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Impact of brewing process operations on phytate, phenolic compounds and in vitro solubility of iron and zinc in opaque sorghum beer

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Abstract

Opaque sorghum beer is a significant component of the diet of millions of poor people in rural Africa. This study reports the effect of traditional brewing operations on its level of micronutrients, especially iron and zinc. The example of a West African sorghum beer, *tchoukoutou*, in Northern Benin was studied. The beer was characterized and the impact of process unit operations on phytate, phenolic compounds, and Zn and Fe in vitro solubility was evaluated. The major microorganisms involved in the beer fermentation were *Saccharomyces cerevisiae* and heterofermentative lactobacilli. The manufacturing process reduces the phytate content by nearly 95%, particularly during germination, mashing-boiling and fermentation. The level of reactive phenolic groups increased as a result of germination and fermentation as well as from a shift in dry matter composition. Simultaneously with these modifications, an increase of Fe solubility was observed, and a correlation between phytate and Fe solubility ($R^2 = 0.85$) was established. No clear correlation could be established between the Zn solubility and the phytate content of the products. During beer manufacturing, significant losses of minerals occur particularly during soaking and mashing/filtration; thus the quantity of minerals available to consumers is restricted. Improvements aiming to minimize such losses are highly desirable.

Keywords: Sorghum; Brewing; Tchoukoutou; Solubility; Iron; Zinc

1. Introduction

Iron (Fe) and zinc (Zn) deficiencies are a major public health problem in developing countries. Chronic micronutrient deficiencies, particularly of Fe and Zn, cause child mortality, impaired mental and physical development, decreased work output, and contribute to morbidity from infections (Dossa, Ategbo, de Koning, van Raaij, & Hautvast, 2001; Gibson, 1994; Oikeh, Menkir, Maziya-Dixon, Welch, & Glahn, 2003). Therefore, an increase of bioavailable Fe and Zn in the indigenous diets is highly desirable.

Opaque sorghum beers are popular alcoholic beverages in Africa. In the West Africa region they are known as *tchoukoutou* in Benin, *dolo* in Burkina-Faso, *pito* in Ghana, and *burukutu* or *otika* in Nigeria (Kayodé, Adégbidi, Linnemann, Nout, & Hounhouigan, 2005; Odunfa, 1985). The beers have a sour taste, a relatively high dry matter content (5–13 g 100 ml^{-1}) and low alcohol content (2–3 ml 100 ml^{-1}), which make them suitable beverages for adults and teenagers (Agu & Palmer, 1998; Briggs, Boulton, Brookes, & Stevens, 2004). They are largely consumed by the poorest people and significantly contribute to the diet of millions of consumers. The beers are mostly prepared with Guinea corn (*Sorghum bicolor*) but other cereals such as millet or maize can be used as adjunct or as substitutes (Kayodé et al., 2005). The manufacturing process consists of malting (soaking, germination, sun drying), brewing (mashing, boiling, filtration) and fermentation. Depending on the geographic location, variations may occur in the process (Haggblade & Holzapfel, 1989; Odunfa, 1985).

The nutritional attributes of eight commercial sorghum beers have been reported by Novellie and De Schaepdrijver

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(1986) as follows: protein 5.4 g l^{-1}, ash 1.13 g l^{-1}, carbohydrate 47.6 g l^{-1}, iron (Fe) 1.4 g l^{-1} and zinc (Zn) 1.4 g l^{-1}. This suggests that such beer can significantly contribute to macronutrient supply to the diet, considering the rather large quantity that is consumed daily in certain locations (Briggs et al., 2004; Kayodé et al., 2005). At present, little information is available on the micronutrient availability in African opaque beers. The unit process operations (e.g. soaking, germination and fermentation) involved in the preparation of opaque sorghum beers have been reported to reduce the levels of phytate, a major inhibitor of Fe and Zn availability (Graf, 1986; Mahgoub & Elhag, 1998; Traoré, Mouquet, Icard-Vernière, Traoré, & Trèche, 2005). In addition, several studies demonstrated that germination and fermentation affect condensed phenolic compounds (PC) (Bvochora, Danner, Miyafuji, Braun, & Zvauya, 2005; Obizoba & Atii, 1991; Subramanian, Murty, Sambasiva, & Jambunathan, 1992) which also reduce mineral bioavailability by chelation. Thus, considering the prominent role of germination and fermentation in the process, increased solubility of Fe and Zn could be expected in indigenous African beers.

