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Khyopeh, a traditional fermented yak meat product of Sikkim

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The preparation of naturally fermented meat product is an integral part of socio-cultural practice of different ethnic groups of people dwelling in the Himalayan regions of India, Nepal, Bhutan and Tibet in China. This study is aimed at documenting the traditional preparation of *khyopeh*, a naturally fermented meat product of yak prepared by ethnic people of Sikkim and its food safety. This is the first report on *khyopeh* with emphasis on its traditional method of preparation and food safety

Keywords: *Khyopeh*, Sikkim, ELISA, *Staphylococcus*, Yak

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Meat is considered as highly nutritious and has become an essential component of human diet being a rich source of valuable proteins, minerals, vitamins, fats and micronutrients[1]. The consumption of meat in daily meal is common dietary culture of some ethnic people residing in the Himalayan regions of India, Nepal, Bhutan and China (Tibet)[2]. Due to high content of moisture and protein in the meat, it is easily susceptible to microbial spoilage[3], hence to prevent the spoilage and to prolong the shelf life of perishable raw meat, it is either dried or fermented or smoked[4]. The domestic livestock of Sikkim in India mostly includes cattle, pig, goat, yak etc. which are commonly used for milk, milk products, and meat. Among these livestock, yak (*Bos grunniens*) is reared in alpine and subalpine scrub lands between 2,100 to 4,500 m altitude in the Himalayas for milk products and meat[5]. In Sikkim, 88.3% of people are non-vegetarian and 11.7% are vegetarian, which depicts an increase in demand of meat and its product[6]. Some ethnic meat products of Sikkim have been documented earlier such as are *kargyong, satchu, sukakomasu* and *chilu*[7]. However, the unlisted naturally fermented meat product called *khyopeh* has not been documented yet. This paper aims to give information on the indigenous knowledge of preparation of *khyopeh* in North district of Sikkim.

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Materials and methods

Documentation and data collection

Field survey was carried out at Lachung village of North Sikkim for a period of three months from October 2017 till December 2017. Data collection was done based on structured questionnaire, interviewing the people involved in traditional preparation of *khyopeh* and personally analyzing the preparation procedures. The interviewees were local elders of respective village who have had proper traditional knowledge of preparation, their culinary skills and socio-economy of the products.

Sample collection

A total of 5 samples of *khyopey* were collected from North Sikkim and taken into the laboratory in a sterile polythene bags and stored at -20°C for microbial analysis. The pH was determined directly by a digital pH meter (Thermo Scientific Instruments, Waltham, Massachusetts, USA) and moisture content was measured using OHAUS MB45 Moisture Analyzer (OHAUS, Parsippany, New Jersey, USA).

Microbiological analysis

Sample (25 g) was homogenized with 225 mL buffered peptone-water (CM 509 Oxoid Ltd., Basingstoke, UK) in a Stomacher (Seward, Thetford, UK) and serial dilutions of homogenate (10^1 to 10^9) were prepared for microbiological analysis. Decimal dilutions (1 mL) of the sample homogenate were inoculated in 3 M Petrifilm TM Aerobic Count Plate (3 M Company, Maplewood, Minnesota, US), Baird–Parker agar (Hi Media, India) plates supplemented with potassium tellurite and egg yolk emulsion for *Staphylococcus* species[8], Violet Red Bile Glucose agar (VRBGA, Hi Media Ltd.) for *Escherichia coli* and Coliform bacteria[9], (*Bacillus cereus* agar (Hi Media, Mumbai, India) for *Bacillus cereus*[4] and bile esculin azide (BEA) agar for *Enterococcus* species. The plates were incubated at 35°C for 24-48 h. On Baird Parker agar, convex, black, shiny colonies with narrow white margin surrounded by clear zone were regarded as *Staphylococcus* species and on VRBGA, Coliform bacteria formed small red colonies (~1 mm diameter), with or without a red precipitate. Colonies that showed black pigmentation on the BEA agar were regarded as *Enterococcus*[10]. The results were expressed as colony-forming units per gram (cfu/g). The isolates were preliminary identified based on Gram stain, cell morphology, catalase test, IMViC (Indole, Methyl Red, Voges-Proskauer and Citrate Utilization tests, carbohydrate fermentation and other tests[11].

Enzyme Linked Immuno Sorbent Assay (ELISA) test

ELISA tests were performed for *Staphylococcal* enterotoxins, *Bacillus diarrhoeal* enterotoxins and *Salmonella* in *khyopeh* samples using *Staphylococcus* Enterotoxin Assay (SET Total) (r-biopharm, Germany)[12], *Bacillus* diarrhoeal enterotoxin visual immunoassay (3 M Microbiology, USA)[13]and *Salmonella* visual immunoassay (3 M Microbiology, USA)[14]according to manufacturer's instructions.

