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Antimicrobial Activities of *Kilbu* and Tamarind Pulp Extracts Used in Traditional Medicine and Cereal Gruel in Cameroon

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Abstract: A challenge study was used to evaluate the antimicrobial effects of *Kilbu* and Tamarind pulp Extracts (TE) on *E. coli* in fermented maize gruel. A 5×3×2×2 factorial experiment with keeping time, addition level, seasoner source and Groundnut Butter (GB) level as the variables was performed. Fermented flour samples enriched with GB (0, 20%w/w) were processed into gruel treatments, seasoned with *Kilbu* (0, 1, 2%w/v) or TE (0, 10, 20%v/v). After cooling to 35±2°C, each treatment was inoculated with *E. coli* (~10^6 cfu g^{-1}). Microbial loads (*E. coli* and aerobic mesophilic counts) and physico-chemical determinants (texture, pH, dry matter and sugar contents) were analysed during storage (0, 2.5, 5, 7.5 and 10 h). Results showed that pH and microbial loads were affected by the variables. After enrichment by GB, pH varied from 5.1 to 6.2. Addition of *Kilbu* increased the pH (> 3.5 units). An inverse trend was observed with TE. Both seasoners have shown antimicrobial properties, though their efficacy was reduced with increasing time. Based on the results, these tropical resources could be used to control microbiological risks associated with weaning food under tropical conditions.

Key words: Traditional additives, antimicrobial activities, *E. coli,* infant gruel

INTRODUCTION

In developing countries, the beginning of the weaning process in human infants has been associated with an increase in diarrhoeal episodes as a result of consumption of contaminated complementary foods. Diarrhoeal diseases are the major food-borne infections[1] and high incidences of these diseases have been reported in children between the ages of 6 to 18 months[2,3]. It has been estimated that each year more than 1.5 billion episode of diarrhoea occurs in children less than five year of age in developing countries, resulting in over three million deaths[4]. More than half of these infections may be transmitted through food[5].

Simple processing and handling techniques can be used to eliminate, reduce or control microbial contamination of complementary foods. However, in low-income households, setting adequate food processing and handling may be constrained by lack of economic resources. Possible approaches to overcoming these constraints are the use of pre-prepared food-mixtures or food subjected to lactic acid fermentation. The unavailability and high cost of pre-prepared weaning foods has fuelled search for formulas that can be affordable, attractive and nutritious[1]. However, microbial contamination risks still remain and increasing the nutrient content may better the microbial growth in gruel, in the absence of refrigeration or hot-holding facilities[6]. Lactic acid fermentation has also been recognized as an affordable method to reduce contamination with food-borne pathogens[1-7]. Interest on the use of fermentation techniques in food has been revived since there is now a restriction on the use of chemical additives for preservation. Fermentation is a natural biological process that can be easily adopted in developing/tropical countries where storage (refrigeration) or cooking (wood, butane gas or petroleum) facilities are lacking. However the extent to which pathogens are inhibited in fermented foods depends on a variety of parameters including microbial strain, initial level of contamination, time/temperature, amount of acid produced and buffering capacity of the food.

In north Cameroon as in other sub-Saharan areas where traditional gruels served as medicine vehicles, cereal processing into gruel requires various seasoning steps. Lactic fermentation and natural additives among which groundnuts, tamarind fruits and *Kilbu* are often used as seasoners[8-10]. *Kilbu* is a natural alkaline salt,

