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Debaryomyces Hansenii: Harnessing its Signification in Kumaun Himalaya's Traditional Meat Products

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ABSTRACT

The article delves into the prevalence of yeast *Debaryomyces hansenii*, as identified by using phenotypic identification following the method of Kreger-van Rij and Kurtzman and Fell alongside biochemical assays is notably higher in traditionally prepared meat products as compared with other yeast genera from the different pockets of Kumaun Himalayan region. This surveillance is harmonious with the observation from studies carried out on meat products by other researchers across the globe. Despite the traditional preparation methods of meat products adopted by ethnic people of the Himalayas, the occurrence of this yeast plays a significant role in enhancing the shelf life and highlights potential avenues for improving food security in resource-constrained environments. Notably, documenting the indigenous knowledge of traditional meat processing techniques among ethnic groups residing in remote areas of the India-Nepal border is arduous. Understanding the microbial ecology of these items is crucial because it provides insight into the natural processes that support their preservation. The existence of *D. hansenii,* a yeast that has been detected by biochemistry and phenotypic analysis, is rigorously examined in this paper.

Keywords: Debaryomyces hansenii, Ethnic, indigenous knowledge, food security, Kumaun Himalaya

Introduction

The meat industry is heavily impacted by the shifting eating habits of consumers around the world due to population growth and rising urbanization. The processing of meat can be divided into two categories: primary and secondary. Whereas the latter typically requires multiple additional processing stages, the former typically uses animal carcasses or entire meat primals. (Soladoye, 2021). Meat processing is the combination of chemical curing, microbial fermentation and drying which not only prolongs the shelf-life of perishable raw meat but also gives stable, safe, ready-to-eat end products (Bacus, 1984; Rantsiou and Cocolin, 2006). Microorganisms are mostly present in or on the raw materials, ingredients, utensils, and environment, and are selected through adaptation to the substrate for fermentation (Hesseltine, 1983; Tamang, 1998). Microorganisms are known to transform the chemical constituents of raw substrates during fermentation into acceptable food products with improved flavour, aroma and texture, enhancing nutritional value and other health benefits (Steinkraus, 1996; Stiles and Holzapfel, 1997).

The ethnic residents of Kumaun Himalaya use their traditional expertise of preparation to cook and consume a variety of meat products. By seeing how adeptly they prepare sausage-like goods from unsightly animal parts such as leftovers, organ meats, fat, blood, etc., it is reasonable to assume that the Himalayan people are capable of storing raw meat. The prokaryotic microbes known to be present in fermented

sausages are crucial to the ripening and fermentation process, such as staphylococci, micrococci, and lactic acid bacteria, which are not mentioned in the article that is being presented. In contrast to yeast, the role of bacteria in the production of meat products is very well understood. The main focus of the paper is the identification and prevalence of *Debaryomyces hansenii* by phenotypic analysis in conjunction with biochemical tests. The abundance of *D. hansenii* in sausages and dry-meat products indicates a prevalence of this yeast and can generate volatile and aromatic compounds, enhancing consumer acceptance. This yeast can improve the characteristics of sausages and dry-meat products (Ramos-Moreno et. al. 2022). The most prevalent type of yeast discovered in the casings of several meat products from the southern Spanish region of Valle de los Pedroches was *D. hansenii* which included "lomo," "chorizo," and "salchichon" (Jose, Ramos et. al. 2017). It has been found that the genus has the potential as a biocontrol agent against unwanted fungi in the food and the use of the strains can be a natural alternative to chemical preservatives in food (Chacón-Navarrete et. al. 2022). To improve the finished product's aromatic quality, *D. hansenii* is frequently utilised as a starter in meat fermentations. It plays a part in giving meat products their desired scents. Meat products experience fluctuations in temperature, pH, and osmotic pressure among other abiotic stress conditions during fermentation. For the fermentation of meat, *D. hansenii* is a good option because it can thrive in these stressful environments (Belloch et. al. 2022). Selectable phenotypic traits, like the capacity to thrive in the presence of abiotic stress and produce pleasant scents, can be found in *D. hansenii* isolates, making them ideal starters for meat products (Belloch et. al. 2022).

In sausages and other dry-meat products, it has been found that *D. hansenii* prevalence over other yeast is ambiguous. The use of *D. hansenii* as a biocontrol agent and the production of new meat products by reducing preservatives may be the research avenues that will enhance current knowledge and help in developing more ecological products. (Ramos-Moreno, 2021).

Kumaun hills of Uttarakhand are situated in the Central Himalayan region at the tri-junction of Nepal, Tibet (China) and India with a total population of about 8479,562 (Census, 2001). Five major tribes, i.e., the Tharus, the Jaunsaries, the Buxas, the Bhutias and the Rajis numbering 179,002 inhabit the Central Himalayan region of Uttarakhand. The Tharus, the Buxas and the Jaunsaries are agriculturists while the Bhutias are pastoralists and the Rajis are mostly hunters and gatherers. The eight major Bhutia groups are the Johari, Jeethora, Darmi, Chaudansi, Byabsi, Marchha, Tolcha and Jad, and are scattered over eight main river valleys known as Johar, Darma, Byans, Chaudans (Pithoragarh district in Kumaun), Mana, Niti (Chamoli district in Garhwal), Nilang and Judun (Uttarakashi district in Garhwal) (Nandy et. al. 2006). The ethnic people of the Kumaun Himalaya prepare and consume different products of meat which is indigenous to that region. With the prevalence of *D. hansenii* as a natural substitute for chemical preservatives, the goal of this study is to isolate various strains of yeast species from various meat products native to the Kumaun Himalayan region and characterise them based on their morphological and biochemical characteristics. Though they are unaware of the potential of microorganisms for natural preservation, these ethnic groups have been preparing and consuming these items for millennia.

