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Isolation and Identification of Microorganisms Associated with Yoghurt Enriched with Coconut Milk During Refrigerated Storage

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Abstract

Current trends and evolving consumer preferences present significant opportunities for innovation in fermented dairy products. This study investigated the microbial flora associated with yoghurt enriched with coconut milk during refrigerated storage. Yoghurt samples were produced by blending 800 g of reconstituted powdered cow milk with six litres of water, followed by the addition of coconut milk in varying proportions (10%, 20%, 30%, and 40%), alongside a control sample made with 100% cow milk. After fermentation, all samples were stored under refrigeration for two weeks. Microbiological analysis showed no bacterial growth at week zero in any sample. However, viable bacterial counts emerged in weeks one and two, ranging from 3.0×10^3 to 5.0×10^3 cfu/ml and 5.3×10^3 to 11.2×10^3 cfu/ml, respectively. Yeast and mold counts ranged from 2.6×10^3 to 4.0×10^3 cfu/ml in week one and 3.6×10^3 to 5.8×10^3 cfu/ml in week two. Coliform bacteria were not detected in any sample throughout the storage period. Bacterial isolates identified included *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Staphylococcus epidermidis*, and *Klebsiella pneumoniae*. Fungal isolates comprised *Aspergillus* spp., *Fusarium* spp., *Candida* spp., and *Saccharomyces* spp. The study

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concludes that yoghurt enriched with coconut milk can serve as a vehicle for probiotic delivery, offering enhanced nutritional benefits and contributing to consumer health and well-being.

Keywords: Coconut Milk; Microbial Flora; Yoghurt; Fermentation; Enrichment; Refrigerated Storage

INTRODUCTION

Yoghurt is a fermented dairy product obtained through an anaerobic fermentation of lactose in milk by relevant micro-organisms (Yilmaz *et al., 2003*). The microbial fermentation process resulted in the production of acetaldehyde, diacetyl, lactic, and acetic acids, which are responsible for the characteristic flavour of yoghurts (Sanful, 2009). Yoghurt, apart from being a probiotic carrier, is a rich and known source of quality protein, calcium, milk fat, potassium, magnesium, and vitamins B2, B6, and B12 (Vos et al., 2009). The fact that most of the lactose in milk precursor is being converted to lactic acid by the bacterial culture during fermentation makes yoghurt suitable for people who are moderately lactose intolerant (Yaakob,et al.,2012).

Apart from being nutritionally rich in protein, vitamins, and minerals, yoghurts offer several health benefits, some of which include the prevention of antibiotic associated diarrhoea and helping with a variety of gastro-intestinal conditions (Mazahreh and Ershidat, 2009). Other notable roles attributable to probiotic bacteria in dairy fermentations include the production of flavour compounds such as acetaldehyde in yoghurt and cheese, and other metabolites such as extracellular polysaccharides that will provide a product with the organoleptic properties desired by the consumer, the preservation of the milk by the generation of lactic acid and probably other antimicrobial compounds, the provision of special therapeutic or prophylactic properties against cancer, and the improvement of the nutritional value of food, this for example includes the synthesis of vitamins or the release of free amino acids (Mohammadi-Gourajiet al. 2019). Recent researches are shifting focus to diverse components in dairy foods, particularly fermented dairy products. Probiotics and prebiotics are evolving nutritional concepts in the development of dairy functional foods. Probiotics are defined as live microbial food ingredients which beneficially affects the host animal by improving its intestinal microbial balance (Mohamed Ahmed et al. 2021). While prebiotics are non-digestible foods that

make their way through our digestive system and help desirable gut bacteria to grow and flourish ((Shori et al. 2022).

Coconut *(Cocos nucifera L.),* a versatile fruit of the family *Arecaceae,* provides nutritious sources of meat, Juice, milk and oil. It is classified as a "functional food" because it provides many health benefits beyond its nutritional content, due to its fiber and oil content (Sanful, 2009). The oil is known to contribute to improved insulin secretion and the utilization of blood glucose; reduce symptoms associated with mal absorption syndrome and cystic fibrosis; help to relieve symptoms associated with crohn's disease; ulcerative colitis and stomach ulcers; improve the utilization of essential fatty acids and protect them from oxidation(Sanful, 2009).