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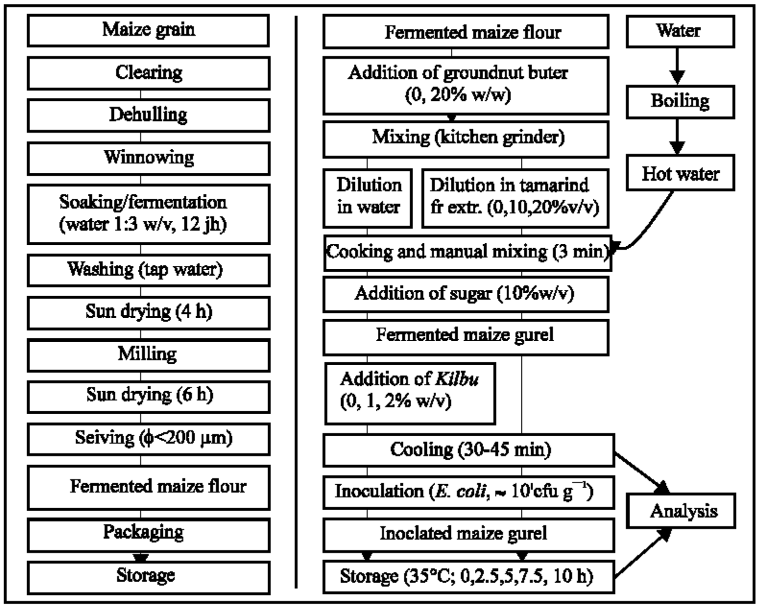

Fig. 1: Flowshart of the fermented maize flour (a) and gruel (b) production

consumed in gruel as a traditional medicine for diarrhoeal treatment[10]. To the best of our knowledge, no study has so far been carried out to determine these ingredients antimicrobial properties. The present study was therefore carried out to determine the influence of *Kilbu* and tamarind pulp extracts on the fate of microorganisms in a fermented maize gruel contaminated with *E. coli*.

MATERIALS AND METHODS

Material: Maize grains, groundnut butter, dried tamarind fruits (*Tamarindus indica* L.), *Kilbu* (natural alkaline salt) and sugar were purchased from the Garoua market (North Cameroon).

Bacterial strain, media and inoculum preparation: The *E. coli* strain was previously isolated from a maize gruel kept at 35±2°C for 10 h. This sample was manufactured by a commercial processor from Garoua. The isolate was maintained at 4°C on Eosin Methylene Blue (EMB-Difco, Detroit, USA) agar slops and transferred by loop inocula at 24h intervals to 50 mL of Nutrient Broth (Difco) at 37°C to reach 10^7-10^8cfu mL^{-1}. The preparations were refrigerated at 4°C prior to use.

Tamarind fruit and *Kilbu* pre-treatments: Tamarind fruits were washed in tap water and peeled using a stainless steel knife. After thorough cleaning, Tamarind pulp Extracts (TE) were obtained after boiling peeled fruits in water 20%w/v for 3 min. *Kilbu* was thoroughly ground in a kitchen mill (type HR1701, Phillips, Brazil) to pass through a 200 µm sieve. *Kilbu* powder was sterilised (120°C, 10 min) in an oven (Memmert, UL 40, Germany) and cooled at ambient temperature prior to use.

Maize processing into fermented gruel and experimental design: The specifications and methods of routine maize processing into fermented flour and gruel including various physical stages and ingredients previously identified[10] were modelled within a laboratory-based system to mimic normal traditional processes Fig. 1. A 5×3×2×2 factorial experiment was performed, considering the following variables: keeping time, addition level, seasoner source (TE and *Kilbu*) and groundnut butter-GB-level. Fermented maize flours (pH 4.9) alone or enriched with GB 20% w/w were thoroughly mixed using a kitchen grinder. The blends were divided into two groups. One group was dissolved in tap water 5% w/v, sweetened sucrose, 10% w/v and boiled for 3 min. The gruel samples were seasoned with *Kilbu* 0, 1, 2% w/v. In the other group, the previous blends were diluted in TE (0, 10 and 20% v/v) for gruel production. After cooling to 35±2°C, the treatments were inoculated with *E. coli* ˜10^6cfu g^{-1} for physicochemical analysis and microbial challenge testing[11] during storage (0, 2.5, 5, 7.5 and 10 h) at 35±2°C.