Materials And Methods

Culture media used

Following culture medium were used for culturing and analyses of microorganisms from the collected samples: Potato Dextrose Agar (PDA); HiMedia; India, Yeast-Malt Extract (YM) Agar; HiMedia; India, Yeast-Malt Extract (YM) broth; HiMedia; India, Plate Count Agar (PCA); HiMedia; India, Yeast Morphology Agar; HiMedia; India, Sterile ascospore agar; HiMedia; India, Malt-Extract agar; HiMedia; India, Yeast Nitrogen base; HiMedia; India.

Methodology

Survey

A field survey was conducted in different places of the Kumaun Himalaya to document the indigenous knowledge of ethnic people nestled on the border between India and Nepal on meat processing. The information gathered from ethnic communities representing Bhutias of the region. Description of each meat product including a traditional method of preparation, preservation, culinary, mode of consumption and socio-economy were well documented.

Collection of samples

Together 16 samples of traditional meat products were also collected from different places of Kumaun Himalaya in Dharchula and Pithoregarh district of Uttarakhand. Samples were collected aseptically in pre-sterile poly bags as well as sterile bottles and were, sealed and labelled. Samples were stored at -20° C for further microbial and biochemical analyses.

Microbiological analysis

Ten grams of sample was homogenized with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender

(400, Seward, UK) for 1 min. A serial dilution (10^{-1} to 10^{-8}) in the same diluent was made.

Different strains of yeast were isolated on PDA and YM agar supplemented with 10 IU/ml benzylpenicillin and 12 µg/ml streptomycin sulphate, respectively; and plates were incubated aerobically at 28° C for 72 h.

TVC

Total viable count (TVC) was determined in the PCA plates which were incubated at 30° C for 48-72 h. Colonies of all microorganisms were selected randomly or all sampled if the plate contained less than 10 colonies according to (Leisner et. al. 1997). The purity of the isolates was checked by streaking again on fresh agar plates of the isolation media and sub-culturing on corresponding agar medium, followed by microscopic examinations. Microbiological data obtained were transformed into logarithms of the numbers of colony-forming units (cfu) per g of sample. Identified strains of microorganisms were preserved in respective media using 15 % (v/v) glycerol at −20° C.

Characterization of yeast Isolates

Cell morphology

Cell morphology and mode of vegetative reproduction of yeast were observed following the method of Yarrow (1998) using a phase contrast microscope (Olympus CH3-BH-PC, Japan). Sterile yeast morphology agar slants were inoculated with an actively growing (24 h-old) yeast culture and incubated at 28° C for 3 days. Dimensions of cells were measured with a standardized ocular micrometre.

Pseudo- and True-mycelium

For observation of pseudo-mycelium and true-mycelium of yeast isolates, the slide culture method described by (Kreger-van Rij, 1984) was followed. A petri dish, containing a U-shaped glass rod supporting two glass slides, was autoclaved at 121° C for 20 min. The glass slides were quickly removed from the glass rod with a flame-sterilized pair of tweezers and were dipped into the molten PDA after which they were replaced on the glass rod support. The solidified agar on the slides was inoculated very lightly with yeast isolates in two lines along each slide. Four sterile coverslips were placed over part of the lines. Some sterile water was poured into the Petri dish to prevent the agar from drying out. The culture was then incubated at 28° C for 4 days. The slides were taken out of the Petri dish and the agar was wiped off from the back of the slide. The edges of the streak under and around the coverslips were examined microscopically for the formation of pseudo-mycelium or true-mycelium.

Characteristics of asci and ascospore

Sterile ascospore agar slants were streaked with actively grown yeast cultures, incubated at 28° C for 3 days and examined at weekly intervals for up to 4 weeks for observation of asci and ascospores. A heat-fixed smear was flooded with 5 % w/v aqueous malachite green for 60 seconds, heated to steam 3 to 4 times over the flame followed by counterstaining with safranin for 30 sec and observed under the microscope (Yarrow, 1998).

Reduction of nitrate

Yeast cultures were grown in 5 ml nitrate broth incubated at 28° C. After 3, 7 and 14 days, 1 ml of the culture was mixed with 3 drops of the reagent for nitrate reduction test and observed for the development of a red or yellow colour, indicating the presence of nitrate. A small amount of zinc dust was added to the tube that was negative even after 14 days and observed for the development of red colour, indicating the presence of nitrate, i.e. absence of reduction (Yarrow, 1998).

Growth at 37° C

Slants of malt-extract agar were inoculated with actively grown yeast isolates and incubated at 37° C for 4 days and observed for growth (Yarrow, 1998).

