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Staphylococcal ecosystem of kitoza, a traditional malagasy meat product

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ABSTRACT

Kitoza is a traditional meat product from Madagascar manufactured with strips of pork or beef. The process includes a first step of salting and mixing with spices followed by sun-drying or smoking step. As salting and drying select coagulase-negative staphylococci (CNS), our aim was to identify the CNS species in kitoza with the objective in the future of developing indigenous starters. Microbial analyses revealed that the only pathogenic bacterium enumerated was *Staphylococcus aureus*, which was found in 54% of the samples. The level of *Enterobacteriaceae* revealed a rather good hygienic quality of these products. CNS were confirmed in all the samples at high levels ranging from 5 to 7 log cfu/g. Identification of CNS species in a large collection of 829 isolates revealed 9 identified species, 7 for beef and 8 for pork kitoza. There were significant difference in the distribution of CNS species according to the type of meat and the process. *Staphylococcus saprophyticus* was the dominant species for sun-dried or smoked beef and sun-dried pork kitoza (73–75%), while for smoked pork kitoza *Staphylococcus equorum* (26%), *S. saprophyticus* (23%), *Staphylococcus succinus* (23%) and *Staphylococcus epidermidis* (17%) co-dominated. Some CNS could be used as indigenous starters in particular to compete against *S. aureus*.

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1. Introduction

Salting, smoking and drying are among the oldest methods of meat preservation. In tropical countries, where the climate and environmental conditions promote the rapid degradation of meat and where there is sometimes a lack of adequate facilities for the storage of raw meat, traditional processing techniques are often based on the use of these single or combined operations. They lead to a wide variety of products such as biltong in South Africa (Petit et al., 2014), kilishi in Nigeria (Kalilou et al., 1998), boucané in Réunion Island (Poligne et al., 2001), charqui in Brazil (Pinto et al., 2002) and kitoza in Madagascar (Santchurn et al., 2012).

Kitoza is a traditional meat product from Madagascar manufactured with strips of either pork or beef. It is produced in rural and urban regions by artisans and families following traditional processes and sold in local markets. In a first step of the process, miscellaneous pieces of pork and beef meats are cut into strips approximately 2–4 cm thick and 20–50 cm long and are salted with coarse salt mixed with spices such as garlic, pepper, and ginger. Then, a sun-drying and/or smoking step is carried out.

Depending on the products, a wide range of water activity (a_w) and salt content have been recorded. They varied from low a_w (0.65–0.68) and high NaCl concentration (5.5–7.9 g/100 g) in dry commercial biltong to higher a_w (0.85–0.89) and lower salt content (3.8–5.6 g/100 g) in moist commercial biltong (Petit et al., 2014). The a_w of beef and pork jerky varied from 0.83 to 0.79 and the a_w of traditional làcon was 0.90 (Lorenzo et al., 2015; Yang et al., 2009). Also, the pH of these products varied from 5.0 to 6.2 (Lorenzo et al., 2015; Petit et al., 2014; Yang et al., 2009). The total microbial viable count is usually high in dried salted meat products, for instance 6 to 9 log CFU/g in biltong or in làcon, and the salt-tolerant microbiota was the dominant population.

Coagulase-negative staphylococci (CNS) are halotolerant and thus one of the dominant microbiota of salted/dried/fermented meat products (Leroy et al., 2015; Lorenzo et al., 2015; Pinto et al., 2002). In fermented sausages, CNS enhance color stability, prevent rancidity by inhibiting oxidation of unsaturated free fatty acids and release aromatic substances (Coppola et al., 1997; Papamanoli et al., 2002; Talon and Leroy, 2006). In jerked beef, a derivative of charqui meat, staphylococci represent the dominant microbiota and the inoculation of *Staphylococcus xylosus* as starter culture leads to products preferred by the sensory panel (Pinto et al., 2002). Meat-associated CNS are able to produce bacteriocins that may contribute to bioprotection against meat pathogens, in particular *Clostridium botulinum* and *Staphylococcus aureus* (Sanchez Mainar et al., 2016). For instance, species usable as starter cultures

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such as *S. xylosus* and *Staphylococcus equorum* have been shown able to produce bacteriocin (Laukovà et al., 2010; Sanchez Mainar et al., 2016). Thus, CNS could contribute both to the sensory qualities and the bioprotection of meat products (Talon and Leroy, 2006).

No data were available about identification of CNS from kitoza and the aim of this study was to identify these bacteria at the species level in kitoza made from beef or pork and either sun-dried or smoked and collected from producers at different sites. These genetic resources can be used to develop indigenous starters that could be applied to product biopreservation. At the same time, a global view of the microbiology of kitoza is given as well as some of the physicochemical properties important for microbial quality.

2. Materials and methods

2.1. Samples

A total of 54 kitoza samples ready for consumption were collected in butcheries, markets or supermarkets in or near Antananarivo (Madagascar). They included 27 samples of beef kitoza (13 smoked, 14 dried) and 27 of pork kitoza (14 smoked, 13 dried).

The kitoza products were manufactured according the two steps described in introduction with a lot of variation in the process. Samples were kept at 4 °C before microbial analyses and stored at −20°Cfor physicochemical analyses.

2.2. Physicochemical analyses

The salt content was measured with the Model 926 Chloride Analyzer (Sherwood Scientific, Cambridge, UK) after 2 h of cold extraction in 0.3 N nitric acid. Water activity was measured at 25 °C with a Fast-lab water activity meter (GBX, Romans, France). The pH was measured with a TitroLine® easy titrator (SI Analytics GmbH, Mainz, Germany) after homogenization of 3 g samples with 27 mL of distilled water for 30 min. These analyses were carried out on the 54 samples in duplicates.