Coconut milk is a nutritious food product consumed all over the world. (Narataruka et al. (2010) defined coconut milk as a sweet, milky white cooking base obtained, by mechanical extraction, from the endosperm of mature coconut, with addition of water. Coconut also serves as a raw material for the development of dairy-like products such as yoghurt (Belewu et al., 2010; Edem and Elijah, 2016 a). According to Yaakob et al. (2012), the nutritional content of coconut milk is superior to cow milk. Coconut milk has about 35% fat, 54% moisture and 11% solid non-fat ((Sanful, 2009b)., and is equally rich in minerals and vitamins.

To date, enhancing health and nutrition provision of probiotics to less-affluent communities in a locally sustainable way is still a major challenge. The use of indigenous foods as potential vehicles for transmission of probiotics has been given little attention even though it is an option with great potential in developing countries.

Broad Objective

The aim of this study is to produce yoghurt enriched with coconut milk during refrigerated storage so as to ascertain its safety.

Specific Objectives : The specific objectives are to :

1. isolate and identify the microbial floral associated with yogurt enriched with coconut milk during refrigerated storage

2. determine the safety of the coconut milk enriched yogurt.

MATERIALS AND METHODS

Raw Materials Procurement

The coconut fruit and commercial powdered full cream peak) milk was obtained from Wukari new market, Taraba state Nigeria. The Yogourmet freeze-dried starter culture containing *Streptococcus thermophillus* and *Lactobacillus delbrueckiisubsp. Bulgaricus* was purchased from a chemical supermarket in Aba, Abia state Nigeria.

Sample Preparation

Extraction and Preparation of Coconut Milk

The method as described by Sanful 2009 was used with some modification. The coconuts were cracked open and the water poured and stored in a refrigerator. Two kilogram (2 kg) of coconut flesh was scrapped off of the brown skin. The meat was cut into smaller pieces to facilitate blending. The flesh was blended with the coconut water and 2 litres of water for 2 minutes. It was passed through a double folded cheese cloth of 0.25 mm mesh size to get the coconut milk and stored in a container in a refrigerator (4°C). Fig 1.0

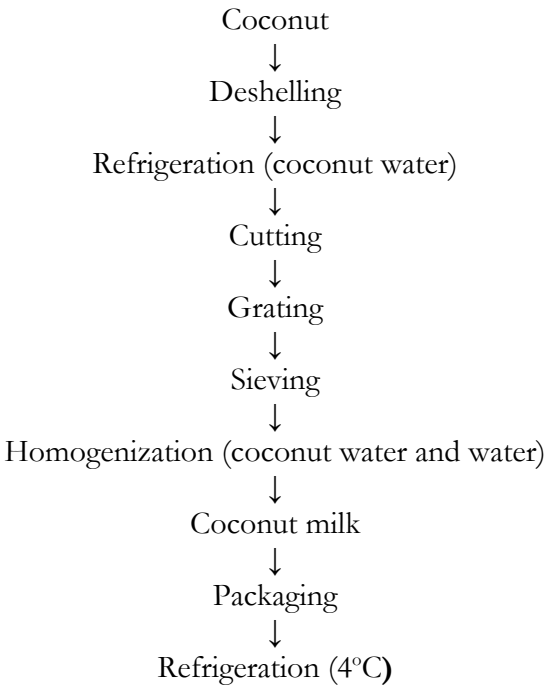

Fig 1: Process flow diagram for Extraction of Coconut Milk

Source: Sanful, 2009.

Re-Preparation of Cow Milk

Eight hundred gram (800g) of powdered cow milk (peak) was homogenized with 6 liters of water to produce equivalence of fresh milk (Sanful, 2009).

Experimental Treatments

The yoghurt samples were prepared from blends of cow milk and coconut milk and the sample was divided into four batches as stated below:

i. Sample A: 100 % cow milk (500ml)
ii. Sample B: 90 % cow milk (1350ml) and 10 % coconut milk (150ml)
iii. Sample C: 80 % cow milk (1200ml) and 20% coconut milk (300ml)
iv. Sample D: 70 % cow milk (1050ml) and 30% coconut milk (450ml)
v. Sample E: 80% cow milk (900ml) and 40% coconut milk (600ml)
Making a total of 4500ml of cow milk and 1500ml of coconut milk

Preparation of Fresh Yoghurts

The method of Sanful (2009) was used. Fifty gram (50 g) of sugar were added to the sample of the reconstituted milk and mixed thoroughly to homogenize. The mixtures were heated in a water bath at 85 ˚C and maintained at this temperature for 30 minutes. The pasteurized milk blends were cooled to 42 ˚C and each was inoculated with 10 g of yoghurt culture (*S. theophrmillus* and *L. bulgaricus*) and incubated at 28 ± 2 ˚C for a period of 16 hours to produce fresh yoghurt. Different composition of the yogurt and coconut milk were blended and stored in the refrigerator for three weeks and analyses were carried out at weekly interval.