Sugar fermentation

Yeast isolates were grown at 28° C on YM agar slants for 3 days. Tubes of 10 ml of fermentation basal medium (Wickerham, 1951) supplemented with 2 % w/v sterile sugars containing inverted Durham tubes, were inoculated with the above yeast culture and incubated at 28° C and were shaken regularly to observe gas accumulation in the inverts (Yarrow, 1998).

Sugar assimilation

Yeast isolates were grown at 28° C on YM slants for 3 days. Tubes containing a 5 ml mixture of yeast nitrogen base and carbon source were inoculated with cultures and incubated at 28° C for 3 to 7 days. The control test tube was made by adding 0.5 ml of yeast nitrogen base in 4.5 ml of sterilised distilled water (devoid of any carbon source). Assimilation of carbon sources was observed by comparing with the control (Yarrow, 1998).

Identification of Yeast

Yeast isolates were identified at the genus level according to (Kreger-van Rij, 1984), (Kurtzman and Fell, 1998) and (Yarrow, 1998).

Technological Properties of yeast isolates

Titratable acidity

The titratable acidity of the sample was calculated by titrating the filtrates of a well-blended 10 g sample in 90 ml carbon-dioxide-free distilled water with 0.1 N sodium hydroxide to the endpoint of phenolphthalein (0.1 % w/v in 95 % ethanol) (AOAC 1990).

Moisture

The moisture content of the batters was calculated by drying 2.5–3.0 g of a well-mixed sample at 135 ± 1° C for 2 h to constant weight (AOAC 1990).

Ash

A sample (~2 g) was accurately weighed into a previously dried and weighed porcelain crucible and placed in a muffle furnace, preheated to 550° C for 3 h. The crucible was transferred directly to desiccators, allowed to cool to room temperature and weighed immediately (AOAC 1990). The process of heating for 30 min, cooling and weighing was repeated until the difference between two successive weighings was ≤ 1 mg.

Fat

The fat content of the sample was determined by ether extraction using a glass soxhlet (AOAC 1990). The flat-bottomed flask was oven-dried and kept in a desiccator for cooling. The weight (W_1) of the round-bottomed flask was taken. A cellulose thimble (dry and fat-free) was taken in which ~ 2 g of sample was placed and put in the soxhlet. Fat was extracted by using petroleum ether with a boiling range of 40-60° C, on a heating mantle at 60° C for 5 h. The flat-bottomed flask was dried for 1 h at 100° C to evaporate ether and moisture, cooled in a desiccator and weighed (W_2). Fat was calculated: Fat (%) = $W_2 - W_1$/Sample weight × 100.

Protein

The total nitrogen of the sample was determined following the method described in AOAC (AOAC 1990). Approximately 1 g of sample was taken in a digestion flask, 0.7 g catalyst ($CuSO_4$: K_2SO_4, 1:9) and 25 ml of concentrated H_2SO_4 were added to it. The flask was heated gently until frothing ceased, boiled briskly until the solution became clear and then continued boiling for about 1 h. The solution was transferred quantitatively to a round-bottomed flask, and mixed with approximately 100 ml of distilled water and 25 ml 4 % w/v aqueous Na_2S to precipitate mercury. A pinch of zinc granules to prevent bumping and a layer of 40 % w/v NaOH were added carefully. The flask was immediately connected to a distillation apparatus and the tip of the condenser was immersed in standard 0.1 N H_2SO_4 containing about 5 drops of methyl red indicator. The flask was rotated to mix the contents thoroughly and heat until all the ammonia had been distilled. The receiver was removed and the tip of the condenser was washed with distilled water. The remaining acid in the receiver was titrated with standard 0.1 N NaOH solutions. The blank determination on reagents was considered for correction.

Nitrogen was calculated in percentage. Total nitrogen (%) = [(ml of standard acid × N of standard acid) – (ml of standard NaOH – C.F.) × N of standard NaOH] ×1.4007/weight of the sample (g). Correction factor (C.F.) = (titre of standard NaOH against blank – ml of standard acid).

Protein content was determined by multiplying the total nitrogen value by 6.25 (AOAC, 1990). Protein (%) = Total Nitrogen (%) × 6.38

Carbohydrate

The carbohydrate content of the samples was calculated by difference: 100 – (% protein + % fat + % ash) (Standal 1963).

Food Value

The food value of each batter sample was determined by multiplying the protein, fat and carbohydrate contents by the factors 4, 9 and 4, respectively, and adding all the multiplication values to get kcal per 100 g (Indrayan et. al. 2005).

Statistical analysis

The statistical data were analysed by determining the standard deviation (SD) as described by (Snedecor and Cochran, 1989).

Results

An extensive field survey was conducted in different regions of Uttarakhand for the collection of information and samples: Dharchula, Pangu, Rumjum, Marchal and Munsiyary, Sosa in the Pithoregarh district of Uttarakhand. Based on personal observation and interviews with the producers, three major types of meat products from Uttarakhand were documented. A total of 16 samples from the Kumaun Himalaya of various types of meat products were analyzed for the isolation of yeast strains. In all traditionally prepared meat products, the population of yeasts was 10^3 to 10^7 cfu/g. Following strains of yeast species were recovered: *Debaryomyces hansenii, Candida albicans, C. fermata* and *C. humicola*.

Description of meat products i.e. Chartayshya, Jamma and Arjia, including a traditional method of preparation, preservation, culinary, mode of consumption and socio-economy were well documented below (Rai, 2008).