Physico-chemical and microbiological analyses: Physico-chemical properties (pH, texture as penetration index, dry matter and sugar contents) were analysed using official methods[12]. Microbiological analyses were carried out using the serial dilution plate count method on a 1 mL

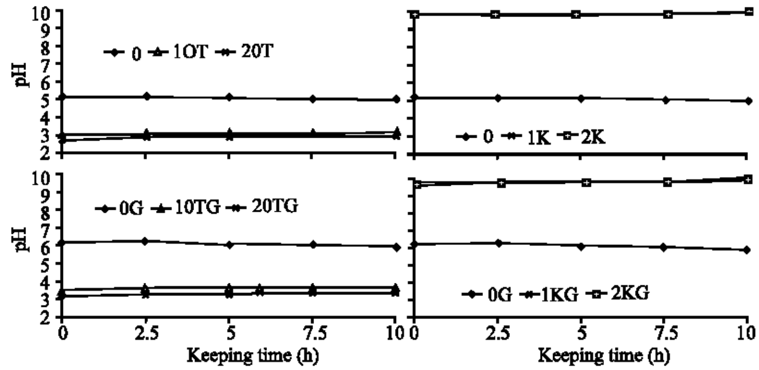

Fig. 2: pH changes in fermented maize gruel as affected by additive treatment and keeping time (Table 1 for treatment details)

Table 1: Physico-chemical properties of maize gruel treatments (means (sd))

Treatments	Dry matter content (g 100 mL^{-1})	pH	Penetration index (mm/10^{-3} sec)	Sugar content (g 100 mL^{-1})
0	14.9 (0.8)	5.1 (0.0)	41.1 (0.0)	11.5 (0.1)
0G	15.0 (0.6)	6.1 (0.0)	42.6 (0.1)	11.5 (0.0)
1K	15.2 (0.6)	9.7 (0.0)	41.0 (0.0)	12.2 (0.1)
2K	16.8 (0.5)	9.7 (0.0)	40.9 (0.1)	13.0 (0.1)
1KG	15.7 (0.4)	9.1 (0.0)	41.9 (0.1)	12.5 (0.1)
2KG	17.0 (0.4)	9.7 (0.0)	41.4 (0.1)	13.5 (0.1)
10T	15.3 (0.5)	3.0 (0.0)	40.9 (0.1)	11.8 (0.0)
20T	15.1 (0.5)	2.8 (0.0)	40.2 (0.0)	12.2 (0.1)
10TG	15.2 (0.5)	3.4 (0.0)	41.9 (0.1)	11.5 (0.0)
20TG	15.2 (0.5)	3.1 (0.0)	41.4 (0.1)	12.5 (0.1)

Treatments: 0. control, 0G. 20%groundnut butter (w/w), 1KG. 20%groundnut butter (w/w) + *Kilbu* 1% (w/v), 2KG. 20%groundnut butter (w/w) + *Kilbu* 2% (w/v), 1K. *Kilbu* 1% (w/v), 2K. Kilbu 2% (w/v), 10TG. 20%groundnut butter (w/w) +Tamarind extracts 10% (v/v), 20TG. 20%groundnut butter (w/w) +Tamarind extracts 20% (v/v), 10T. Tamarind extracts 10% (v/v), 20T. Tamarind extracts 20% (v/v)

sample. For each sample, 30 g were homogenized in 270 mL sterile diluent (0.1% peptone, 0.8% NaCl) using a lab blender. Appropriate 10-fold dilutions were plated onto EMB and PCA agars. The petri dishes were incubated aerobically at 44°C for 24 h and 30°C for 48-72 h, respectively for *E. coli* and aerobic mesophilic counts[13]. .Plates showing between 30 and 300 colony forming units (cfu) were counted and results converted to $\log_{10}$ cfu g^{-1} sample. Averages of two or tree replicates were reported for each treatment.

RESULTS AND DISCUSSION

Dry matter and pH profiles: Dry matter content and pH of prepared gruels are presented in Table 1. Dry matter of blends containing *Kilbu* slightly increased with substitution level whereas insignificant changes appeared after tamarind pulp extracts (TE) addition. Gruel enrichment with Groundnut Butter (GB) increased pH from 5.1 to 6.2. Mixing TE into maize gruel contributed to an important reduction of the pH of the samples prior to storage. An inverse trend was observed after addition of *Kilbu*. The change decrease or increase was less pronounced in the blends containing GB and lower levels of other additives. The high increase in pH obtained after *Kilbu* substitution could be attributed to the alkalinity induced in the mixture by the seasoner. A similar result was described after addition of lime in maize during nixtamalization[14-15]. The decrease in pH observed after addition of TE could be related to the high organic acid content of tamarind pulp[16]. Figure 2 presented the pH profile in gruel treatments. Contrary to the control, pH of gruel treatments did not vary during the keeping time. Hence with both seasoners, there was no or insignificant biochemical changes in gruels during the storage beside the microbial infestation.