The present study was conducted to characterize *tchoukoutou*, the major opaque sorghum beer of Benin and to assess the impact of the different process unit operations of *tchoukoutou* making on phytate, PC and Fe and Zn in vitro solubility, as an index for their bioavailability.

2. Materials and methods

2.1. Processing and sampling

The processing trials took place in *Parakou*, a small town in the northern region of Benin, where *tchoukoutou* is commonly produced and consumed. Three batches of red sorghum grain, selected and purchased by processors from a local market, were processed into *tchoukoutou* at three commercial production sites according to the traditional brewing practices, as follows. On average, 27 kg of grain were soaked overnight at room temperature (Processor 1: 9 h, Processor 2: 11 h, Processor 3: 12 h), germinated (P1: 85 h, P2: 74 h, P3: 72 h), sun-dried at ambient temperature of about 30–37 °C (P1: 15 h, P2: 15 h, P3: 7 h), ground in a disc mill, mashed in water by gradually heating until the boiling point was reached after 2 h, soured during an overnight rest, filtered, boiled (P1: 9 h, P2: 8 h, P3: 6 h), cooled, inoculated and fermented overnight (P1: 13 h, P2: 14 h, P3: 13 h). These ranges of values for processing parameters, may lead to fluctuation in data. We highlighted this by expressing values as means with standard deviation. At the end of the fermentation, beer samples were collected in screw-capped bottles, packed in an insulated icebox, transported to the laboratory and analysed immediately for yeasts and lactic acid bacteria (LAB) content (Hounhouigan, Nout, Nago, Houben, & Rombouts, 1993). The remaining beer samples were freeze-dried and stored at −20 °C for further analyses. In addition, samples were withdrawn from each process step, dried in an oven, ground in a *Retsch* mill (type ZM 1, Retsch, Haan, Germany) fitted with a 0.5 mm screen and stored at −20 °C until analysis.

2.2. Microbiological analysis

Duplicate samples of *tchoukoutou* (10 ml) were diluted in 90 ml sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1000 ml distilled water, pH = 7.0) and homogenized with a Stomacher lab-blender (type 400, London, UK). Decimal dilutions were plated. Total counts of aerobic mesophilic bacteria (TC), LAB, yeasts and Enterobacteriaceae were enumerated as described by Hounhouigan et al. (1993).

The characterization of LAB was performed as follows. Representative colonies of LAB were randomly picked from higher dilutions and purified by successive sub-culturing on de Man Rogosa and Sharpe Agar (MRSA, CM 361, Oxoid, Hampshire, England) containing 1 g l^{-1} natamycin (Delvocid, DSM, The Netherlands) with incubation in anaerobic jar (Anaerocult A, Merck KGaA, Germany). The isolates were tested for cell morphology, Gram stain, catalase and oxydase reactions. Gas production from glucose was tested after incubation at 30 °C for 48 h in MRS broth (CM 359, Oxoid, Hampshire, England). Growth tests were performed at 15 °C for 5 d and 45 °C for 1 d in MRS broth. Carbohydrate metabolism of isolates was tested on API 50 CH strips with API 50 CHL medium (BioMérieux, Lyon, France) following the manufacturer's instructions. The identification of LAB was done using the IBIS software (Intelligent Bacteria Identification System, The Netherlands) (Wijtzes, Bruggeman, Nout, & Zwietering, 1997).

For yeasts identification, isolates representing the three production sites were purified by successive sub-culturing on malt extract agar (MEA, CM 59, Oxoid). Preliminary confirmation was based on microscopic observation. The isolates were tested for the fermentation of sucrose, lactose, glucose and raffinose, as well as the assimilation of selected nitrogen sources, i.e. nitrate, ethylamine, L-lysine, cadaverine, and creatine. The assimilation of carbon sources was performed using API 20 C AUX strips (BioMérieux, Lyon, France) according to the manufacturer's instructions. The Diazonium Blue B reaction, a test to differentiate between ascomycetous and basidiomycetous yeasts, was performed as described by Kurtzman, Boekhout, Robert, Fell, and Deak (2003). The on-line available software (http://www.cbs.knaw.nl) of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands was used for yeasts identification.

2.3. In vitro digestion of samples

Step 1—the enzymatic degradation—of the in vitro digestion method described by Kiers, Nout, and Rombouts