Antibiotic Susceptibility Test

Antibiotic susceptibility tests of isolates grown on Mueller Hinton agar plates (Hi Media, Mumbai, India) were performed with 24 antibioticsusing the Kirby–Bauer disk diffusion method[15] following guidelines of Clinical Laboratory Standards Institute[16]. Isolates with the standard strain *Staphylococcus aureus* MTCC 96 were incubated at 37°C for 24 h and the diameters of the zones of inhibition were measured (CLSI 2017).

Results and discussion

Method of traditional preparation

Khyopeh is an ethnic fermented yak meat product, which is prepared in the North district of Sikkim. The ethnic *Lachungpa* community of Sikkim who resides in the northern parts of Sikkim mostly practices the preparation and consumption of *khyopeh*. The preparation of *khyopeh* is seasonal which is prepared only in the month of December every year as the yaks are being slaughtered for the Buddhist festivals. The main ingredient used for preparation of *khyopeh* is parts of liver, lungs, fats, intestines and innards. During traditional method of preparation of *khyopeh*, yak meat with its fat are chopped finely, and mixed with required amount of salt. The meat mixtures are stuffed into the rumen (stomach) of yak, and are tied up with rope. It is then hanged in a bamboo stripes above the kitchen oven or at attic for smoking and drying for 4 to 6 months or even for a year to make *khyopeh* (Fig. 1). *Khyopeh* is soft or hard and brownish in colour (Fig. 2). It is eaten as raw or cooked with nettle leaves, locally called *sishnu* (*Urtica dioica* L.) in the main meal with boiled rice in North Sikkim.

Socioeconomic importance

The practice of preparing *khyopeh* is quite rare and confined to Lachung village of north Sikkim which are quite far from the urban localities. Hence, it is not found in the local markets of Sikkim. It is usually prepared for home consumption and festivals. It is believed by the villagers that yak meat products have an immense medicinal potential. Yaks graze on herbs especially *Cordyceps sinensis*, locally called as *yarsagumba*, which is found only in high altitude of mountains and is believed to be an excellent potent for strengthening the immune system.

Food safety

Microbial load of five samples of *khyopeh* was analyzed- mesophilic aerobic count (10^6-10^7 cfu/g),

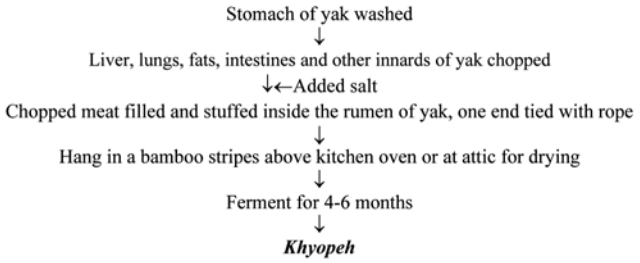

Fig. 1 — Traditional method of preparation of *khyopeh* in North Sikkim.

Fig. 2 — *Khyopeh*, an end product of fermented yak meat inside the rumen of yak.

Staphylococcaceae count (10^4-10^5 cfu/g) and Enterobactericeae count (<10^4 cfu/g). The pH and moisture content of *khyopeh* was 5.8 to 6.1 and 1.6-3.5%, respectively. Based on phenotypic characterization (Table 1), *Staphylococcus*, *Escherichia* and *Enterococcus* were tentatively identified. *Bacillus cereus* was not detected in any sample. ELISA tests were found to be negative for all bacterial toxins tested. Antibiotic sensitivity tests were performed on representative strains of *Staphylococcus*, *Enterococcus* and *Escherichia* (Fig.3). *Enterococcus* was resistant to six antibiotics, *Staphylococcus* was resistant to Oxacillin and *Escherichia* was found to be sensitive to all antibiotics tested except amoxicillin/clavulanate.

Microbiological analysis of samples *khyopeh* tested for food safety revealed presence of low population of *Staphylococcus* species and Enterobacteriaceae by plating method supported by the ELISA tests which also showed negative test for Staphylococcal enterotoxin, *Bacillus* diarrhoeal enterotoxin and *Salmonella.* Similar results were reported in traditional Greek fermented sausage[17]. Antimicrobial susceptibility test indicated that *Staphylococcus* strain KHST1was sensitive to all the antibiotics except oxacillin. However, the isolate may be considered as an ORSA (Oxacillin resistant *Staphylococcus aureus*) if

