

## Short Communication

## Assessment of the microbiological quality of *koozh*, a fermented millet beverage

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***Koozh* is a fermented beverage made with millet flour and rice, and consumed by ethnic communities in Tamil Nadu, India. Six street vended samples were assessed for the total bacterial count (TBC), lactic acid bacteria (LAB) count, yeast-mould count (YMC), coliforms at 35°C and pathogens. The *koozh* pH ranged from 4.3 to 4.9 with high titratable acidity. Although no *Staphylococcus* sp. and *Listeria* sp. were found, high colony counts of *Clostridia* sp., *Salmonella* sp. and *Shigella* sp. were present in some samples. The LAB was dominant as compared to TBC, YMC and coliforms. Pathogens were detected, indicating contamination following processing in the traditional fermented food.**

**Key word:** Fermented millet, street food, lactic acid bacteria, pathogens, microbiological quality, contamination.

### INTRODUCTION

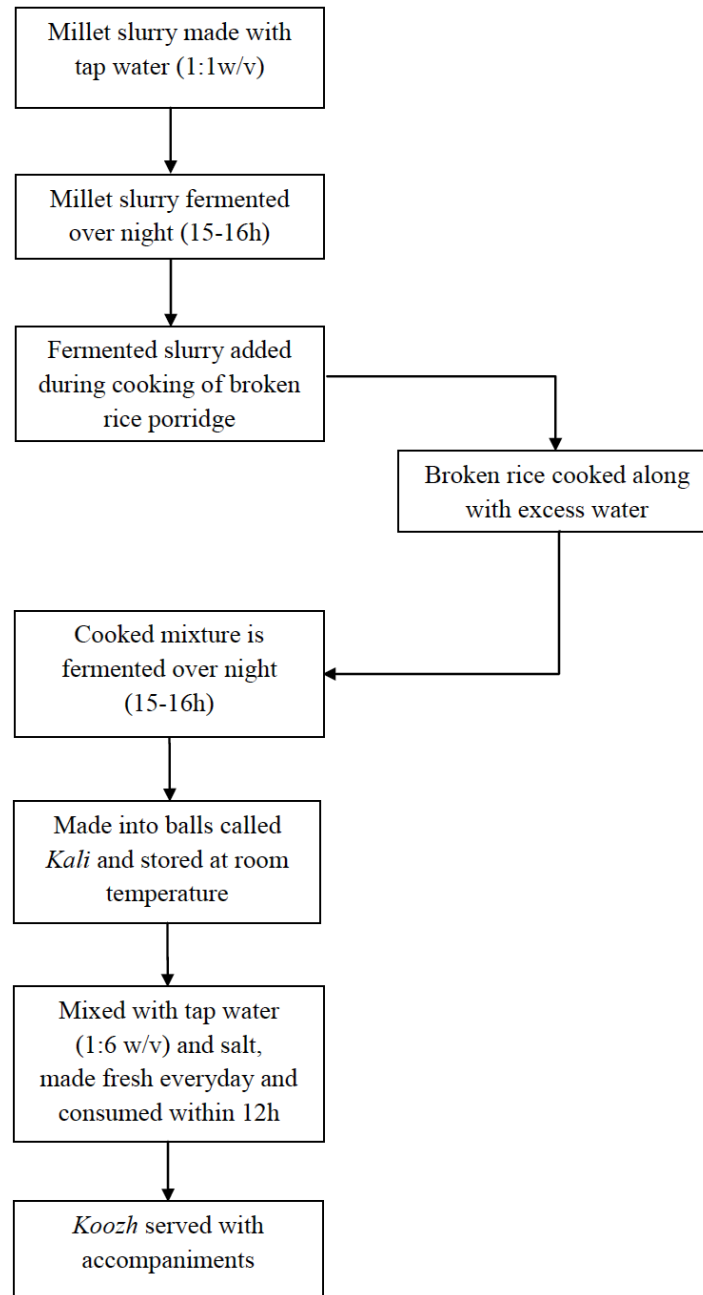
Millet is an important minor cereal in tropical and subtropical regions and India is the largest producer. Many traditional fermented products are made from millets both in African and Asian countries. *Koozh* (Tamil term for porridge) is a ready-to-eat (RTE) food/energy beverage either made from finger millet- *Eleusine coracana* (*Kezhvaragu*) or pearl millet- *Pennisetum glaucum* (*Kampu*), broken rice flour (*noyee*) (Kumar et al., 2010).