Chartayshya

Chartayshya (Photo 1) is a traditional chevon meat product of the Kumaun Himalayas, consumed by Bhutias of Dharchula and Munsiyari in the district of Pithoregarh of Uttarakhand. This product is preferred by the people of Darma, Chawdas

and Byans Valley of Dharchula. It is prepared mainly during the religious festival called 'Kolatch' (worshipping ancestral spirit).

Traditional method of preparation

Red goat meat (chevon) is cut into small pieces of 3-4 cm, mixed with salt, sewed in a long thread hung on the bamboo stripes or wooden sticks and kept in the open air in the corridor of the house for 15-20 days (Fig. 12). It can be kept at room temperature for several weeks for future consumption. In Western Nepal, a similar product called *sukha sikhar* is prepared from chevon.

Raw chevon meat
↓
Cut into small pieces; marinated with salt
↓
Smoke or dry for 15-20 days
↓
Chartayshya

Fig.1: Flow sheets of the traditional method of *Chartayshya* preparation in Kumaun

Mode of consumption

Curry is made by frying in edible oil with tomato, ginger, garlic, onion and salt.

Socio-ethnical importance

The ethnic people of the Kumaun Himalaya prepare *chartayshya* curry, especially during the *kolatch* festival (worshipping the ancestral spirit) and offer it to ancestors before eating. It is not sold in the Market.

JAMMA/ GEEMA

Jamma or *Geema* (Photo 2) is a traditional fermented sausage of the Kumaun Himalayas prepared from chevon meat. These products are also consumed by the Bhutias of Dharchula and Munsiari of Pithorgarh district.

Traditional method of preparation

Red goat meat is chopped into fine pieces; ground finger millet (*Eleusine coracana*), wild pepper locally called 'timbur' (*Zanthoxylum* sp.), chilli powder and salt are added and mixed. A small amount of fresh animal blood is also added. The meat mixture is made semi-liquid by pouring water and stuffed into the small intestine of a goat of about 2-3 cm in diameter and 100-120 cm in length with the help of a funnel and tied to both ends of the long intestine (Photo 10). It is pricked randomly to prevent bursting while boiling. After boiling for 15-20 min, stuffed intestine are smoked above the kitchen oven for 15-20 days (Fig. 13) or it can be eaten as such. The method of preparation of the product is similar to that of *kargyong* in the Sikkim Himalaya.

Mode of consumption

It is consumed as curry by mixing with onion, garlic, ginger, tomato and salt. It is also deep-fried and is eaten with local alcoholic beverages. Sometimes, *Jamma* may be eaten as cooked sausage. It is not sold in the local market.

Goat meat and ingredients
↓
←Add blood and water
↓
Stuff into the small intestine; seal, prick
↓
Boil for 15-20 min
↓
Smoke/dry for 15- 20 days
↓
Jamma/ Geema

Fig.2: Flow sheet of the traditional method of *Jamma* preparation in Kumaun

ARJIA

Arjia (Photo 3) is also a traditional sausage prepared from chevon meat. It is also an important food of the Kumaun.

Traditional method of preparation

The preparation method of *arjia* is similar to *jamma*, however, a mixture of chopped lungs of goat, salt, chilli powder, 'timbur' (*Zanthoxylum* sp.) and fresh animal blood are stuffed into the large intestine of goat, instead of the small intestine, and boiled for 15-20 min. Pricking of a stuffed large intestine is necessary to prevent bursting while boiling. It is dried/smoked for 15-20 days above the kitchen oven (Fig. 14).

Goat meat, goat lungs, chilli, garlic
↓
←Add blood and water
↓
Stuff into the large intestine; boil for 15-20 min
↓
Dry/smoke for 15- 20 days
↓
Arjia

Fig.3: Flow sheet of the traditional method of *Arjia* preparation in Kumaun

Mode of consumption

Arjia is consumed as curry or deep-fried sausage along with the main meal. It is not sold in the local market.

