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Full Length Research Paper

Effects of pasteurisation on survival patterns of microorganisms and vitamin C retention in kunun-zaki

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The effects of pasteurisation on the survival patterns of the microflora as well as vitamin C retention in kunun-zaki fortified with vitamin C was syudied. A laboratory sorghum based kunun–zaki was fortified with 90 mg/l of vitamin C. The content was subjected to pasteurisation at 70°C for different time intervals ranging from 0 - 30 min. Samples of the beverage were then taken at 5 min intervals during pasteurisation and were analysed for microbial load and residual vitamin C content by 2,6-dichloroindophenol titration method. The results showed that there was a gradual decline in vitamin C retention and steep decline in the microbial load reduction during pasteurisation. The result also showed that at the 20th min of pasteurisation, 80% of microbial isolates had been eliminated leaving only *Bacillus Subtilis* and *Saccharomyces cerevisiae* as the surviving organisms. The D_{70} -value of the most heat resistant organism *B. subtilis* was found to be 6.5 min.

Key words: Kunun-zaki, pasteurisation, D-value, vitamin C, *Bacillus subtilis*.

INTRODUCTION

Employment of modern food processing and preservation techniques to enhance the nutritional value and to prolong the shelf stability of food has remained the key instrument to fighting insurgent global food crises, hunger and malnutrition. Food fortification with vitamins (vitaminization) is the intentional and regulated addition of vitamins to food products that are found lacking in certain vitamins. Vitamin C (ascorbic acid) is a white crystalline compound of a simple structure that is closely related to the monosaccharides and which is naturally synthesized in certain plants like the citrus family and by some microorganisms. Vitamin C is needed for the formation and maintenance of intercellular substances or tissues, building resistance to infections and in the absorption of calcium and iron. Humans need a daily intake of 30 - 45 mg per day while deficiency of this vitamin results in a nutritional disorder referred to as scurvy (Fisher and Bender, 1985). In developing countries (including Nigeria) where the standard of living is poor, the need to fortify beverages such as kunun-zaki which is widely consumed by the populace should be a strategy to overcoming vitamin C deficiency that is prevalent among the rural poor.

Pasteurization is an age-long practice of subjecting beverages or drinks to high temperatures of below 100°C (60 – 90°) with the aim of destroying the vegetative cells and without altering the organoleptic quality of the food (Adams and Moss, 1997). It has been proven successful in dairy products, fruit juices as well as beer and other drinks. The proximate composition of kunun-zaki as determined by Edward-Inatimi et al. (1988) showed kunun-zaki to be generally of low nutritional status aside from providing substantial carbohydrate value. Kunun-zaki is a popular Nigerian cereal based non-alcoholic beverage consumed mostly in Northern Nigeria and neighboring Chad and Niger republics. Among the many challenges of indigenously fermented foods which include the slow and crude methods of their production, is the fact that these foods, (including kunun-zaki) have very low shelf stability, as they readily undergo microbial-induced spoilage within 2 to 3 days. Egbere et al. (2008) had isolated and characterized microorganism associated with the spoilage of kunun-zaki which include *Saccharomyces cerevisiea*. Studies on pasteurization of kunun-zaki as undertaken by Inyang and Dabot (1997), Olasupo et al. (2000) and Gaffa et al. (2004) all indicated

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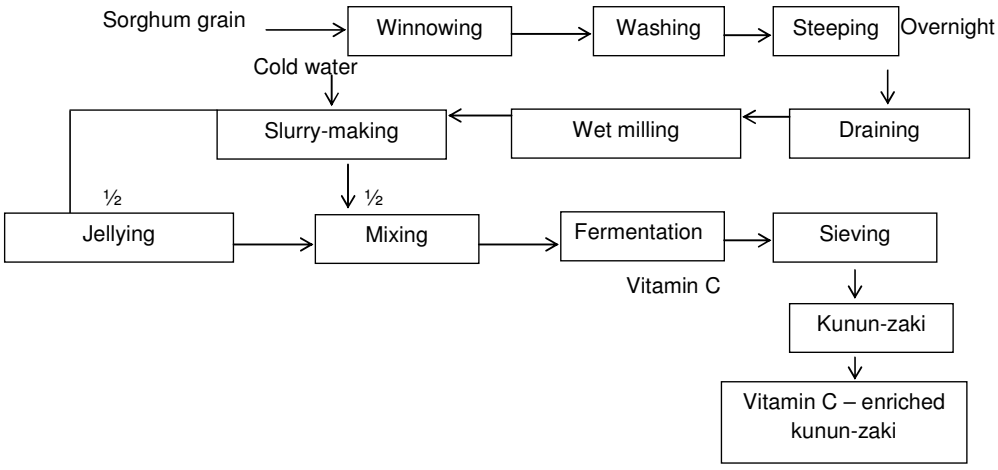