Reconstituted powered cow Milk (CM)
↓
Dosing of ingredients (sugar)
↓
Pasteurization (85^0C, 30 minute)
↓
Cooling (42^0C)
↓
Inoculation (yoghurt culture)
↓
Incubation (16h, 45^0C)
↓
Fresh yoghurt
↓

Filling
↓
Blending of yoghurt with coconut milk
↓
Refrigeration storage (4^0C)

Fig 2. Flow Chart for Fresh Yoghurt Composite Preparation

Source: (Sanful (2009).

Microbiological Analysis

Isolation and Enumeration

Total viable bacterial cells were determined using the method as described by (Obasi et.al; 2019). Serial dilution (10 fold) was carried out (1:10, 1: 100,1:1000…10,0000). 0.1ml of appropriate dilutions (10-2 and 10-4) was placed on various agar plates using pour plate method and incubated at 37^0C for 18-24 hours for total aerobic bacteria and coliform count. For fungi

0.1ml amount of appropriate dilutions (10-2 and 10-4) was poured into the plates of potato dextrose agar and incubated at room temperature at 28 ± 1^0C for 3 to 5days. All enumeration were expressed as colony forming unit (cfu/ml).

Purification and Maintenance of Microbial Isolates

Bacteria and yeast isolates were transferred into fresh agar medium of isolation and incubated at 37^0C for 24hours.Pure colonies of bacteria and yeast were maintained and stored at 4^0C until needed.

Characterization and Identification of Isolates

Bacteria isolates were characterized and identified based on their microscopic appearance, colonial morphology, gram staining and biochemical tests. The isolates were identified by comparing their characteristics with those of known taxonomy, as described by Bergey's Manual of Systematic Bacteriology (2005) and Obasi et.al; 2019). The microbiological identification procedures included: motility test, color /pigmentation on culture plates. The biochemical tests included : Oxidase, Catalase, Coagulase, Urease, Indole, Citrate Utilization Test and triple sugar ion fermentation (TSI).

Statistical Analysis

All analyses were conducted in duplicate. All the data obtained were subjected to one way analysis of variance (ANOVA) and the difference among the means were determined using the Duncan multiple range test ($p<0.05$). Data analysis were carried out using the Statistical Package for Social Sciences (SPSS) Version 20.0 and the results was presented as mean with standard deviation.

RESULTS AND DISCUSSION

Microbial Evaluation of Yogurt Enriched with Coconut at Refrigeration Temperature (4ºc)

The Table 1. shows the bacterial cells for week 0, in sample A-E recorded no growth. But in the 1st and 2nd week there were viable count. This ranged from $3.0x10^3$ to $5.0x10^3$ and $5.3x10^3$ to $11.2x10^3$. The increase in the bacterial cell count were still acceptable and did not exceed the codex alimentarius standards for yoghurt permitting a minimum of 10^7cfu/g in the finished product (Codex standard for fermented milk, 2003). The yeast and mold count for week 1 and 2 ranged from $2.6x10^3$ to $4.0x10^3$ and $3.6x10^3$ to $5.8x10^3$. Coliform count was zero for all samples during storage. The result indicates that the total viable count level in the stored yoghurt samples increased during storage. The result obtained also revealed that yoghurt with highest coconut milk (40%) shows higher increase in growth ranging from $3.0x10^3$ to $11.2x10^3$cfu/ml on cow milk (plain yoghurt). This increase can be as a result of the addition of natural functional ingredient (coconut milk) used in our study which contain some components, essential fatty acids, vitamins, minerals, and others, that can assist to increase the growth of bacteria (Mohamed Ahmed et al. 2021; Wijesekara et al. 2022). This result is also in agreement with the findings of Isam et al., (2011) who reported similar trend during storage of the yoghurt enriched with coconut.