Table 1 — Phenotypic characterization of bacteria isolated from khyopeh

Isolate code	Pigment production	Catalase	Motility	Urease reaction	DNase	Sucrose	Voges - proskauer	Methyl red	Indole	Xylose	Raffinose	Urease	Rhamnose	Tentative identification
KHSTI	Golden yellow	+	-	+	+	+	+	-	-	-	-	+	-	*Staphylococcus*sp.
KHST2	Cream	+	-	+	-	+	+	-	-	-	-	+	-	*Staphylococcus*sp.
KHST3	Cream	+	-	+	-	+	+	-	-	-	-	+	-	*Staphylococcus*sp.
KHST4	Golden yellow	+	-	+	-	+	+	-	-	-	-	+	-	*Staphylococcus* sp.
KHST5	Cream	+	-	+	-	+	+	-	-	-	-	+	-	*Staphylococcus* sp.
KHENI	Black	-	-	-	-	+	+	-	-	-	-	-	-	*Enterococcus* sp.
KHENII	Black	-	-	-	-	+	+	-	-	-	-	-	-	*Enterococcus* sp.
KHENIII	Black	-	-	-	-	+	+	-	-	-	-	-	-	*Enterococcus* sp.
KHE1	Pink red	+	+	-	-	-	-	+	+	+	+		+	*Escherichia* sp.
KHE2	Pink red	+	+	-	-	-	-	+	+	+	+		+	*Escherichia* sp.
KHE3	Pink red	+	+	-	-	-	-	+	+	+	+		+	*Escherichia* sp.
KHE4	Pink red	+	+	-	-	-	-	+	+	+	+		+	*Escherichia* sp.
KHE5	Pink red	+	+	-	-	-	-	+	+	+	+		+	*Escherichia* sp.

All isolates were Gram +ve, fermented glucose, mannitol, maltose, trehalose, lactose and galactose.
None of the isolates fermented adonitol; coagulase and nitrate reductions were positive and citrate reduction was negative for all isolates, respectively.

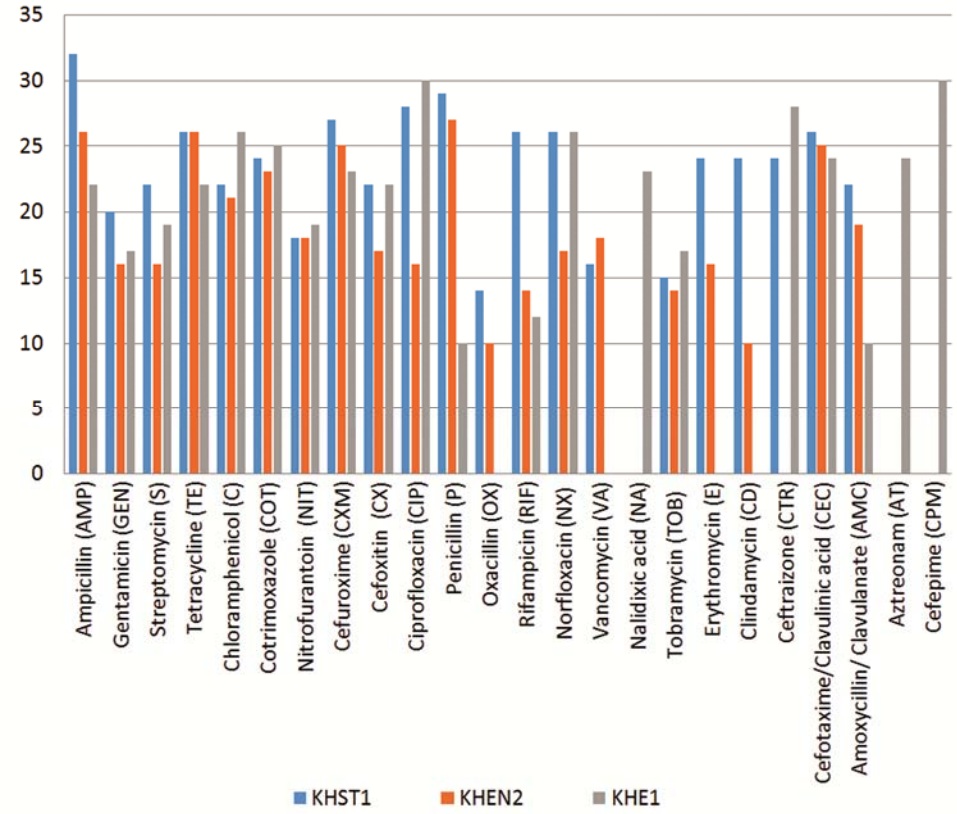

Fig. 3 — Antibiotic sensitivity tests of bacteria isolated from *khyopeh*

the isolate is resistant to multiple agents like trimethoprim-sulfamethoxazole combination, clindamycin, erythromycin, quinolones, tetracycline, and aminoglycosides[18]. *Escherichia* strain KHE1 was also found to be sensitive to most of the antibiotics except amoxicillin-clavulanate. The amoxicillin-clavulanate resistant isolates were also found in sausages in Malaysia[19]. *Enterococcus* strain KHEN1 was resistant to many antibiotics like oxacillin, cotrimoxazole, ciprofloxacin, cefoxitin, rifampicin and clindamycin. The results showed that consumption of *khyopeh* is safe by ethnic people of Sikkim.

Conclusion

Consumption of traditionally processed dried, smoked and fermented meat products without using starter cultures and chemicals by the ethnic people in Himalaya region is a common practise since centuries. *Khyopeh* holds the importance in preserving the traditional knowledge of preparation of rare and minor naturally fermented yak-meat product in Sikkim. There has been no report of food poisoning in Sikkim by consuming *khyopeh*. This is the first report on unique ethnic meat product *khyopeh* from the Himalayas.

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