Figure 1. The modified laboratory method of kunun-zaki production (Egbere et al, 2008).

that kunun-zaki could be improved upon from 2 days to a period of 14 – 27 days either by pasteurization or the use of sodium benzoate or both.

Vitamin C is known to be thermo-labile and equally susceptible to oxidation on exposure to atmospheric oxygen. It is converted to an oxidized form known as dehydroascorbic acid (Ryle and Kyla, 1994). The need to improve on the vitamin C content and as well make kunun-zaki shelf stable and safe to consumers has necessitated this research. While achieving these aims it becomes critical to strike a balance between nutrient retention and microbial load reduction/elimination. The concept of thermal death time or decimal reduction time D-value which refers to the time in minutes that is required to reduce the microbial load of an organism by 90% (or by one log cycle) is a mathematical tool that can be used in understanding the degree of heat resistance of a notorious spoilage microbial agent during pasteurization.

MATERIALS AND METHODS

Sample purchase

The raw materials, sorghum bicolour, malted rice, dry and raw sweet potato chips and cloves used for the laboratory preparation of kunun-zaki were purchased form the Kwararafa new market in Jos metropolis, Plateau State, Nigeria.

Laboratory preparation of Kunun-Zaki

The laboratory method of preparing kunun-zaki as described by Egbere et al. (2008) using *Sorghum bicolour* grains as the main ingredient was carefully followed (Figure 1).

Vitamin C fortification

A Known quantity of (180 mg) while crystals of vitamin C powder was weighed out using a laboratory weighing balance (Beam) and introduced into 2 l of freshly prepared kunun-zaki. The content was thoroughly mixed and kept aside for use in pasteurization studies.

Pasteurization of vitamin C enriched kunun-zaki

The vitamin-enriched kunun-zaki was distributed into sterile plastic bottles (200 ml each) which were then screw-capped and labeled. The sample (in bottles) in duplicates k0 (unpasteurized) k5, k10, k15, k20, k25 and k30 were subjected to different heat exposure times (in a water-bath set at 70^{o}C) for 0, 5, 10, 15, 20, 25 and 30 min, respectively. The sample bottles were removed consecutively from the water-bathed medium and immediately cooled by immersion in cold water to attain room temperature.

Determination of vitamin C

The 2,6-dichlorophenol – indophenol titrimetric method for determination of vitamin C content in foods as described by the American Association of Analytical chemists (AOAC, 1980) was followed. This method is based on the principle that ascorbic acid is easily oxidized by the coloured dye, 2,6-dichlorophenol – indophenol to dihydroascorbic acid.

Isolation and identification of microbial isolates

Pure influxes of the microbial isolates were obtained by repeated culturing and the isolates were identified using cultured, morphological, and microscopic and biochemical characteristics as described by Fawole and Oso (1988).

Determination of the D-value

The most heat resistant bacterium at the 30th min of pasteurization was determined to be *Bacillus subtilis*. Pure culture of *Bacillus subtilis* was obtained by repeated sub-culturing. A loopful of the bacterium was inoculated into 10 ml of sterile peptone water and incubated at 37^{o}C for 24 h. The presence of turbidity indicated growth of the organism. The stock culture (10 ml) was inoculated into 990 ml sterile kunun-zaki (autoclaved) and mixed properly. The kunun-zaki was then redistributed (in 200 ml each) into sterile plastic bottles and then subjected to pasteurization at 70^{o}C for

Table 1. Effects of pasteurization at 70°c on vitamin c and microbial load reduction in vitamin c fortified kunun-zaki.