The result of isolation and identification of bacteria from yogurt enriched with coconut milk are shown in Table 1. The result revealed the strains of isolates of bacterial species from sample A-E, these included *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Staphylococcus epidermidis, Klebsiella pnuemoniae.* It is known that these bacteria may be available in environmental samples from soil, and plants and if there is a contamination to milk during milking and processing, it can lead to the growth of these micro flora in fermented milk products because they are tolerant of a range of pH values (Ahmed et al; 2023). The

prevalence for all the bacterial isolated is within the range of 20%.The yeast and mold isolated from the samples includes species of *Aspergillus, Fusarium, Camdida and Saccharomyces.*

It has been discovered that milk and milk products have been destroyed by the secretion of extracellular enzymes by psychrotrophic bacteria during long period of refrigerated storage (Samarzija. *et al*., 2012). The most common psychrotrophs in these product are gram-negative rods which produces a variety of enzymes that cause chemical deterioration of milk resulting in off flavor. Gram-negative psychrotrophs do not survive pasteurization thus their occurrence in heat treatment product are attributed to post pasteurization contamination even though the bacteria are destroyed in pasteurization their enzymes are not inactive and may continue to degrade milk product (Ahmed et al.,2023).

Table 1. The Total Aerobic Plate Count of Bacterial Cell, Yeast, and Mold (cfu/ml) In Yoghurt Enriched with Coconut during Refrigeration Storage.

Storage period (weeks)	Sample A	Sample B	Sample C	Sample D	Sample E
Zero(0)	ND	ND	ND	ND	ND
1	5.0×10^3	3.7×10^3	3.9×10^3	3.2×10^3	3.0×10^3
2	5.3×10^3	5.6×10^3	5.4×10^3	6.3×10^3	11.2×10^3
Yeasts and Mold count					
Zero(0)	ND	ND	ND	ND	ND
1	2.6×10^3	4.4×10^3	3.0×10^3	4.8×10^3	4.0×10^3
2	3.6×10^3	5.2×10^3	4.2×10^3	5.8×10^3	5.6×10^3
Coliform count					
Zero(0)	ND	ND	ND	ND	ND
1	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND

Sample A; control, yoghurt without coconut milk, sample B, C, D, and E; yoghurt with 10%, 20%, 30%, 40% coconut milk. CCM: coconut milk, CM: cow milk. ND = No growth.

Table 2. Morphological and Biochemical Characteristics of Bacterial Strains Isolated from Yoghurt Enriched with Coconut during refrigerated storage

ISOLATES CODE	CS	CA	RXN	OXI	CIT	GLU	SUC	LAC	IND	COA	H_2S	GAS	CAT	IDENTITY (ORGANISM)
WEEK 1														
A	RODS	SINGLE	-	-	-	+	+	+	+	+	-	+	+	*Escherichia coli*
B	RODS	SINGLE	-	-	+	+	+	+	-	+	-	+	+	*Enterobacter aerogenes*
C	COCCI	CLUSTER	+	-	+	+	+	+	-	+	-	+	+	*Staphylococcus epidermidis*
D	COCCI	CLUSTER	+	-	+	+	+	+	-	+	-	+	+	*Staphylococcus epidermidis*
E	COCCI	CLUSTER	+	-	+	+	+	+	-	+	-	-	+	*Staphylococcus aureus*
WEEK 2														
A	COCCI	CLUSTER	+	-	+	+	+	+	-	+	-	-	+	*Staphylococcus aureus*
B	ROD	SINGLE	-	-	+	+	+	+	-	+	-	+++	+	*Klebsiella pnuemoniae*
C	RODS	SINGLE	-	-	+	+	+	+	-	+	-	+	+	*Enterobacte aerogenes*
D	RODS	SINGLE	-	-	+	+	+	+	-	+	-	+++	+	*Klebsiella pneumoniae*
E	RODS	SINGLE	-	-	-	+	+	+	+	+	-	+	+	*Escherichia coli*

KEY: RXN = Reaction, OXI = Oxidase, CIT = Citrase, GLU = Glucose, SUC = Sucrose, LAC = lactose, IND = indole, COA = coagulase H_2S = Hydrogen Sulphide, CAT = Catalase, - = Negative, + = positive, GR = gram's reaction, S/N = serial number, CS= Cell Shape, CA= Cell Arrangement

