



# Pathogenic and Spoilage Causing Microorganisms Associated with Traditional Seafood Product *Jaadi* Manufactured in Southern and Western Coastal Belt of Sri Lanka

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** The objective of this study was to investigate on microbial related food safety issues and microorganisms associated with the production of Sri Lankan traditional seafood *Jaadi*.

**Place and Duration of Study:** Food Technology Section, Industrial Technology Institute, Colombo, Sri Lanka, between December 2013 to April 2016.

**Methodology:** Seven *Jaadi* processing centers along the Southern and Western coastal belt of Sri Lanka were evaluated for its compliance to food safety, in terms of environment, processing techniques, hygiene and sanitation, physiochemical and microbiological quality of the final product. Samples drawn from all sites were analyzed for its water activity, salt content, pH and microbiological quality. Microorganisms associated with *Jaadi* production were isolated in selective media, followed by phenotypical, biochemical and molecular biological characterization.

**Results:** The pH, water activity and salt content of *Jaadi* samples ranged between 3.60-5.85, 0.73-0.82 and 24.82-40.47%, respectively. Thirteen bacterial strains and one fungal strain detected were strains found to be responsible for human pathogenesis and food spoilage. These include, *Bacillus cereus* strain I (MN726935.1), *Bacillus cereus* strain II (MN901259.1), *Bacillus haikouensis* strain I (MN901262.1), *Bacillus haikouensis* strain II (MN726976.1), *Bacillus licheniformis* (MN726987.1), *Acinetobacter baumannii* (MN901499.1), *Bacillus pumilus* strain I (MN901264.1), *Bacillus pumilus* strain II (MN901263.1), *Bacillus paralicheniformis* (MN901167.1), *Bacillus thuringiensis* strain I (MN901165.1), *Bacillus thuringiensis* strain II (MN901257.1), *Bacillus cereus* (MN901161.1), *Staphylococcus saprophyticus* (MN901156.1) and *Trichoderma longibrachiatum* (MN907169.1). Presence of such organisms clearly proclaims the poor hygienic practices and risks related to food safety in this traditional processing technology.

**Conclusion:** *Jaadi* processing facilities of Southern and Western coastal belt of Sri Lanka needs scientific and technical knowledge to upgrade their processing in order to assure food safety and product quality.

**Keywords:** *Jaadi*; pathogens; spoilage causing microorganisms; traditional seafood product.

## 1. INTRODUCTION

Sri Lanka is a tropical island in the Indian Ocean possessing a territorial sea of 21,500km<sup>2</sup> and having maritime claim of Exclusive Economic Zone up to 370km from the coastline [1]. The entire uninterrupted coastline (1770km), pre-occupied with marine fishing, coastal as well as off-shore, has significant impact on both social and economic status of the populace [2]. Whilst capture fisheries contribute > 95% of the gross national fish production, fish contributes >60% of the mean annual protein intake of the people hence the vital source of protein in their diet [3]. Country's seafood industry has emerged as an excellent exporter of a wide variety of fish and fishery products, prominently to the European Union, USA and Japan, earning foreign exchange worth 200Mn USD in 2020 [4]. Apart from fresh fish, primary-processed fish and other novelties, traditionally processed popular fishery products with cultural and ethnic identities are playing a dynamic role in food supply [5].

Traditional fish processing techniques continue as practices to preserve the excessive harvest. Reduction of water activity ( $a_w$ ) and/or alteration of pH are adopted to increase the shelf-life of processed product. Food ingredients with proven anti-microbial activity (i.e., sea salt, *Garcinia cambogia*, spices, and vinegar), firewood smoking, curing, sun-drying and pickling was practiced to improve organoleptic characteristics of such products. Even though food safety was assured in modern processing techniques i.e., canning, traditionally processing methods still continue with wide acceptance of their habituated taste and aroma [6]. However, to sustain in a

market economy, various malpractices such as use low quality fish, incorrect handling procedures, admixture and adulteration, using non-permitted additives, non-food grade containers, insufficient time period for curing and drying, improper packaging and storage, etc., have posed several food safety issues. Improper food safety procedures followed by traditional fish processors, are a significant challenge to this growing industry [7]. Lack of adequate infrastructure, unawareness on food safety contaminants, i.e., pathogenic microorganisms are major limitations for marketing these products both locally and globally [8].

*Jaadi*, introduced by Portuguese colonialists in the 16<sup>th</sup> century, has gained popularity, acquiring a niche market to locals and tourists, along the Southern and Western coastal belt of the island. *Jaadi* is a cured raw fish immersed in liquid extruded from fish during storage. The liquid by-product called *lunijja* or *Jaadi* water is currently used as a food additive (flavor enhancer) and can further improve into fish sauce [9]. The shelf-life is occasionally further extended by sun-drying. A number of fish varieties including, skipjack tuna (*Katsuwonus pelamis*), sardinella (*Sardinella longiceps*), Spanish mackerel (*Scomberomorus commerson*), striped tuna (*Euthynnus pelamis*), herring (*Amblygaster sirm*), trevally (*Caranx, stellatus*), ribbon fish (*Trichiurus savala*) and Indian mackerel (*Rastrelliger kanagurta*) are used in *Jaadi* production [10]. Salt (NaCl) is used in the osmotic dehydration of the flesh, while acidifying agents such as *Garcinia cambogia* fruit (*Goraka*) containing hydroxy-citric acid and tartaric acid are used to reduce the pH in smoked fish, are used to inhibit the growth of spoilage and pathogenic microorganisms. During

