sample NXS003 starch and *B. thurengiences* yielded cyclodextrin which when added to goat milk yoghurt at 0.5% showed lower cholesterol (1.67 mg at day 6) than other sample analyzed. In cassava varieties, 419 starch and *B. licheniformis* yielded a cyclodextrin that resulted in lowest cholesterol at 0.3% (419-303=1.10 mg at day 6).

Table 3. Effect of different levels of cyclodextrin (0.3%, 0.5%) isolated from sweet potato starch source and *Bacillus* Spp on cholesterol content of goat milk yoghurt at days 2, 4 and 64°C storage (mg/100 g)

Sample	Storage time (day)		
	2	4	6
Control	4.08[a]	4.05[a]	3.9[a]
Pot-103	4.02[b]	2.95[b]	1.72[b]
Pot-105	4.01[b]	2.72[c]	1.54[b]
Pot-203	4.00[b]	2.30[d]	2.10[b]
Pot-205	4.02[b]	2.25[d]	2.00[b]
Pot-303	4.03[b]	3.00[b]	1.98[b]
Pot-305	4.02[b]	2.15[e]	1.82[b]
LSD	0.124	0.147	0.063

pot(x-igbariam) is white skinned sweet potato, 1,2,3 denotes CG Tase from B. cereus, B. thuringences, and B. licheniformis respectively while 03 and 05 are the levels of cyclodextrin. Means with different superscripts are significantly different at P≤.05

Sweet potato and *B. cereus* also yielded a cyclodextrin that lowered cholesterol level than other treatments (pot-105=1.54 mg) when applied at 0.5%. Cholesterol reduction was not significantly affected by percentage cyclodextrin used. This result was in line with the findings of Favier et al., [27] which reported that β-CD has the potential to decrease plasma cholesterol. This result also confirms the work of other investigators (Graille et al. [28] Lee et al. [6], and kwak, et al.) [7].

3.2 Effect of Cyclodextrin Level, Carbon Source and *Bacillus spp* on Fat, Protein, Reducing Sugar and Total Soluble Solid Content of Goat Milk Yoghurt For 7 Days at 4°C

Effect of different levels of cyclodxtrin, *Bacillus spp* and starch source on fat is shown in Plates 1, 2, and 3: The control recorded highest *(p≤ .05)* fat while addition of cyclodextrin has led to decrease in fat content. Fat content reduced with increased level of cyclodextrin. Cyclodetrin from different carbon source and different *Bacillus* species showed similar effects on the fat content of yoghurt products. Ha et al., [17]