Photo 1. *Chartayshya*

Phot Increase Indent

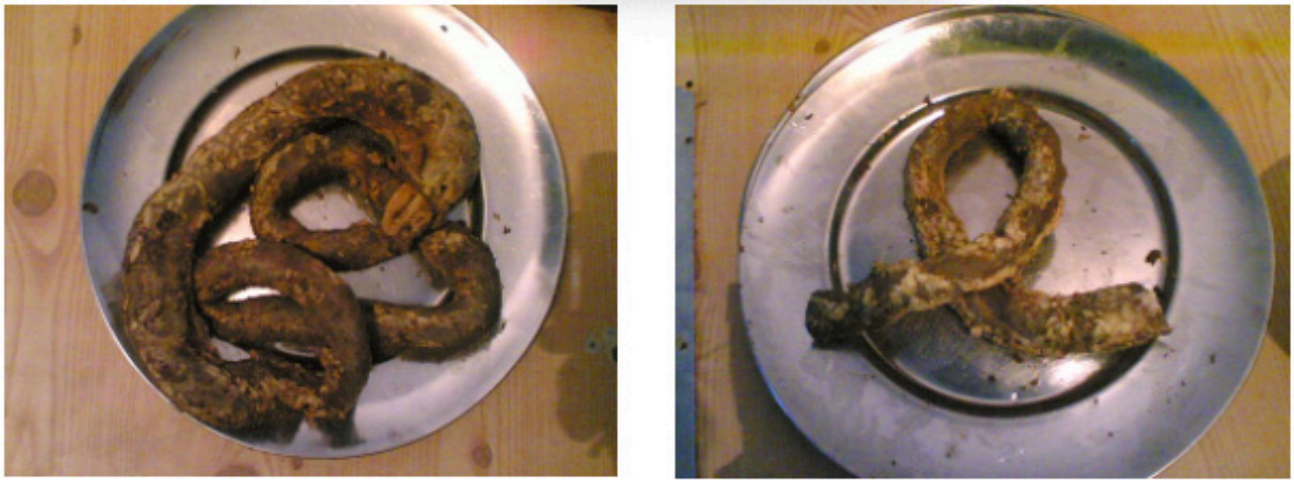

Photo 3. *Arjia*

Photo 1,2,3: Traditional Meat products of Kumaun Himalaya

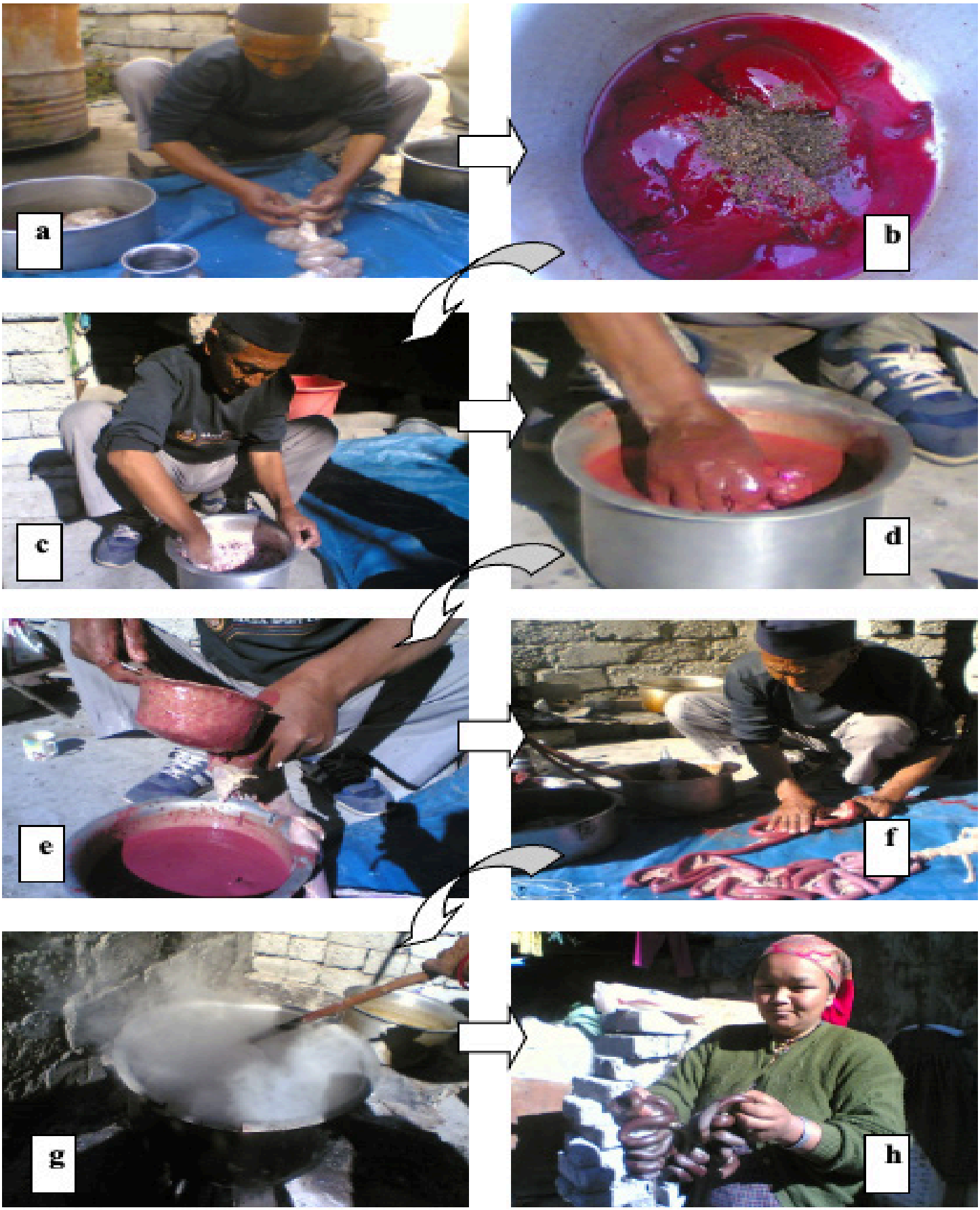

Photo 4: Traditional meat processing in Kumaun Himalaya: (a) Cleaning of the intestine; (b) Seasoning; (c) Mixing and kneading; (d) Semi-liquid ingredients; (e) Stuffing into the intestine; (f) Pressing ingredients into the intestine; (g) Cooking; (h) Freshly prepared *Jamma* (Rai, 2008).