2.3. Microbial analyses

Microbial analyses of the 54 samples were performed on selective media in duplicate. The total counts, yeasts and molds, staphylococci, lactic acid bacteria (LAB), *Enterobacteriaceae,* coagulase positive staphylococci and *S. aureus, Listeria monocytogenes* and *Salmonella* were analyzed as described by Lebert et al. (2007). In particular, the presence of *S. aureus* isolated from Baird Parker agar supplemented with tellurite yolk egg was confirmed by the Pastorex Staph-Plus test, a latex agglutination test for the identification of *S. aureus* (Bio-Rad, Marnes La Coquette, France). For LAB, the MRS media was incubated under anaerobic conditions. In addition, *Bacillus cereus* was numerated on *Bacillus cereus* Selective Agar (Oxoid, USA), incubated at 30 °C for 18–48 h; *Clostridium perfringens* on Tryptose Sulfite Agar (Biokar, France), incubated at 37 °C for 20 h and confirmed with Lactose Sulfite medium (Biokar, France) at 46 °C.

A collection of 829 isolates of staphylococci was constituted by selecting for each sample an average of 15 colonies on countable plates of Mannitol Salt Agar (MSA, Bio-Rad, France, Leroy et al., 2010). These isolates were cultivated on Brain Heart Infusion (BHI, Difco, USA) at 30 °C overnight. They were then stored at −80 °C in BHI broth containing glycerol 20% before identification.

2.4. Identification of the CNS isolates

Firstly, all the isolates were grown on BHI agar (24 h, 30 °C). Amplifications were performed from one colony picked from the agar plate of each isolate with the primers TstaG422 (5′-GGC CGT GTT GAA CGT GGT CAA ATC A-3′) and Tstag765 (5′-TTA CCA TTT CAG TAC CTT CTG GTA A-3′) from the *tuf* gene allowing the identification the *Staphylococcus* genus, as described by Martineau et al. (2001).

Secondly, the isolates belonging to the *Staphylococcus* genus were further identified at the species level by a species-specific oligonucleotide array as described by Giammarinaro et al. (2005). The primers D1 (CCITAYICITAYGAYGCIYTIGARCC) and D2 (ARRTARTAIGCRTGYTCCCAIACRTC) were used to amplify the *sodA* gene by PCR. This PCR product was labeled with digoxigenin, heat denatured and hybridized with the species-specific oligonucleotide probes fixed to a nylon membrane. The hybridized targets were detected with the Dig color detection kit (Roche, Meylan, France).

2.5. Statistical analysis

The statistical analyses were performed with the XLStat program, version 2016 (Addinsoft, Paris, France). Physicochemical parameters and microbial counts of the four types of kitoza were compared using analysis of variance. Tukey's HSD least significant difference was used with a level of significance of 95%. To test for independence between combinations of *Staphylococcus* species and type of kitoza, the Chi-square (χ^2) test was used.

3. Results and discussion

3.1. Physicochemical characteristics

Kitoza belongs to a wide variety of low and intermediate moisture meat products obtained after salting, drying, and sometimes smoking. In our study, the average salt content and the average water activity of the kitoza products varied from 2.64 to 4.15 g/100 g and from 0.83 to 0.96, respectively (Table 1). There were significant differences in water activity ($P < 0.05$) between smoked and dried products, the smoked ones being less dry than the dried ones. Significant differences in salt content were only noticed between pork kitoza samples, the dried ones being more salty than the smoked ones. The water activity of smoked kitoza was of the same order as that of traditional làcon (Lorenzo et al., 2015) while that of dried kitoza was of the same order as that of biltong, a salted dried product (salt content from 3.8 to 7.9 g/100 g) made from beef, kudu, springbok or chicken (0.65–0.89) (Petit et al., 2014). Similarly, the water activity of traditional fermented sausages from Greece, Spain, France or Argentina varies from 0.79 to 0.91 (GarcíaFontán et al., 2007a; García Fontán et al., 2007b; Leroy et al., 2015).

The pH of the beef kitoza samples, either dried (5.67) or smoked (5.87) (Table 1), was in the same range as the pH of fresh beef (5.5 to 5.9; Laurent, 1981). The pH of the smoked pork kitoza (6.08, Table 1) was in the range of the pH of the fresh raw pork meat (5.7 to 6.2; Laurent, 1981), while the pH of the dried pork kitoza (6.45, Table 1) was higher. Thus, in pork samples, the process affected the pH as dried pork products had a significantly higher pH ($P < 0.05$) than the smoked ones (Table 1). Similar pH ranges were found in biltong samples (5.00 to 6.26) (Petit et al., 2014), beef and pork jerky (5.53–6.07) (Yang et al., 2009) and traditional dry cured làcon (5.00 to 6.00) (Lorenzo et al., 2015).

Table 1
Physicochemical parameters of kitoza.

Sample	Water activity Mean ± SD	Salt content (g/100 g) Mean ± SD	pH Mean ± SD
Smoked beef ($n = 13$)	0.94 ± 0.02^{a}	$2.83 \pm 0{,}81^{a,b}$	$5.87 \pm 0.17^{b,c}$
Dried beef ($n = 14$)	0.86 ± 0.06^{b}	$3.61 \pm 1.21^{a,b}$	5.67 ± 0.18^{c}
Smoked pork ($n = 14$)	0.96 ± 0.02^{a}	2.64 ± 1.13^{b}	6.08 ± 0.55^{b}
Dried pork ($n = 13$)	0.83 ± 0.07^{b}	4.15 ± 1.85^{a}	6.45 ± 0.31^{a}

n: number of samples; SD: standard deviation; ^{a,b,c}: mean values in the same column not followed by a common letter differ significantly ($P < 0.05$).

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