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QUALITY AND STORAGE CHARACTERISTICS OF SOME COLD-STORED YOGHURTS

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Abstract: **One of the basic functions of the food research scientist in the modern era is the identification of both beneficial food sources as well as the prevention of foods from being route of infectious diseases. Yoghurt is a well-known biologically active foods widely consumed the world over. Its long known beneficial roles could be linked to the presence of high concentrations of live bacteria they contain which provides various metabolites in the human diet. However, due to factors such as inadequate storage facility, this important food material can, apart from losing the essential nutrients, could become vehicle for the transmission of some important human disease-causing agents. In this present study, four samples (Y1-Y4) of vended, commercially available yoghurts were evaluated for their functional, technological and microbial properties during a Twenty-eight-day storage time. Some qualities of the yoghurt samples were determined by measuring the pH level during the storage period while forced syneresis were measured as technological property. The total microbial counts were determined by culturing aliquot samples on nutrient agar and other appropriate selective media. The results showed that at day 0 of storage, the pH of the products ranged from 4.65 down to 4.31, while those of the 28th day storage ranged from 4.53 to 4.01. The bacteria content increased from 2.53x10^{7} on day 1 to 3.16x10^{7} on the 14th day of storage then decreased to 5.2x10^{6} on the 28th day. The cell-free supernatant of some of the samples produced zones of inhibition greater than 14mm against three indicator bacteria strains *E. coli*, *Salmonella* and *Staphylococcus aureus.* There was maximum acceptability of the product based on the panelists' assessment observed on the 28th day of sample storage. The use of cold storage enhanced the properties of yoghurt as could be observed in this work.**

Keywords: **Outbreak, contamination, deterioration, Nutrient and storage.**

1. INTRODUCTION

In recent years, there has been a series of reports and efforts to increase both the awareness and production of different varieties of customized yoghurts. This important 'functional foods' continue to experience attention owing to consumer-driven changes in life styles in terms of the kind of foods people are willing to consume (Oberman, 1985).

Various factors have been attributed to the consumption of yoghurts. They include the organoleptic characteristics, nutritional versatility, dietary and the medical importance. Yoghurt is obtained from the fermentation of dairy milk using

some selected acidifying bacteria in a synergetic combination. The bacteria, basically Lactobacillus bulgaricus and Streptococcus thermophilus, releases organic acids and characteristic aroma into the fermented products as well as inhibiting spoilage bacteria contaminants in the final product thereby increasing its shelf life (Kamenik *et al*., 2021). Depending on consumers' choice, however, other bacteria such as Lactobacillus acidophilus and Bifidobacterium strains are now been added as supplements to the yoghurt products. This new generation yoghurt is often preferred by many dedicated consumers.

The physiological, chemical as well as the overall acceptance of any yoghurt product is directly dependent or affected by the appropriate selection of the raw materials used for the production as well as the production and storage methods/conditions because these goes a long way to affecting the organoleptic characteristics of the products on which consumer demand tendencies depends (Aly *et al*., 2004). The storage time and conditions also significantly affect physicochemical, sensory and microbiological characteristics of any given yogurt. For instance, while some yoghurtss have short shelf life of three weeks and less, others might be stored for longer periods with fairly stable nutrition and sensory characteristics. Yoghurt products that are not properly stored experiences decrease in nutritional qualities, deterioration of organoleptic properties as well as decrease in the number and ratio of the probiotic beneficial mutual microorganisms due to the toxic environment produced by some fermentation microorganisms that continue to grow in the poor storage conditions (Escalante *et al*., 2012). In a number of retailed yoghurt products in Nigeria, the storage temperature of dairy products has been found to greatly affect and therefore determine the quality of the products finally available to the end-users. Through awareness and education that could be made available from research such as this, retailers and consumers could be made to know that there is recommended storage temperature of for longer stability of their product which though is usually from 1°C to 8°C, but could be increased by storing at 4°C or less. (Hassan, 2010). The aim of this study was therefore to determine the impacts of cold-storage of yoghurts on the quality and other characteristics.

2. MATERIALS AND METHODS

Collection of Yoghurt Samples

Four popular samples of vended, commercially available yoghurts were randomly purchased at some retail shops within the Federal Capital Territory (FCT), Abuja, Nigeria. The samples were immediately transported to the microbiology laboratory in cold coolers for analysis (Al Muzahid and Mondal, 2014).

Sterilization of Media and Ware

All the Glass wares used in this work were sterilized with hot-air oven at 180^0C for one hour and the media were sterilized at 121^0C for 15minutes using autoclave system. Other sensitive materials were sterilized according to specifications applicable (Hassan, 2010).

Sample Analysis

The samples were distributed in sterile package materials to enabled seven-day analysis for the Twenty-eight days storage time at refrigerated and ambient temperatures.The average yogurt composition, according to the manufacturer's information were; pasteurized milk, Sugar, live yogurt bacteria cultures, Nutritional value in 100g of product: Energy (calories), 61.9 kcl; Fat, 1.7g; proteins, 6g and Carbohydrates 5.2g (AOAC, 1990).