Penetration index and sugar profiles: Penetration index and sugar content in gruel after cooking are presented in Table 1. Addition of *Kilbu* and TE have little effect on gruel texture. The penetration index gives an estimation of the gelling tendency of flour very important with respect to weaning food since the starch conversion and functionality are strongly related to material and processing conditions[17]. Changes in penetration index and sugar content in gruel as affected by seasoning conditions and keeping time are presented in Fig. 3 and 4. For both ingredients, penetration index slightly increased with keeping time. This may be attributed to starch hydrolysis in the presence of microorganisms. The sugar

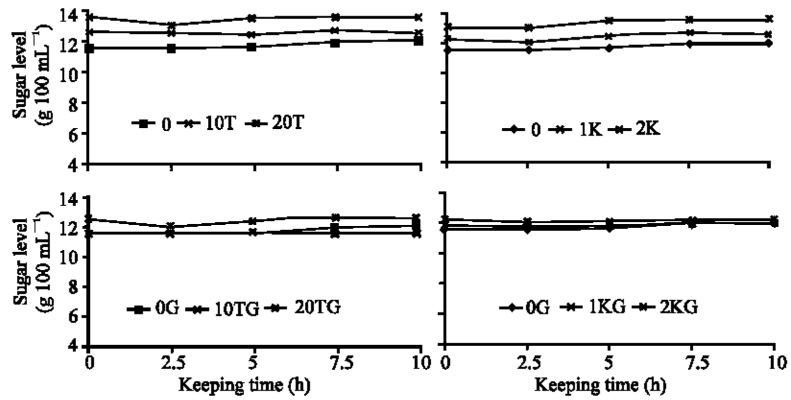

Fig. 3: Sugar changes in fermented maize gruel as affected by additive treatment and keeping time See Table 1 for treatment details

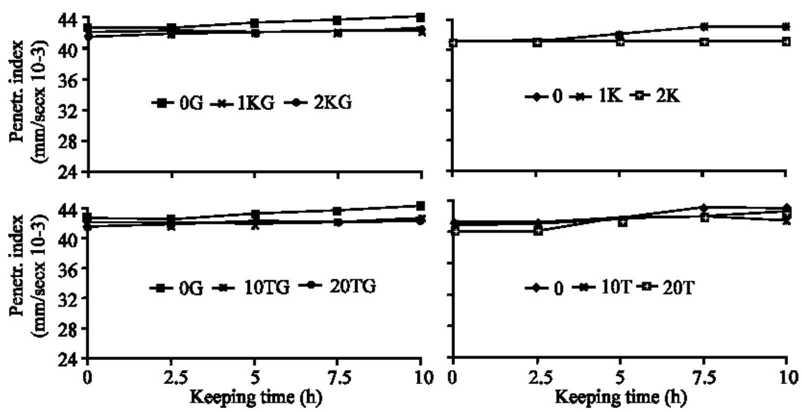

Fig. 4: Penetration index changes in fermented maize gruel as affected by additive treatment and keeping time (Table 1 for treatment details)

content is one of the most important criterion governing gruel taste and acceptability especially for infant feeding. Addition of *Kilbu* and TE slightly increased the sugar content as well. There was a barely perceptible difference in the sugar profile during storage. These observations suggested that the studied variables have little effect on texture and taste that constitute major reference marks for mothers or child minders. Hence, there may be a justification of the consumption of gruel 5 to 10 h after cooking and handling at ambient temperature in north Cameroon[10]. It has been reported that the traditional processing of cereal is often laborious and inconsistent, leading to unstable products under tropical conditions[7-10]. This suggests the necessity to target and study specific quality determinants associated with microbiological risks as far as food borne diseases are concerned. Hence there is a need to investigate on adequate and simple food control techniques under tropical conditions especially during cereal processing for infant feeding.