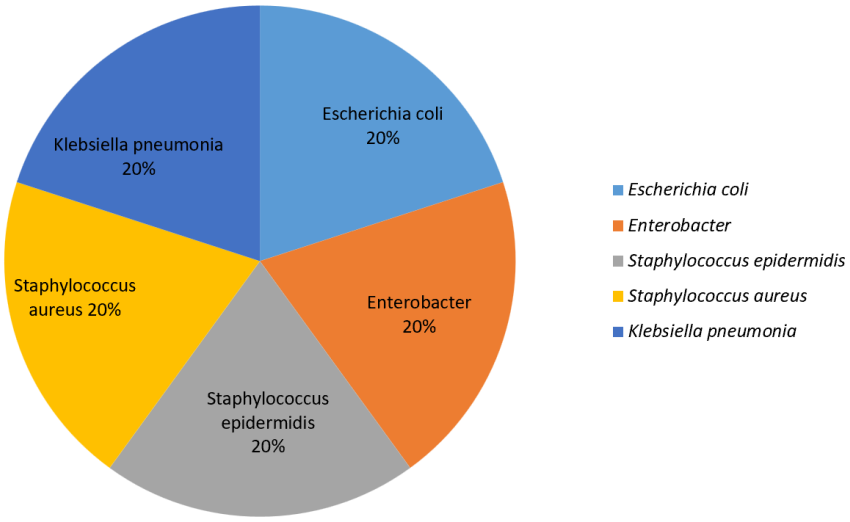

Fig 3. Prevalence of Organisms Isolated from Coconut Enriched Yoghurt during Refrigerated Storage

Table 2 Microscopic and Colonial Morphology of Mold Isolated from Yoghurt Enriched with Coconut Milk

Storage period(weeks)	ISOLATES CODE	MICROSCOPIC	COLONIAL MORPHOLOGY	SUSPECTED ORGANISMS
Zero(0)				
	B	Mycelium septate. Smooth and colourless Conidiophores and spores. Globose conidia head, dark brown in colour.	Wooly, white to yellow then turning black.	*Aspergillus spp*
	C	Smooth and colourless Conidiophores and spores. Globose conidia head, dark brown in colour.	Wooly, white to yellow then turning black	*Aspergillus spp*
	D	Smooth and colourless Conidiophores and spores. Globose conidia head, dark brown in colour.	Wooly, white to yellow then turning black	*Aspergillus spp*
	E	Radiating conidia head. Conidiophores appear rough.	Velvety, yellow , green or brown colony.	*Aspergillus spp*
1				
	A.	Conidiophores appeared singly and some grouped,elongate and cylindrical. Micro conidia are one walled and often numerous in chain	White cottony and fluffy appearance owing to extensive mycelium	*Fusarium spp.*
2				
	C	Micro Conidia are one walled and often numerous in chains. Also elongate and cylindrical. Conidiophores appeared singly and some grouped.	White cottony and fluffy appearance owing to extensive mycelium	*Fusarium spp.*

Table 3. Microscopic and colonial morphology of yeast isolated from yoghurt enriched with coconut milk.

Storage Period (Weeks)	ISOLATES CODE	GRAM RXN	MICROSCOPIC CHARACTERISTICS	COLONIAL MORPHOLOGY	SUSPECTED ORGANISMS
Zero(0)					
	A	+	Thiny oval short cells	White colour, creamy growth	*Candida spp.*
	B	+	Thiny oval short cells	White colour, creamy growth	*Candida spp.*
	C	+	Large oval elongated cells	White colour, creamy growth	*Saccharomyces spp.*
2					
	A	+	Large oval elongated cells	White colour, creamy growth	*Saccharomyces spp.*
	B	+	Large oval elongated cells	White colour, creamy growth	*Saccharomyces spp.*
	C	+	Large oval elongated cells	White colour, creamy growth	*Saccharomyces spp.*

Sample A; control, yoghurt without coconut milk, sample B, C, D, and E; yoghurt with 10%, 20%, 30%, 40% coconut milk.

CONCLUSION

This study has shown that yogurt enriched with coconut milk exhibited an increase in the number of viable bacterial cells during storage. However, the microbial load remained within the acceptable limits set by the *Codex Alimentarius*, which allows a minimum of 10^6–10^7 cfu/g in finished yogurt products. These findings confirm the microbiological safety of coconut milk-enriched yogurt over the observed storage period.

The study contributes to the growing literature on functional dairy-alternative products by demonstrating the compatibility of coconut milk with probiotic viability and product safety. The results also suggest that incorporating natural antibacterial agents such as curcumin and turmeric during storage may further reduce microbial activity, enhancing product safety and extending shelf life.

In light of these outcomes, it is recommended that future research explores thermal treatment techniques for coconut milk yogurt to enhance its microbial stability. Additionally, the development and quality assessment of coconut milk-based ice cream and

cheese products should be undertaken to expand the functional use of coconut milk in dairy alternatives and further promote innovation in plant-based food products.

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