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Production of wara, a West African soft cheese using lemon juice as a coagulant

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Abstract

As an important protein source for West African consumers, wara cheese made from the leave extract of *Calotropis procera* has extremely short shelf life of only 2–3 days [Adegoke, G. O., Nse, E. N., & Akanni, A. O. (1992). Effects of heat, processing time, and pH on the microflora, aflatoxin content, and storability of wara, a soft white cheese. *Die Nahrung*, *36*(3), 259–264; Umoh, V. J., & Solomon, O. (2001). Safety assessment and critical control point of milk product and some cereal beverages in Northern Nigeria. In: *Proceedings of USDA/USAID/NIGERIA international conference on food safety and security*, August 1–3 (pp. 122–127). Ibadan, Nigeria: IITA; Belewu, M. A., Belewu, K. Y., & Nkwunonwo, C.C. (2005). Effect of biological and chemical preservatives on the shelflife of West African soft cheese. *African Journal of Biotechnology*, *4*, 1076–1079; Adetunji, A. O., Alonge, D. O., & Chen, J. (Unpublished). Microbial quality of wara, a southwestern Nigerian soft cheese]. Lemon juice was used in this study as a substitute coagulant during wara manufacture in order to improve the microbial quality of wara. The cheese was manufactured from pasteurized milk inoculated with 10^1 or 10^2 CFU ml^{-1} of *Listeria monocytogenes*. Samples of the milk or cheese were taken along the manufacturing steps and during a 5 d storage period at 15 and 28 °C in order to determine the populations of *L. monocytogenes*, total aerobes, *Enterobacteriaceae*, and psychrotrophs, as well as mold and yeast. On the 4th day of storage, portions of the un-inoculated control cheese from 28 °C were deep fried in vegetable oil, mimicking the practice of West African local cheese processors. The results showed that *L. monocytogenes*, at both inoculation levels, did not survive the manufacture of wara. In samples initially inoculated with 10^1 CFU ml^{-1} of *L. monocytogenes*, the *Enterobacteriaceae* counts decreased from the initial 1.78 to 1.00 Log_{10} CFU g^{-1} with the addition of lemon juice, and became undetectable (<1.00 Log_{10} CFU g^{-1}) at the curdling point as well as during the 5 d storage period at both temperatures. The total aerobic counts increased from the undetectable level on the 1st day of storage to 7.65 and 3.39 Log_{10} CFU g^{-1}, respectively at 28 or 15 °C on the 5th day of storage. The psychrotrophic, as well as the yeast and mold counts increased from the undetectable levels on the 1st day of storage to 7.11 and 5.03 Log_{10} CFU g^{-1}, respectively at 28 °C. At 15 °C however, the population of pyschrotrophs remained undetectable throughout the 5 d storage period whereas, the yeast and molds count increased to 3.08 Log_{10} CFU g^{-1} on day 3 before quickly decreasing to the undetectable levels on the 5th day of storage. A similar trend was observed in cheese made from the milk with an initial *Listeria* inoculation level of 10^2 CFU ml^{-1}. The results of this study showed that lemon juice significantly reduced the populations of the sampled microorganisms, especially the populations of *Enterobacteriaceae*.

Keywords: Wara cheese; Lemon juice; Coagulant; *Listeria monocytogenes*; Spoilage microorganisms

1. Introduction

Wara is an un-ripened cheese consumed in several parts of West Africa. The cheese is prepared by coagulating fresh cow milk with the leaf extract of the Sodom apple (*Calotropis procera*) or pawpaw (*Carica papaya*).

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The preferred coagulant comes from *C. procera* because the cheese made with this coagulant has a sweeter flavor versus the cheese made with the other coagulant. The ingredient in the leaves of *C. procera* that is useful in cheese production is calotropin, an enzyme that curdles milk proteins (Belewu & Aina, 2000).

Wara has an average shelf life of 2–3 d when stored in whey at ambient temperature (approximately 28 °C) (Adegoke, Nse, & Akanni 1992; Belewu, Belewu, & Nkwunonwo, 2005; Umoh & Solomon, 2001) or 4–5 d when placed in cool well water (approximately 15 °C). The cheese is usually deep fried in vegetable oil near the end of its shelf life in order to further preserve the cheese. Due to the lack of household refrigeration facilities in Nigeria and West Africa, attempts have been made in the recent past to include starter cultures or various chemical preservatives such as propionic acid, sodium benzoate, and sorbic acid in the production of wara (Anonymous, 1995; Aworh & Egounlety, 1985; Belewu et al., 2005; Joseph & Akinyosoye, 1997; Sanni & Onilude, 1999). Some of these preservatives have been shown to be effective in inhibiting mesophilic and psychrotrophic bacteria as well as coliforms. However, these preservatives are not easily accessible to the local cheese processors in West Africa. Therefore, the need for a useful and realistic alternative must be met.