Yeast population

A total of 16 from the Kumaun Himalaya of various types of meat products were collected. All the samples were analyzed for the yeast population (Tables 1). In all the traditionally prepared meat products, the total viable count in all the samples analysed was ranging in between 10^4 – 10^7 cfu/g (Table 1).

Table 1. Population of yeast strains in different meat products collected from different places of Kumaun Himalayan region (Log cfu/g sample)

Place	Product	Region	Place of collection	Yeast (Log cfu/g sample)
Kumaun	*Chartayshya*	Dharch-ula district	Pangu (n = 4)	5.1 ± 0.1
		Dharch-ula district	Rumjum (n = 3)	5.3 ± 0.1
		Dharch-ula district	Marchal (n = 5)	4.2 ± 0.1
		Munsyari district	Sosa (n =5)	6.7 ± 0.1
	Jamma	Dharch-ula district	Dharchula (n = 4)	6.2 ± 0.1
		Dharch-ula district	Sosa (n = 3)	5.5 ± 0.1
		Dharch-ula district	Rumjum (n = 4)	5.4 ± 0.1
		Munsyari district	Sosa (n =4)	6.2 ± 0.1
	Arjia	Dharch-ula district	Dharchula (n = 4)	5.1 ± 0.1
		Dharch-ula district	Rumjum (n = 5)	4.3 ± 0.1
		Munsyari district	Sosa (n =4)	6.8 ± 0.1

n = number of samples.

Data represents the means (± SD) of a number of samples.

Grouping of representatives of yeasts

A sizable number of yeasts were recovered from the meat samples analyzed. A total of 298 yeast strains were isolated from meat products collected from different places in the Kumaun Himalayas. The representative strains of yeast were selected randomly from each grouped strain having similar colony appearance, cell shape, type of mycelia and ascospore for detailed identification (Table 2). Representative strains were assigned the strain code number indicating the sample names and source.

Table 2. Grouping of representative strains of Yeast isolated from meat products of the Kumaun Himalaya

Place	**[a]Product**	**Colony**	**Cell shape**	**Mycelium**	**Ascospore**	**Grouped strains**	**Representative strains**	
							Total No.	**Strains code**
Kumaun	*Chartayshya* (33)	Ss	S-O	Pseudo	Spheroidal	25	2	CD:Y2, CD:Y1
		Ss	O-E	Pseudo	Hat	8	1	CD:Y14
	Jamma (22)	Ss	S-O	Pseudo	Spheroidal	16	1	KJ:Y6
		Ds	O-C	True and Pseudo	Hat	6	1	KJ:L13
	Arjia (28)	Ss	S-O	Pseudo	Spheroidal	18	1	KA:Y1
		Ds	O-C	True and Pseudo	Hat	10	2	KA:Y3, KA:Y7

[a]Total number of isolates in each product is given in parentheses.

All isolates are reproduced by multilateral budding.

Ds: dusty surface, Ss: smooth surface, O-C: oval to cylindrical, O-E: oval to ellipsoidal, S-O: spherical to oval.

Characteristics and identity of yeasts

Following the taxonomic keys of Kreger-van Rij (Kreger-van Rij 1984), and Kurtzman and Fell (Kurtzman and Fell 1998), sugar fermentation and assimilation tests of randomly selected representative strains of yeasts were carried out and identified accordingly.

Strains (CD: Y1, CD: Y2, KJ: Y6, KA: Y1) from *Chartayshya, jamma* and *Arjia* showed smooth surfaced colonies with spheroidal ascospores and fermented glucose weakly were identified as *Debaryomyces hansenii*. Strain (CD: Y14) from *chartayshya* was identified as *Candida famata*. Strains (KJ: Y13) isolated from *jamma* were identified as *Candida albicans* whereas strains (KA: Y7, KA: Y3) from *arjia* were identified as *Candida humicola* based on sugar fermentation and assimilation tests (Table 3).

Prevalence of different yeast strains in meat products from Kumaun Himalayan region.The most dominant yeast recovered in all the samples isolated from meat products of the Kumaun Himalaya was *Debaryomyces* sp. In *Chartayshya*, 75.8 % were *Debaryomyces hansenii* followed by *Candida famata* (24.2 %). Out of 22 strains of yeasts isolated from *jamma*, 72.7 % were *Debaryomyces hansenii* followed by *Candida albicans* comprising 27.3 %. *Debaryomyces hansenii*

Table 3. Phenotypic characteristics of yeast isolated from meat products of the Kumaun Himalaya