Determination of Yoghurt Compositions

The AOAC 1990 methods was used for the determination of yoghurt compositions. The total nitrogen was measured by micro-Kjeldahl method (AOAC 1990). Protein was calculated as N x 5.38. Fat was determined by the Gerber method. Ash content was determined by dry ashing of the samples for 24 h at 550°C. Moisture content was determined by drying samples overnight at 105°C (AOAC 1990). Total solids content was determined by gravimetric method by drying the samples in an oven at 105°C for 24 h (AOAC 1990). Crude fiber content was determined according to the acid/alkali digestion method of Southgate (1976). The analyses were performed in triplicates.

pH determination

The pH was measured using a hand-held pH-meter (HANNA-pH 210, Germany).

The Antimicrobial Effects of the Cell-free Culture Supernatants

The cell-free culture supernatants (CFCS) from the yoghurt samples were obtained by centrifugation of a portion of the sample. The supernatant was tested against pathogenic *E. coli* and *Staph aureus* using the agar well diffusion method. The presence of a zone of inhibition (ZOI) around wells was evaluated at different storage days (time) (Bylund, 1995).

Microbiological Analysis

For the microbiological tests, one gram of each yogurt sample was added to 9 ml sterile 0.1% peptone water and shaken to give a 10^{-1} dilution. Then serial dilutions of up to 10^{-6} were made. Aliquots 0.1 ml of 10^{-4} and 10^{-6} were used respectively to inoculate the surface of the appropriate agar media viz; plate count agar for total bacteria counts; MRS agar containing 10mg ml^{-1} nystatin selective for lactic acid bacteria; M-17 agar selective for *Streptococcus*, Brilliant green, xylose lysine decaeboxylate, and bismuth sulphite agar selective for *Salmonella spp.*; Mannitol salt agar- selective for staphylococci; Baird Parker agar selective for *Staphylococcus aureus,* malt extract agar containing 100 µg ml^{-1} chloramphenicol selective for yeasts and Potato dextrose Agar (PDA) containing 100 µg ml^{-1} chloramphenicol selective for moulds, using the spread plate technique. The agar plates were incubated aerobically and anaerobically at the appropriate temperatures for each target organism. After the 24-48 hours of incubation, the discrete colonies were studied and counted as per colony forming unit in reference to the dilution factors and then the appropriate colonies were selected for further identification protocols (Sullivan and Nord, 2002).

Determination of the Forced Syneresis of Yoghurts

The Syneresis of the yoghurt products were determined by the ratio of the whey that separated out of the total weight (100 g) of each yoghurt sample. The obtained yoghurt mix (25g) was weighed in a graduated centrifuge tube and were centrifuged at 3,500 RPM for 10 Minutes at 4^0C. The whey separated from yoghurt sample was determined by comparing the ratio of the whey with the total volume of yoghurt before centrifugation (Amatayakul and Sherkat, 2006).

3. RESULTS AND DISCUSSION

Table 1: The Effects of Storage on the Qualities of Cold-stored Yoghurt

Sample	Storage (Day)	pH	Syneresis (%)	Inhibition (mm)	
				E. coli	*Staph aureus*
Y1					
	0	4.5	2 2	11	09
	7	4.2	2.5	06	11
	21	4.1	2.6	-	-
	28	3.8	3.2	-	12
Y2					
	0	4.7	4.3	07	06
	7	3.9	4.6	-	04
	21	3.7	4.8	13	09
	28	3.7	5.2	-	-
Y3					
	0	4.4	3.2	10	06
	7	4.1	3.4	-	-
	21	4.0	4.2	04	08
	28	4.2	4.1	07	13
Y4					
	0	4.3	0.0	-	-
	7	4.0	0.0	07	09
	21	3.9	2.1	-	-
	28	3.8	2.7	08	-

Y1-Y4 = Yoghurt Samples; - = No Inhibition zone

The tests in this work showed a significant decrease in pH of the storage product at cold temperature. The pH generally decreased from 4.7 on the first day the samples were purchased in sample Y2 day 0 to 3.7 in the sample but on day 21. During the 28-day storage and monitoring exercise, there were generally decrease in the pH of all the samples (Table 1). The results obtained by other researchers differ from the above. Atallah, A (2015 recorded a lower pH value. Other works have also recorded a regular increase in curd acidity. In a similar report, it has been shown that a steady decrease in pH is associated with a prolonged storage time. The increase in the acidity of yogurt during storage is a result of the fermentation activity of microorganisms that make up the yogurt matrix. The active bacteria are able to convert the milk lactose to lactic acid even at low temperatures although at a lower rate.

Syneresis is a defect in yoghurt production and some times affect the quality and acceptance by the consumers. It is the separation of whey from the milk solids. The highest record in this work was in sample Y2 which is 5.2% on the 28th day of storage. The lowest being 0.0 (No syneresis) in sample Y4 on day 2 and the fresh product. The effect of storage on syneresis revealed that the value of syneresis increased with the storage time (Table 1) The results are in conformation with the research of previous workers such as Banerjee *et al*., 2003 in which the rate of syneresis was observed to be directly related to the acidity and therefore is inversely related to pH.