*Koozh* is prepared in an outdoor traditional kitchen common in rural India, using traditional methods that have been followed for generations. A flow chart of the typical process is given in Figure 1. The millet flour is made into slurry with water by hand-mixing on the first day and left to ferment overnight (12-15 h); on the second day, broken rice (20% by weight of millet) is cooked in excess water, into which the fermented (12-15 h) millet slurry is mixed, stirred and cooked to make a thick porridge called *noyee*.

The fermentation of this porridge overnight (24 h) results in *kali*, a semi solid porridge to which the required

amount of potable water is added (1:6 w/v) and hand-mixed with salt to prepare *koozh*. The product may be further mixed with buttermilk (diluted curd a local preparation of yoghurt) to give a thin porridge consistency. The end product has a characteristic fermented flavour as a result of the microbial succession, which develops complex flavours. While *kali* has a shelf life of approximately one week at room temperature (25-30°C), *koozh* has low shelf life and is usually consumed within about 12 h of preparation. It is prepared in homes and offered in temples during special festivals. It is also sold in the streets or by mobile food vendors and consumed by daily-wage earners of Tamil Nadu, India.

*Koozh* is unique in that it is fermented twice- once before cooking and again after cooking, its preparation last for two days. Traditionally, *koozh* is considered nutritious and health promoting, but there is little scientific documentation on its nutritive or microbial composition, other than some studies on the nutritional advantages of fermented millets - finger millet (Antony and Chandra, 1998) and pearl millet (Khetarpaul and Chauhan, 1994).



**Figure 1.** Indigenous preparation of *koozh* from finger millet- *Eleusine coracana* (Kezhvaragu) or pearl millet - *Pennisetum glaucum* (Kampu).

Geetha and Kalaichelvan (2013) had reported the microbial succession and biochemical changes in *koozh* made from finger millet-ragi, pearl millet-cumbu, sorghum and maize. The second fermentation after cooking of the millet in *koozh* preparation makes it an excellent source of live bacteria, while its storage and service may allow contamination. Hence, this study was undertaken to assess the microbiological safety of *koozh* sold as street food.

## MATERIALS AND METHODS

### Sample collection and its microbial load

Finger and pearl millet *koozh* were collected in 250-mL autoclaved, wide-mouthed, screw-capped plastic containers from market places in Salem district (Sa) and Chennai district (Ch), Tamil Nadu, India and immediately transported to the laboratory in insulated food containers with ice packs and analyzed. The finger millet *koozh* samples were collected from street vendors in different Chennai suburbs and tested on the same day according to Cappuccino and

**Table 1.** pH and total load of bacteria, lactic acid bacteria and yeast present in *koozh*.

Type of <i>koozh</i>	Samples and collection location	pH	Log cfu g <sup>-1</sup>			
			TBC	LAB		Yeast
				MRS	M17	
			Mean ± SD <sup>a</sup>	Mean ± SD <sup>a</sup>	Mean ± SD <sup>a</sup>	Mean ± SD <sup>a</sup>
<i>Kampu</i> (pearl millet) <i>koozh</i>	S <sub>1</sub> -Sa	4.91±0.15	8.56±0.38	10.81±0.60	8.32±0.39	8.85±0.89
<i>Kezhvaragu</i> (finger millet) <i>koozh</i>	S <sub>2</sub> -Sa	4.48±0.02	5.95±0.62	8.96±0.72	7.88±0.42	8.47±0.91
	S <sub>3</sub> -Ch	4.33±0.47	8.10±0.37	8.88±0.13	8.03±0.98	8.42±0.38
	S <sub>4</sub> -Ch	4.56±0.55	7.99±0.99	7.96±0.18	7.84±0.89	5.57±0.65
	S <sub>5</sub> -Ch	4.51±0.41	5.72±0.39	8.68±0.47	7.89±0.91	6.22±0.35
	S <sub>6</sub> -Ch	4.68±0.12	7.12±0.49	8.85±0.10	7.91±0.49	7.99±0.13

a: Standard deviation from the mean of three independent estimation; Sa: Salem, Tamil Nadu, India; Ch: Chennai, Tamil Nadu, India.

Sherman (1996) Harrigan and McCance (1998). The total bacterial count (TBC), lactic acid bacteria count (LAB), yeast-mould count (YMC), coliforms at 35°C and pathogens was carried out for all samples. The microbial quality of the *koozh* was assessed based on the norms specified for RTE foods by the Health Protection Agency (HPA 2009).

#### Determination of pH

The sample pH was determined using AP-1 plus pH meter (Susima Technologies, Chennai). The titratable acidity was estimated in *koozh* filtrate by titration with 0.1 N sodium hydroxide to the end point with phenolphthalein indicator (AOAC, 2000).