Previous laboratory studies have confirmed the presence of *Listeria monocytogenes* in milk and milk products processed in Nigeria including ice cream, fermented milk, and local butter (Adetunji, Ikheloa, Adedeji, & Alonge, 2003). The pathogen was isolated from 20% of the 1-d-old wara cheese and cheese storage whey collected from the local cheese vendors in Southwestern Nigeria (Adetunji et al., 2003).

Outbreaks of listeriosis have been linked to the consumption of cheese in many parts of the world (Gellin & Broome, 1989; Goulet et al., 2001; Makino et al., 2005; Wehr, 1989). A Latin-style fresh cheese was found to be responsible for a large listeriosis outbreak in California in 1985 (Linnan et al., 1988), while another outbreak occurring in Japan in 2001, was attributed to the consumption of a wash-type cheese (Makino et al., 2005). Although the first confirmed case of human *L. monocytogenes* infection in Nigeria was reported in 1982 (Onyemelukwe, Lawande, Egler, & Mohammed, 1983), foodborne listeriosis has not been documented. It is known that *L. monocytogenes* is able to survive the manufacture and storage conditions of several cheeses (Anonymous, 2006; Buazzi, Johnson, & Marth, 1992; Carminati, Gatti, Bonvini, Neviani, & Mucchetti, 2004; Cetinkaya, & Soyutemiz, 2004; Erkmen, 2001; Manfreda, Acesare, Stella, Cozzi, & Cantoni, 2005; Yousef & Marth 1990). However, the fate of the pathogen during the manufacture and storage of wara cheese has not yet been investigated.

The leaf extract of *C. procera* has been shown to introduce the microorganisms naturally associated with them into wara cheese (Adetunji, Alonge, & Chen, Unpublished), an alternative coagulant has therefore, been sought in this study. Lemon fruit is readily accessible in West Africa, and its use as a sanitizer and bactericidal agent against bacterial pathogens has been reported (Sengun & Karapinar 2004, 2005). In this study, lemon juice was used as a coagulant to replace the leaf extract of *C. procera* in wara cheese manufacture in order to observe whether the lemon juice would improve the microbial quality and shelf life of wara cheese.

2. Materials and methods

2.1. Preparation of *L. monocytogenes* inoculum

Five strains of *L. monocytogenes* were grown and subcultured on modified oxford agar base (MOX) supplemented with appropriate antibiotic supplements (Becton, Dickinson and Company, Sparks, MD, USA). The inoculated plates were incubated at 37 °C for 24 h. A colony of each culture was transferred into test tubes containing 10 ml tryptic soy broth, respectively then incubated under the same conditions as described above. The populations of *Listeria* in the broth cultures were determined by plating appropriate serial dilutions of the cell cultures on MOX agar supplemented with appropriate antibiotics, followed by incubating the inoculated plates at 37 °C for 24 h. One milliliter of each broth culture was placed into a sterile test tube to make a 5-strain mixture of *L. monocytogenes*.

2.2. Preparation of milk for cheese manufacture

Pasteurized whole milk (32.5 g l^{-1} fat) was purchased from a grocery store in Griffin, Georgia, USA. The milk was maintained at 4 °C in a cooler and transported to the laboratory where it was stored at 4 °C until use. A volume of 2.20 l of the milk was placed into three cooking pots: A, B, and C. The milk in pots A and B were inoculated, respectively with the 5 strain mixture of *L. monocytogenes* at a level of 10^1 and 10^2 CFU ml^{-1}, respectively. The milk in pot C was not inoculated with *Listeria* and was used as a negative control in the study. Both the inoculated and uninoculated milk were then used for wara manufacturing.

2.3. Wara manufacture

The milk described above was heated to approximately 45–50 °C in about 30–40 min. The milk was stirred gently during the heating process using magnetic stir bars. Freshly squeezed lemon juice (49.5 ml) was added to the warm milk (2.2 l), and the milk and lemon juice mixture was heated with intermittent stirring until it reached 95 °C. The milk with added lemon juice was kept at this temperature until it coagulated and the separation of curd and whey became visible. The milk pots were then removed from the heating source, and the curds and whey were ladled or poured into sterile egg separators (8 mm in diameter), which facilitated