Isolate code	Cell Morphology	Cell size (µm)	Mycelium	Ascospore	Nitrate Reduction	Growth at 37°C	Sugars Fermented										Sugars Assimilated															Identity
							Glucose	Galactose	Lactose	Maltose	Raffinose	Sucrose	Starch	Trehalose	Arabinose	Cellobiose	Galactose	Glycerol	Inositol	Lactose	Maltose	Melibiose	Mannitol	Raffinose	Rhamnose	**Sucrose**	**Starch**	**Trehalose**	**Xylose**				
CD:Y1	S - O	l = 3.7 (1.6 - 5.6) b = 2.0 (1.2 - 2.9)	Pseudo	Spheroidal	–	+	+	–	–	–	–	–	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+	+	+		*D. hansenii*
CD:Y2	S - O	l = 4.3 (2.4 - 8.0) b = 3.5 (2.2 - 4.7)	Pseudo	Spheroidal	–	+	+	+	–	–	–	–	–	–	–	+	+	+	–	–	+	–	+	–	–	+	+	+	+		*D. hansenii*
CD:Y14	O - E	l = 7.0 (4.3 -10.1) b = 4.2 (2.7 - 6.3)	Pseudo	Hat	–	–	+	–	–	–	–	+	+	–	+	+	+	+	–	+	+	–	+	+	+	+	–	+	+	+		*C. famata*
KJ:Y6	S - O	l =4.5 (3.5 - 5.2) b =4.0 (3.1 - 4.8)	Pseudo	Spheroidal	–	+	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	+	+	–	–	+	+	+	+		*D. hansenii*
KJ:Y13	O - C	l = 11.1 (2.9 - 18.1) b = 5.6 (2.1 - 8.2)	True, Pseudo	Hat	–	+	+	+	–	+	–	+	–	–	–	–	+	+	–	–	+	–	+	–	–	+	+	+	+		*C. albicans*
KA:Y1	S - O	l = 3.5 (2.6 - 4.5) b = 2.8 (2.0 - 3.5)	Pseudo	Spheroidal	–	+	+	–	–	–	–	–	–	–	–	+	+	+	–	+	+	–	+	+	–	+	+	+	+		*D. hansenii*
KA:Y3	O - C	l = 14.4 (4.8 - 24.0) b = 4.9 (2.5 - 7.2)	True, Pseudo	Hat	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	+	+	+	+	+	–	+	+	+	+		*C. humicola*
KA:Y7	O - C	l = 13.9 (5.6 - 24.7) b = 4.7 (2.3 - 7.4)	True, Pseudo	Hat	–	–	–	–	–	–	–	–	–	–	–	w	+	+	–	+	+	+	+	+	–	+	+	+	+		*C. humicola*

O - E, oval to ellipsoidal; S - O, spherical to oval; o - c, oval to cylindrical; l, length; b, breadth

was dominant in *Arjia* having 64.3 % of the total yeast isolates followed by *Candida humicola* representing 35.7 % (Fig. 4a). The result shows that the yeast belonging to the genus *Debaryomyces* sp. was dominant in almost all the samples analysed (Fig. 4).

(Fig 4. To go here)

Profiles of Yeast isolatesCompiling the identification profiles, the following yeast strains were recovered comprising 2 genera with 4 species: *Debaryomyces hansenii, Candida albicans, C. famata* and *C. humicola* (Table 5).

Table 4. Profile of microorganisms isolated from meat products of Kumaun Himalayan region

Place	Product	Yeast strains
Kumaun	*Chartayshya*	*D. hansenii* and *C. famata*
	Jamma	*D. hansenii* and *C. albicans*
	Arjia	*D. hansenii* and *C. humicola*

Proximate composition

The proximate composition of traditionally prepared meat products collected from the Kumaun Himalayas was analyzed (Table 5). The pH of all these products was between 5.3 and 6.9 with the titrable acidity ranging from 0.3-2.6 %. In all samples analysed, food value ranged from 400.0-634.5 kcal/100g dry matter.

Table 5. Proximate composition of meat products collected from different places in the Kumaun Himalaya

Parameter	Product		
	Chartayshya[1] (n = 6)	*Jamma*[2] (n = 6)	*Arjia*[3] (n = 4)
pH	6.5 ± 0.1	5.5 ± 0.2	6.3 ± 0.1
Titratable acidity % (as lactic acid)	2.1 ± 0.1	1.5 ± 0.1	1.1 ± 0.1
Moisture %	17.4 ± 0.2	65.1 ± 0.6	60.2 ± 0.2
Ash (% DM)	7.8 ± 1.2	5.2 ± 0.5	3.5 ± 0.4
Fat (% DM)	17.0 ± 0.2	4.2 ± 0.5	5.5 ± 0.4
Protein (% DM)	36.6 ± 3.0	7.8 ± 1.0	6.4 ± 0.9
Carbo-hydrate (% DM)	38.6 ± 4.3	82.8 ± 2.0	84.6 ± 1.7
Food value (Kcal /100g DM)	454.0 ± 5.9	400.0 ± 0.7	413.5 ± 1.2

n, total number of samples (n) collected from each source is given in parenthesis.

DM, dry matter.

Data represents the means (± SD) of triplicate of each sample.

[1]Samples collected from Pangu (2), Rumjum (2) and Marchal (2).

[2]Samples collected from Dharchula (2), Sosa (2) and Rumjum (2).