#### Statistical analysis

The microbial count values were tested using paired student's *t* test and the correlation between variables was also determined using GraphPad 6 (SanDiego, Ca, USA) software. The calculated *r* values are interpreted.

## RESULTS AND DISCUSSION

### pH

The pH of *koozh* ranged from 4.3 to 4.9 (Table 1) with acidity ranging from 0.16 to 0.35%, in all samples. This may pose a problem because some samples with pH over 4.5 may allow spoilage or growth of pathogenic microbes. This observation is similar to other millet-fermented products reported in literature: an alcoholic Himalayan beverage from finger millet called *Kodo ko jaanr*, with pH ranging from 3.7 to 4.5 (Thapa and Tamang, 2004); the Northern Ghana's *Koko* sour water from pearl millet with a pH of 3.9 ± 0.1 (Lei and Jakobsen, 2004); a fermented mixture of millet and sorghum flour, called *bushera* of Uganda with 3.7 ± 0.1 pH, and *bushera* made only with sorghum with a pH range of 4.0-4.5 (Muyanja et al., 2003).

### Bacteria, LAB and yeast-mould counts

In all *koozh* samples, LAB were found to be dominant

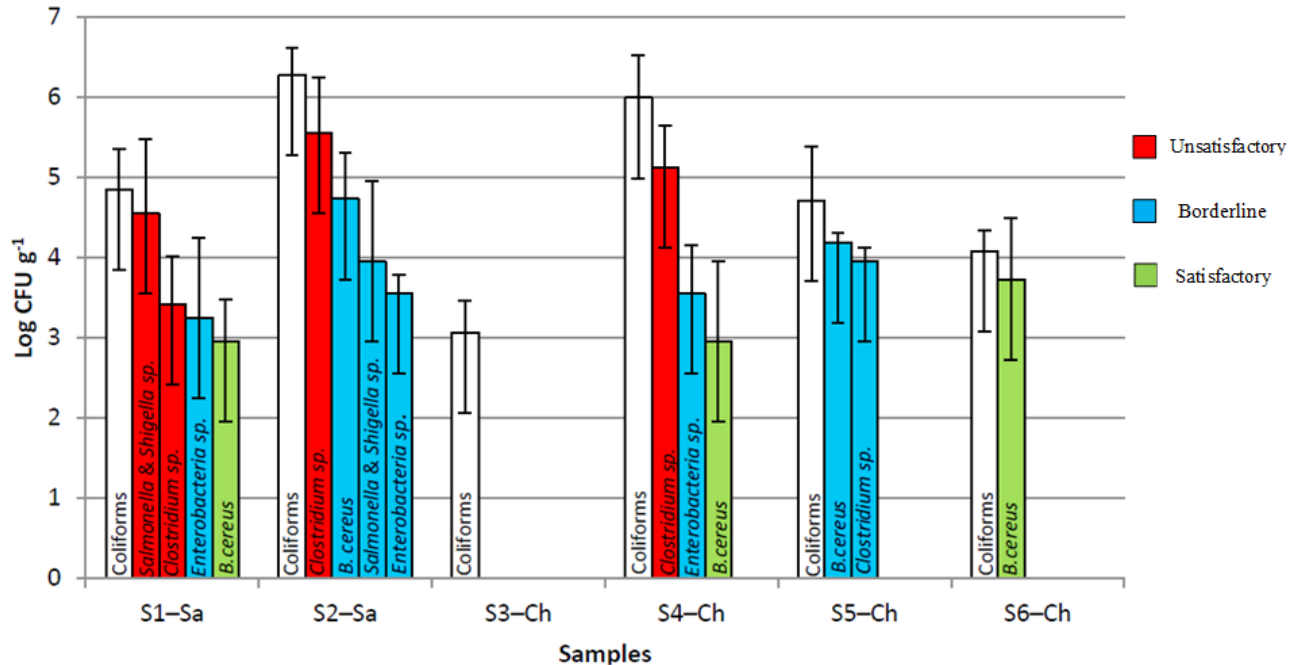
and yeast-mould counts were comparatively lower. LAB counts on MRS showed significant differences ( $p \leq 0.05$ ) with TBC and counts on M17 and yeast counts. The LAB counts on MRS showed a very strong correlation with counts on M17 ( $r = 0.9396$ ) as both are selective media used for LAB enumeration. Yeast counts were strongly correlated with LAB count on MRS ( $r = 0.7205$ ) and moderately correlated with counts on M17 ( $r = 0.6261$ ). Co-metabolism between yeast and LAB may exist, where the bacteria provide the acid environment, which selects the growth of yeast, that in turn; provide vitamins and other growth factors to the bacteria (Steinkraus, 1996).