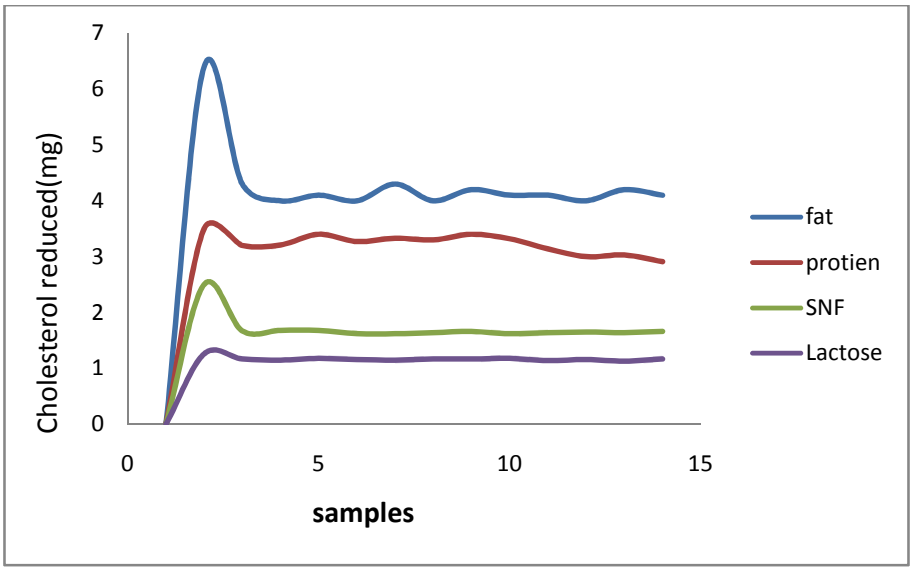

Plate 1. Effect of different levels of cyclodextrin (0.3%, and 0.5%) isolated from cocoyam starch source and *Bacillus spp* on total soluble solid (tss), reducing sugar and other nutritional content of goat milk yoghurt at day 2 (4°c) storage period

SNF = Solid non-fat

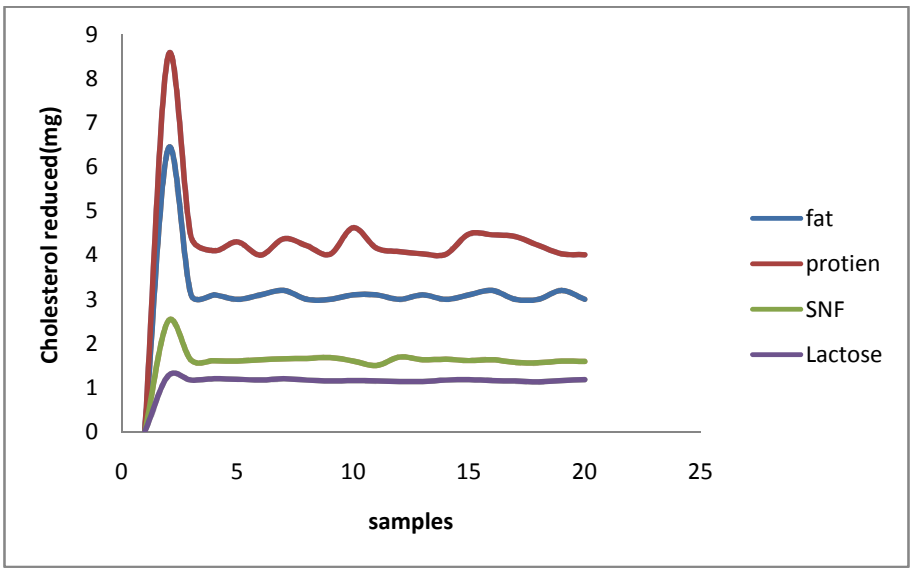

Plate 2. Effect of different levels of cyclodextrin (0.3%, 0.5%) isolated from cassava starch source and *Bacillus spp* on total soluble solid (TSS), reducing sugar and other nutritional content of goat milk yoghurt at day 2 (4°c) storage period (mg)

SNF = Solid non-fat

suggested that the free fatty acids in food may be reduced because they are trapped in cyclodextrin molecule cavities. Kwat et al., [29] reported that cheddar cheese fat content made by milk treated by *B*-CD was lower than control milk samples.

Protein content decreased as the level of cyclodextin increased. This is evident in the turbulence pattern in the graphs shown in Plate 1, 2 and 3 which indicates 0.3 and 0.5% levels. Cyclodetrin from different carbon source and different *Bacillus spp* showed similar effects on