[3]Samples collected from Dharchula (2) and Rumjum (2)

Discussion

A thorough investigation of traditionally prepared meat items was carried out in several Kumaun Himalayan terrains. Information was sought directly from the local people of the respective places on the types of indigenous meat products, they prepare and consume, their traditional method of preparation, culinary skills and socio-economy of the products. They use their indigenous knowledge of the preservation of perishable meats without using starter culture and chemicals. (Tamang et al. 2007). Traditional Chevon meat products like jamma and arjia are created similarly to the Sikkim Himalayan kargyong but with different raw materials. Chartayshya is a dried meat product. Before serving meals, they present cooked chartayshya to the ancestral spirit for worship. (Rai et al. 2009; Oki et al. 2011). In meat products, the presence of *D. hansenii* presents a number of opportunities related to traditional preservation methods, cultural importance, health advantages, enhanced texture, flavour development, natural preservatives, and food security (Yoo et al. 2023). During dry ageing, it has been discovered that *D. hansenii* enhances the flavour and tenderness of inferior beef. The application of *D. hansenii* and other microbial cultures to traditional fermented meat products can enhance their safety and quality while also offering possible health advantages to customers (Rossi et. al. 2023.). To sustain themselves amid the food shortage, ethnic groups may have developed these preservation methods. The history of the Himalayan ethnic people's use of meat products is not well understood, but documentation on their traditional methods of preserving and preparing highly perishable raw meats offers important new insights into this topic. Instead of employing starter cultures or adding sodium nitrites or nitrates, all three meat products are naturally cured. It is reasonable to conclude that the Himalayan people are adept at preserving raw meat from their prowess in making sausage-like goods out of less appetising animal components such scraps, organ meats, fat, blood, etc. Hence, sausage making may be first considered as a use for leftovers of meat of the region.

A sizable number of yeast isolates were recovered from the meat products obtained from Kumaun Hills with TVC ranging between 10^4 – 10^7 cfu/g. This article concentrated on phenotypic characterization and biochemical assays for yeast identification because these methods are equally relevant as they offer complementary insights into the variety, ecology, and applications of yeast in a variety of fields, such as food fermentation and biotechnology. Based on the detailed phenotypic characterization and biochemical analysis, the following yeasts were isolated and identified as *Debaryomyces hansenii, Candida famata, C. albicans and C. humicola*. *Debaryomyces* spp. was the dominant among the yeast isolated from traditionally prepared meat products of the Kumaun Himalaya. Although bacteria are considered to

have a dominant role in meat fermentation, the contribution of yeasts is nevertheless significant (Romano et al. 2006). A diversity of yeast species has been isolated from fermented sausages and cured *hams* produced in different countries with little exception (Martín et al. 2006; Tamang and Fleet, 2009). The findings correlate with the work of (Comi and Cantoni, 1980) that the yeast of the genus *Debaryomyces* predominates on dry sausages. (Simoncini et al. 2007) have reported the occurrence of *D. hansenii, Candida famata* from Italian dry-cured *ham*. *Debaryomyces hansenii* is reported from a traditional South Italian processed sausage along with bacteria (Belloch et. al. 2022).

Rossmanith et al. (1972) observed that the curing colour and flavour could be improved by the addition of selected *Debaryomyces* strains to the sausage mixture. The prevalence of *D. hansenii* in all batches of Greek dry salami suggests its potential use as a starter culture in Greek dry salami (Metaxopoulos et al. 1996). (Coretti, 1977) reported that the contamination of *D. hansenii*, lactobacilli and micrococci gave the best result in the production of fermented sausages. *D. hansenii* has also been reported to possess antimicrobial properties against *Penicillium verrucosum* and *Penicillium nordicum* which is known to grow in dry-cured meat and produce ochratoxin A (OTA). OTA has been associated with carcinogenic, immunotoxic, hepatotoxic, etc. effects. *D. hansenii* may act as a protective agent against moulds producing OTA in cured meats (Andrade et. al. 2014 and Peromingo et. al. 2018).

Isolation, enrichment, purification, characterization, proper identification and authentic nomenclature of microorganisms involved in fermented foods are important aspects of microbial systematic which also ensure the quality control and normalised production of fermented foods (Tamang and Holzapfel, 1999). The isolated, identified and preserved microorganisms from lesser-known meat products may contribute significant information on the unknown microbial gene pool as a genetic resource**.** Understanding the microbial ecology of these items is crucial because it provides insight into the natural processes that support their preservation.

Conclusion

Warm-blooded animals' meat is particularly vulnerable to microbial deterioration. In the Himalayas, drying, smoking, or fermenting perishable meat is an impressive phase in the traditional meat preparation and preservation process. These meat items keep for several months without needing to be refrigerated, so you may eat them whenever you want. Due to their technological or functional characteristics, the dominant yeast strains that were separated from all of the meat products may have a major and complex role in the traditional fermentation process. Additionally, some of these strains may operate as a protective agent against moulds that produce poisonous substances. The prevalence of yeast, *Debaryomyces hansenii* in traditionally cured meat products of Kumaun Himalaya, India as compared with other yeast genera. The noteworthy occurrence of this yeast insinuates its potential as a natural conservant, thereby enhancing the shelf-life of these meat products. This surveillance is harmonious with the observation from studies carried out by other researchers across the globe. This indigenous knowledge is priceless for understanding local food practices and augmenting food security in such resource-constrained environments. The prevalence of yeast genera not only emphasizes its significance in traditional meat preservation but also underscores prospects for further exploration in ameliorating food security strategies. This integrates how to use traditional knowledge to grapple with food security concerns in comparable settings with sustainable food preservation techniques. Additionally, the accidental use of *Debaryomyces hansenii* by the local indigenous population may also be a reflection of the researcher's deliberate manipulation of the species' value in the commercial market to further their own objectives.

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Conflict Of Interest

None.

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