The pH of samples correlated strongly with LAB counts on MRS ( $r = 0.7250$ ) apparently due to the acid production by LAB, and correlated moderately with counts on M17 ( $r = 0.5950$ ), while with yeast the correlation was low ( $r = 0.2327$ ). Moulds were absent in all samples tested. Thus, it can be concluded that LAB and yeasts mediate *koozh* fermentation.

### Bacterial pathogen (hazard) classification

The isolated pathogens were *Bacillus cereus*, *Clostridium* sp., *Enterobacteria* sp., *Salmonella* sp. and *Shigella* sp. Coliforms at 35°C were found in all samples (Figure 2) and the count was positively correlated with TBC ( $r = 0.7216$ ) and are probably from contamination of the water added and the preparation involving hand-mixing. Their presence is associated with poor hygiene, and therefore, indicates a potential health risk. The presence of coliforms has been reported in other traditional fermented products due to its survival in acidic environment (Steinkraus, 1996).

Coliforms had moderate correlation with pH ( $r = 0.5521$ ) and a negative correlation with yeast ( $r = -0.4992$ ) indicating inhibitory action of the microbes on coliforms. Simango (1995) compared the contamination in a fermented cereal gruel (*Mahewu*) with that of a non fermented thick maize meal pap (*Sadza*) in Zimbabwe. After storing for 4 h, both foods were found to have 50%



**Figure 2.** Pathogens present in *koozh* samples classified according to HPA (2009). Sa: Salem, Tamil Nadu, India; Ch: Chennai, Tamil Nadu, India.

*Escherichia coli* contamination but after 24 h no *E. coli* was found in the *Mahewu*. Simango and Rukure (1991) stated that this is due to further fermentation that leads to the inhibition of these contaminants during longer storage. Traditionally fermented laboratory-prepared sorghum bread had coliform in the first fermentation, but the microbes were not detected in the second fermentation (Gassem, 1999). In *bushera*, coliforms population decreased due to high acidity resulting from the metabolism of LAB (Muyanja et al., 2003).

The hazard classification based on pathogen count according to HPA (2009) for RTE is represented in Figure 2. The presence of *Clostridium sp.*, *Salmonella sp.* and *Shigella sp.* in high numbers made three samples unsatisfactory. In one of these *koozh* samples pH was exceeded 4.5.

Other pathogens like *Listeria sp.* and *Staphylococcus sp.* were not detected in any of the samples. Sample S<sub>3</sub>-Ch alone met the microbial standards, indicating safe and hygienic handling of the product. It was also the product with the lowest pH. Two samples (S<sub>5</sub>-Ch and S<sub>6</sub>-Ch) with *B. cereus* and *Clostridium sp.* at low levels were acceptable.

Studies with other fermented foods show that pathogens are inhibited during the fermentation. Pathogens such as enterotoxigenic *E. coli*, *Shigella flexneri*, *Salmonella typhimurium*, *B. cereus*, and *Campylobacter jejuni*, when inoculated were adversely affected during the fermentation of sorghum (Kingamkono et al., 1994). The fermentation of finger millet provides antimicrobial activity against *S. typhimurium* and *E. coli* even after 48

h of fermentation (Antony and Chandra, 1998). The inhibitory effect in fermentation is not only because of lactic acid but other factors like other organic acids, fermentation process, pH and temperature.

Presence of *Clostridium* in heat-treated foods would be due to inadequate cooking or post-processing contamination (HPA, 2009). *B. cereus* survives on processing equipments by germinating prior to sanitization. Handling, storage, or processing can be sources of contamination. Bacteria attaches to surfaces of food equipments, made in polystyrene, hydroxyapatite, glass, rubber and stainless steel might transmit pathogens to food (Mafu et al., 2011). The major contamination is through unclean water used for dilution and hand-mixing. Direct contaminations from unhygienic environment are predominant in market places and in tropical regions were these locations attract house-flies. *Koozh* and accompaniments were found exposed to dust and pathogens; serving utensils were not cleaned properly due to lack of safe running water. All these factors resulted in post process microbial contamination.

## Conclusion

The microbiological quality of *koozh* traditionally considered healthy for its nutritional content, and sold as a street food varies widely. Lactic acid bacteria and yeasts were present in significant numbers. Hazardous levels of pathogens in 3 of 6 samples indicate unhygienic handling, despite the large numbers of LAB. Safety awareness

programmes targeting producers are imperative to eliminate such contamination and ensure safe food.

## ACKNOWLEDGEMENTS

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