Effect of additives on microbial growth and survival

Effect of groundnut butter: In microbiological analysis of food, different microflora associated with the particular foods need to be screened. The major groups investigated were aerobic mesophilic bacteria and diarrhoea pathogens -enteric coliform sp. In this challenge study, *E. coli* was introduced in the gruel before storage as an index of fecal contamination. Furthermore, high numbers of the test-bacteria were added to gruel in order to initiate important bacterial growth, early discernible at the beginning of storage. On small-scale processing, the most likely source of *E. coli* contamination in food is faecal contamination and it would be unlikely that such high levels of contamination would occur after cooking. Therefore, the results presented here represent a 'worst-case scenario' in respect of the persistence of *E. coli* in gruel. After a lag phase of a few h, general steady increases in the populations of *E. coli* were registered in control and GB enriched samples Fig. 5. These steady increases were strictly adhered to by

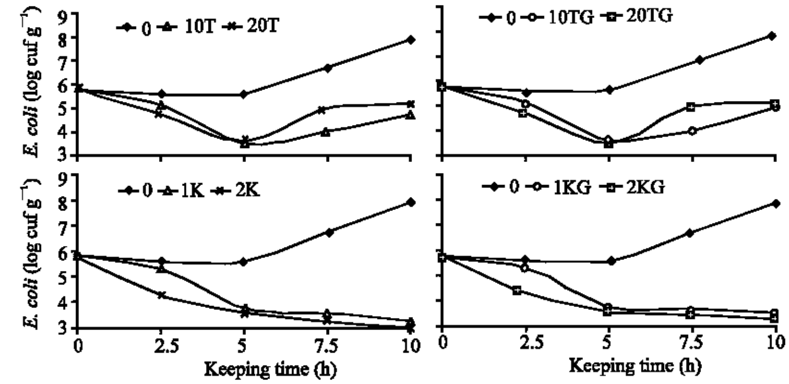

Fig. 5: *E. coli* profile in fermented maize gruel as affected by additive treatments and keeping time See Table 1 for treatment details

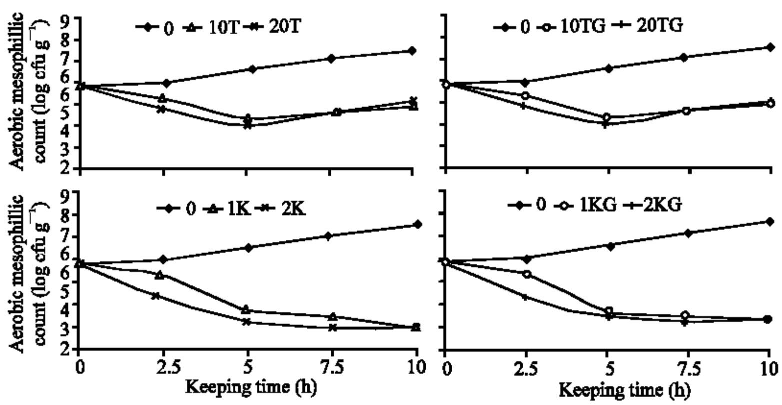

Fig. 6: Aerobic mesophilic flora profile in fermented maize gruel as affected by additive treatment and keeping time (Table 1 for treatment details)

aerobic mesophilic flora Fig. 6. These results were indicative of favourable conditions (temperature, pH and nutrient content) of control and GB enriched fermented gruels. On the other hand these observations were contrary to the microbial stability of fermented porridges as described by other authors[18-19]. Similar insignificant effects were described in fermented maize gruel marketed in northern[10] and southern Cameroon .[20]It has been established that microbial contamination during food storage was a function of time and temperature, which may allow survival and/or proliferation of microorganisms if storage conditions were sub-optimal[2]. Hence the extent to which pathogens were inhibited in fermented foods depends on the microbial strain, initial level of contamination, temperature, amount of acid produced, and buffering capacity of the food.