The inhibitory effects of the cell-free supernatants of the yoghurt samples were also tested against *E. coli* and *Staph aureus* with the inhibitory effects ranging from 6mm to 11mm. some samples however did not have any inhibitory effects (Table 1). This is in agreement with the work of Pleijsier *et al*., 2003 that lactic acid bacteria produce several metabolic products during fermentation that are antagonistic and bactericidal to other microorganisms. The antagonistic activity helps the dominant organisms to maintain their niches. This phenomenon is applied in the natural preservation of several fermented food products by wading off the spoilage and pathogenic microorganisms.

Table 2: The Microbiological Quality of the Cold-Stored Yoghurts

Sample	Storage (Day)	Microbial Occurrence					
		TPC	LAB	Salm.	Staph	Yeast	Mold
Y1							
	0	$3.2x10^7$	$2.4x10^7$	ND	06	$1.3x10^2$	ND
	7	$2.5x10^7$	$3.2x10^6$	ND	09	$1.8x10^1$	ND
	21	$1.4x10^7$	$2.1x10^6$	ND	02	$2.3x10^1$	$1.2x10^1$
	28	$1.2x10^7$	$2.0x10^6$	ND	ND	$1.6x10^1$	$1.1x10^1$
Y2							
	0	$4.1x10^7$	$2.6x10^7$	02	ND	ND	ND
	7	$3.4x10^7$	$2.5x10^7$	ND	06	$1.5x10^1$	ND
	21	$3.2x10^7$	$2.2x10^7$	ND	08	$1.6x10^1$	$1.1x10^1$
	28	$2.9x10^7$	$2.9x10^6$	ND	ND	$1.6x10^1$	$1.3x10^1$
Y3							
	0	$3.5x10^7$	$4.4x10^6$	05	ND	ND	ND
	7	$2.1x10^7$	$3.4x10^6$	02	ND	21	03
	21	$2.0x10^7$	$3.1x10^6$	ND	ND	29	03
	28	$2.0x10^7$	$2.9x10^5$	ND	ND	41	07
Y4							
	0	$4.1x10^6$	$2.6x10^6$	ND	ND	1.0x101	ND
	7	$2.3x10^6$	$2.4x10^5$	ND	ND	1.3x101	ND
	21	$2.4x10^6$	$4.4x10^5$	ND	ND	ND	ND
	28	$2.3x10^6$	$3.4x10^4$	ND	ND	ND	ND

ND = None Detected

The yoghurt samples that were collected and analysed showed high Bacterial counts (Table 2). The highest was in the fresh sample Y2 which was $4.1x10^7$ Cfu/ml. The lowest however was found in sample Y1 on the 28th day of cold storage which was $1.2x10^7$. The higher initial total plate count values in this study might be due to the serious deficiencies and compromises in basic production hygiene. Lack of understanding of clean and hygienic food production environment have been attributed to many disease outbreaks.

There was generally a steady decrease in the record of lactic acid bacteria in all the samples. For instance, in sample Y2, the fresh sample prior to storage was $2.6x10^6$ to $2.9x10^6$ at the end of the cold storage time. This observation indicating decrease in the number of living (probiotic) bacteria in yoghurt products during storage represents an important information for consumers of the products.

Salmonella and Staphylococcus bacteria were either not detected of they occurred in low values (Table2). This is a positive note as far as the food is concerned. It is in record that Staphylococcus and *Salmonella* are top etiologies in foodborne illness outbreaks associated with foods even at low temperature which present a high food risk.

While some yoghurt samples did not have yeast nor mold growth both from fresh and 28-day stored products, others had low occurrence either on fresh or towards the end of storage time. Total yeast and mold count in yoghurt samples ranged from none detected (Y4) to $1.3x10^2$ (fresh Y1 sample). The occurrence of yeasts or molds in yogurt is an indicative of poor sanitary practices in manufacturing or packaging of yoghurts. Though yeasts could be part of fermentation microorganisms, they could also be source of spoilage and disease-causing agent especially those that grows at low temperatures (Deak, 2009). Fungi are known to be more tolerant to extreme conditions than bacteria. This is an important information and knowledge especially where undesirable metabolites are been produced that could be responsible for alteration of colours and tastes. It should be note that food spoilage due to the presence of yeast has increased as a result of mild preservation processes required for developed standards of food quality (Davies et al., 2013).

4. CONCLUSION AND RECOMMENDATIONS

In conclusion, the overall picture of yogurt quality in the study area as measured by the chosen parameters actually indicated the need for adequate knowledge of food quality control and management. The level of bacteria, yeast and mold counts indicated that some form of contamination must have occurred during the production of the yoghurt samples. It is hereby recommended that small and large-scale yoghurt producer to maintain adequate hygienic conditions to make sure that standard condition of food production is provided to ensure wholesome food products with reduced the spoilage and pathogenic microorganisms.

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