Time (min)	Vitamin C (mg/L)		Surviving bacterial population (cfu/ml)	Surviving fungal population (cfu/ml)
	Retained	% Loss		
0	90.00	n.a.	9.4×10^5	4.1×10^4
5	70.00	22.22	8.1×10^4	3.7×10^3
10	67.40	25.11	4.8×10^3	8.3×10^2
15	51.30	43.00	1.4×10^3	3.3×10^2
20	44.00	51.11	3.9×10^2	6.4×10^2
25	36.00	60.00	1.6×10^2	Negligible
30	27.60	69.33	3.1×10^1	Negligible

n a. = Not applicable.

different heating times 0 (control) 5, 10, 15, 25 and 30 min, respectively. Each of the pasteurized samples were subjected to serial dilution and plated out to determine the number of surviving *Bacillus* at 35 – 37°C for 24 h of incubation.

To determine the D-value, a graph of the logarithms of number of the bacterium was plotted against the various heating time intervals at 70°C. The reciprocal of the curve was taken and this according to Adams and Moss (1999) represented the D-value which was equivalent to the time required to decrease the number of the surviving *Bacillus* by 90% (or by one log cycle).

RESULTS AND DISCUSSION

Effects of pasteurization on microbial load

The results in Table 1 show that there was a corresponding decline in both the bacterial and fungal loads of kunun-zaki. However, the effect was found to be more pronounced on the fungi than on bacteria, for the fact that at the 25th min of pasteurization fungal load was already negligible. The pattern of microbial load reduction or death of microbial cells at the pasteurization temperature could be described as being logarithmic; steeply increasing with respect to time of it were to be plotted linearly in a graph. This supports the theory that microbial cell death is exponential and so obeys the first order process that could be mathematically represented (Adam and Moss, 1999). The reduced microbial load on exposure to heat on pasteurization is obviously the cause for prolonged shelf life of pasteurized kunun-zaki from 2 days to a period of 21 days (Inyang and Dabot, 1997).

Effect of pasteurization on vitamin C

There was marked reduction in vitamin C content of pasteurized kunun-zaki, with the loss increasing with increase in pasteurization exposure time. This decrease or loss in vitamin C is supported by previous reports that pasteurization of milk (normally at 72°C for 16 s could lead to more than 50% loss in vitamin C and even up to 80% loss of other water soluble vitamins (Lipinski, 2003). Despite the substantial loss of vitamin C during pasteurization the residual amount of the vitamin at the 25th min of exposure to still being 36 mg/100 ml could still serve as a vitamin C source to consumers considering the fact that most fresh fruits such as lime, banana pineapple and tomatoes have less than 36 mg/100 ml in their respective juices.

The use of pasteurization in preservation of vitamin C fortified kunun-zaki and other beverages should be strongly advocated for despite the economic loss of vitamin C. This is because the effects of gastrointestinal diseases on humans both in terms of health and economic view points would still outweigh the loss of the vitamin particularly in tropical Africa where most ideal foods are produced under unregulated conditions. Furthermore, pasteurization of kunun-zaki would not only eliminate pathogenic organisms but elongate the shelf-life of the beverage.

The results of the survival levels of microbial isolates during pasteurization of kunun zaki (Table 2 and Figure 2) showed that there was a gradual elimination of the microorganisms. At the 10th min of pasteurization only *Enterobacter cloacae* and *Corynebacterium* were eliminated. By the 15th min of exposure about half of the organisms had been destroyed while at the 25th min only *B. subtilis* was left and still persisted up to the 30th min of pasteurization. Generally *Bacillus* organisms are heat-resistant because of their spore forming ability. *B. substilis* is known to be a producer of certain antibiotics (e.g. bacitracin) and commercial amylase enzyme (Torora et al., 1992). Its presence in kunun-zaki could serve as a starch hydrolyzing and an acid-producing agent.

The D-value of *B. substilis* being 6.5 min (Figure 3) infer that for every 6.5 min of exposure to heat by pasteurization at 70°C, 90% of the microbial population would be reduced. This value compares with that of *Escherichia coli* (6.7 min) after treatment with 1.0% paracetic acid and hydrogen peroxide (Mazzola et al., 2003).

Conclusions

The challenge of food processing and preservation technology has always been that of being able to strike a

Table 2. Survival of microbial isolates during pasteurization of kunun-zaki at 70°C.

Pasteurization time (min)	Survival of microorganisms during pasteurization								
	A	B	C	D	E	F	G	H	I
0	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+
10	+	+	+	-	-	+	+	+	+
15	+	-	-	-	-	-	+	-	+
20	+	-	-	-	-	-	+	-	-
25	+	-	-	-	-	-	-	-	-
30	+	-	-	-	-	-	-	-	-

A = *Bacillus subtilis*; B = *Lactobacillus plantarum*; C = *Leuconostoc mesenteriodes*; D = *Enterobacter cloacae*; E = *Corynebacterium* spp.; F = *Streptococus* spp.; G = *Saccharomyces cerevisiae*; H= *Aspergillus niger*; and I = *Mucor* sp.