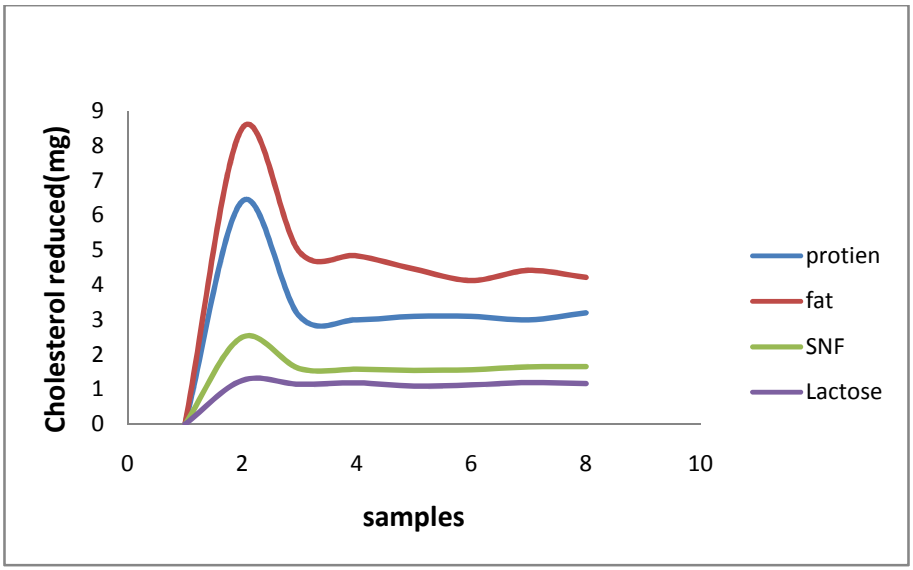

Plate 3. Effect of different levels of cyclodextrin (0.3%, 0.5%) isolated from sweet potato starch source and *Bacillus spp* on total soluble solid (TSS), reducing sugar and other nutritional content of goat milk yoghurt at day 2 (4°c) storage period (mg)

SNF = Solid non-fat

the protein content of yoghurt products. Maskooki et al., [30] reported in their work that there was a direct relation between increasing concentration of cyclodextrin and decreasing level of protein content of milk protein. Kim et al., [31] have also reported the protein reduction as a result of cholesterol removal by cyclodextrin. Ha et al. [16] confirmed that the protein of ream was reduced during reduction of cholesterol by 4%-10% cyclodextrin due to entrapment of amino acid in cyclodextrin cavity. On the other hand, the outer surface of cyclodextrin macromolecules has affinity to absorb the negative charges that cover the protein surface. The process of cholesterol reduction process such as mixing, separation, and centrifugation could lead to decrease of protein content of the yoghurts.

Reducing sugar measured as lactose ranged from 1.13 mg/g -1.18 mg/g. Addition of cyclodextrin reduced the Reducing sugar content of yoghurt samples. Level of cyclodextrin (0.3 and 0.5%) did not show any specific trend of effect on Reducing sugar. Also, the starch source and *Bacillus* spp for each cyclodextrin did not affect level of reduction in Reducing sugar. This result is in line with the findings of Ha et al., [16] which reported that reduction of lactose as a result of CDs treatment was very negligible.

Total soluble solid (TSS) was highest in control (2.5mg/g) and lowest in 30572-205 (1.56 mg/g). Addition of cyclodextrin to goat milk yoghurt decreased the total soluble solid in the products and as level of cyclodextrn increased, the TSS also decreased. This result is in line with the work of Maskooki et al., [30] which reported that the non-fat solid decreased as a result of cyclodextrin treatment. This could be as a result of cyclodextrin reduction of protein and lactose. Ha et al., [16] confirmed the decreasing of some nutritional materials during cholesterol reduction.

4. CONCLUSION

This study showed the ability of cyclodextrin from Different Carbon Sources and *Bacillus spp* at different concentration to remove cholesterol and their effect on the main components of goat milk yoghurt. The ability of this cyclodextin has been proved and the results confirmed. The results showed that all cyclodextrins was effective in reduction of cholesterol in goat milk yoghurt. Cyclodextrin from cassava starch 419 and *B. licheniformis* at 0.3% level (419-303 = 1.10 mg) resulted in a goat milk yoghurt with lowest level of cholesterol among all the samples analysed. As the storage period progressed, the cholesterol levels also reduced as a result of cyclodextrin treatment.

Cholesterol reductions was not significantly affected by percentage of cyclodextrin. There was a direct relationship between increase in cyclodextrin and decreasing level of fat, protein, reducing sugar and total soluble solid of cyclodextrin treated goat milk yoghurt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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