Effect of TE and *Kilbu*: After addition of traditional pap seasoners, bacterial growth was clearly delayed as shown in Fig. 5 and 6. During storage, two phases were observed. Between 0 and 5h (phase I), the microbial reduction was rapid and higher for increased additive concentration. From 5 to 10h (phase II), the microbial death rates were reduced with *Kilbu* while microbial growth resumed with TE. *Kilbu* appeared more active than TE for both types of flora meanwhile growth inhibition was slightly lower in samples containing GB. GB, being richer in proteins, could be expected to raise the pH and support growth of more microorganisms than maize alone. These observations tend to confirm the anti-diarrhoeal activity of *Kilbu*, a common belief in north Cameroon[10]. TE antagonistic effect may be due to acidification generated after addition in maize gruel. Similar results were obtained in acidified infant formulas produced by fermentation or by direct addition of lactic acid[21]. The *Kilbu* antagonistic effect could be related to the high mineral content of the alkaline salt. It has been reported that in media containing high concentration of salt (e.i. NaCl), a variety of responses may be detected, including in gram-negative species, a decrease in the concentration of periplasmic oligosaccharides as well as an altered proportion of the outer membrane proteins[22]. Such mechanisms may be responsible for the microbial growth reduction. However this appeared less effective swith increasing time and could be due to an adaptation to the stress. These results confirm that simple processing and handling techniques can be used to eliminate or control microbial contamination of complementary foods[1]. The persistence and growth of *E. coli* after 10 h in mixtures maintained at 35°C suggested that the hygienic test-bacteria may be controlled more effectively if initial contamination level during processing and storage was maintained at low levels. Despite the numerous benefits of fermentation, it has been recognized that not all pathogens are susceptible to its effects and other methods of control are necessary. An example is given by some enteropathogens, such as *E coli* O157:H7 showing acid resistance[23]. In the tropical areas with facilities for livestock (cattle, pig, goat, poultry) the situation may be more critical since nearly half of all infectious microbial agents of humans have an animal reservoir[24]. Available data indicated new and increasing problems caused by many microbial genera and species including *Salmonella*, *Campylobacter*, *Yersinia*, Enterohemorrhagic *E. Coli* (EHEC) and *Listeria monocytogenes*[25]. However, a hierarchy of hazards is now established and some pathogens are more common and therefore pose a greater risk. The extent to which the risk of food borne illness is reduced during the production of any particular food will thus differ depending on the type of hazard being considered, the nature of the raw material, the precise details of the process used, handling and storage conditions. Recent surveys in North Cameroon revealed that for infant feeding, traditional gruel production requires shorter times avoiding fermentation and seasoning steps. Moreover many street-vended gruels used as complementary food were unsafe 3 to 4 h after cooking although the sale or storage were prolonged after 5 h [10]. Therefore the time required for household processing and the holding period before consumption are other disadvantages as earlier revealed[2]. These practices may be detrimental to the hygienic profile of the gruel and so to consumer's health under tropical conditions. Hence, easy-to-prepare, nutritious and shelf-stable, cereal based formulations should be designed to effect improvements in the traditional technologies and products.

CONCLUSION

Seasoning conditions effects have been studied in order to evaluate the possible changes in microbiological and physico-chemical profile in fermented maize gruel during storage at 35°C. This study confirmed that keeping time is a principal factor influencing the microbial growth. This effect is conditioned by factors such as bacterial level as well as ingredient type and substitution level. Holding time was however more effective and brought about an increase in aerobic mesophilic bacteria and *E. coli* due to favourable conditions (lack of antagonistic components, temperature, pH, moisture content, nutrient and infestation level). Despite the numerous benefits of lactic acid fermentation, not all pathogens are susceptible to its effects. This confirms the possible hygienic risk associated with fermented cereal-based weaning foods under tropical conditions. Addition of *Kilbu* as well as tamarind pulp extracts at various concentrations may increase the shelf life of maize gruel while maintaining some physico-chemical properties. These ingredients are equally important influencing factors on the quality profile of gruel. Additions of *Kilbu* or tamarind pulp extracts provided mixtures with improved shelf life and as such constitute potential ingredients for the production of shelf stable cereal-based weaning foods. These antagonistic effects need to be tested on other pathogens such as *Salmonella* sp., *Staphylococcus* sp. or *Clostridium* sp. The nutritional and sensory effect of these traditional ingredients is currently being evaluated in the preparation and consumption of cereal gruel.

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