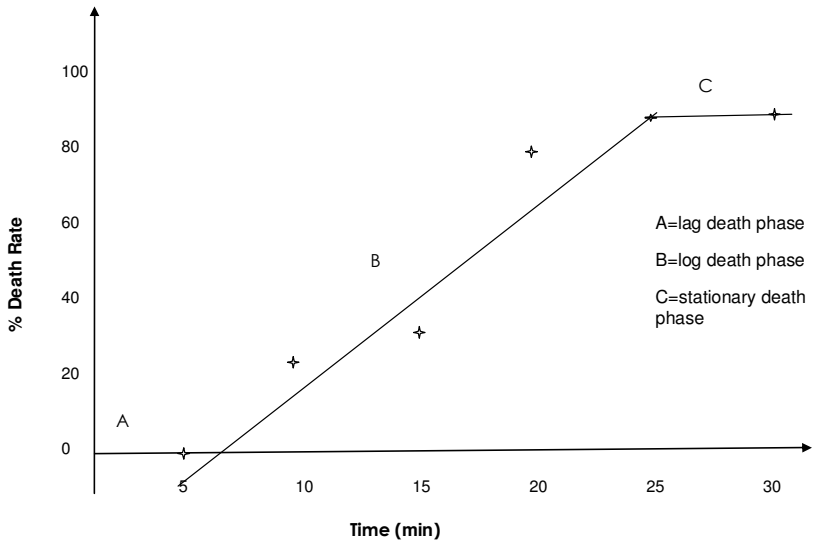

Figure 2. Death rate of microorganisms in kunun-zaki during pasteurization with respect to time.

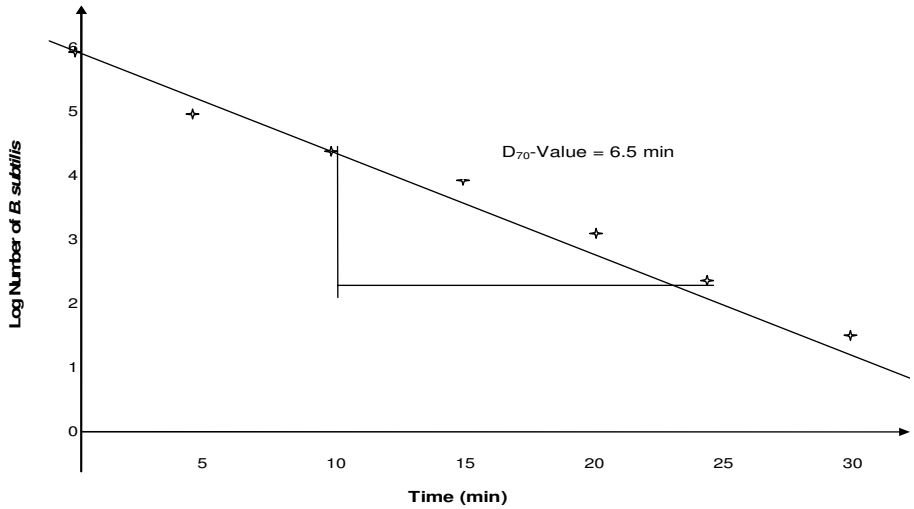

Figure 3. D_{70} Value of *Bacilus subtilis* in pasteurized kunun-zaki.

compromise between microbial load reduction cum shelf-life elongation and nutrient retention. In this study, it could be concluded that pasteurization at 70°C could effectively eliminate majority of the microbial agents associated with spoilage of kunun-zaki. One of such notorious spoilage agent being *S. cerevisiae* (Egbere et al., 2007) was effectively eliminated at the 25th min. It is then implied that pasteurization of vitamin C fortified kunun-zaki at 70°C for 25 min would yield a product that could be relatively shelf stable, free of pathogens and still possess an appreciable amount of residual vitamin C.

The D-value obtained for *B. subtilis* in this study is a useful tool for industrialists who may want to can the beverage. Total elimination of the *Bacillus* organism could be achieved by either canning (ultra high temperature sterilization) or by a combination pasteurization and use of chemical preservatives.

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