commercial operations of *Jaadi* production contamination with spoilage and pathogenic bacteria, fungi and maggots [9] makes the product unsafe for human consumption. The industry predominantly operates in the Southern and Western coastal belt of the island with little or no data on product quality [11]. The objective of this study was to investigate the microbial related food safety issues associated with the production of traditional seafood *Jaadi* and also identifying and characterizing of pathogenic and spoilage causing microorganisms associated with *Jaadi* manufactured in the Southern and Western coastal belt of the Island.

## 2. MATERIALS AND METHODS

### 2.1 Assessment of Processing Facilities, Sample Collection

Seven well-organized, regularly operating *Jaadi* processing facilities, within 200km stretch along the Southern (No.5) and Western (No.2) coastal belt from Galle to Chilaw, Sri Lanka were selected for the study, based on a pre-survey. Compliance to Good Manufacturing Practices (GMP) and food safety related to environment, raw material handling, processing techniques and food ingredients used in processing by these facilities were documented. *Jaadi* samples (including *Jaadi* water *lunijja*) made from different varieties of fish were collected from processing facilities, labelled as S1, S2, S3(I), S3(II), S3(III), S4(I), S4(II), S5, W1(I), W1(II), W2(I), W2(II) were collected in stomacher bags (Seward, UK), and stored at  $< 4\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and transported to the microbiology laboratory, Industrial Technology Institute (ITI), Sri Lanka within 2 h. The samples were stored at  $-20\text{ }^{\circ}\text{C}$  prior to analysis.

#### 2.1.1 Analysis of physiochemical characteristics and microbiological quality

Composite samples of each variety were analyzed for its water activity (Aqua lab series 3TE Water activity meter, USA), pH (Eutech Instruments pH 510 pH meter, Singapore) and salt content [12]. Samples were also observed for maggot infestation. To determine the microbiological quality, each sample was serially diluted up to  $10^{-12}$  in sterilized saline (0.85% NaCl, w/v) and analyzed for aerobic plate count [13], Yeasts and Mould count [14], Coliform [15] and *Escherichia coli* [16].

#### 2.1.2 Isolation of microorganisms in selective media, phenotypical and biochemical characterization

Serially diluted *Jaadi* samples were spread on plates containing solidified microbiological media; Nutrient Agar, Pseudomonas Agar, Mannitol salt Agar, Bismuth Sulphite Agar, de Man Rogosa and Sharpe Agar and Potato Dextrose Agar (Oxoid, UK). Plates containing bacteriological and fungal media were incubated at  $37\text{ }^{\circ}\text{C}$  for 48 h and  $25\text{ }^{\circ}\text{C}$  for 72 h, respectively. Isolates grown on selective media were purified and their colony morphology (form, size, shape, surface, texture, color, elevation and margin) was recorded. Further phenotypical characterization was carried out by Gram staining. Biochemical characterization (indole, methyl red, voges proskauer, citrate utilization, catalase, oxidase, starch hydrolysis and urease) of the bacterial isolates was also carried out [17]. Morphological identification of fungal isolates was carried out by staining with lactophenol cotton blue [18].

#### 2.2 Molecular Identification of Isolates

Bacterial isolates with diverse phenotypical and biochemical characteristics were further studied through molecular identification [19]. Isolates were inoculated into their respective selective broth medium, incubated at  $37\text{ }^{\circ}\text{C}$  for 18 h, 2 ml of each isolate was centrifuged ( $14000 \times g$  at  $4\text{ }^{\circ}\text{C}$  for 2 min) to obtain the pellets. To extract genomic DNA, pellets were mixed with Tris EDTA buffer ( $200\text{ }\mu\text{l}$ ), re-centrifuged at  $14000 \times g$  at  $4\text{ }^{\circ}\text{C}$  for 2 min and the procedure was repeated once.  $10\text{ }\mu\text{l}$  of proteinase K ( $100\text{ }\mu\text{g}/\mu\text{l}$ , w/v) was added to each bacterial pellet and mixed well. Sodium dodecyl sulfate (10%,  $10\text{ }\mu\text{l}$ ) was added to the pellets, incubated at  $50\text{ }^{\circ}\text{C}$  for 1 h. After incubation, equal volumes of phenol and chloroform ( $110\text{ }\mu\text{l}$ ) were added to pellets, centrifuged at  $14000 \times g$  at  $4\text{ }^{\circ}\text{C}$  for 2 min. Resulting aqueous layer was flooded with ethanol ( $30\text{ }\mu\text{l}$ ,  $\geq 99.8\%$ , v/v) and sodium acetate ( $15\text{ }\mu\text{l}$ , 3 M), vortex for uniform mixing and incubated in ice for 1 h. After incubation, tubes were centrifuged at  $14000 \times g$  at  $4\text{ }^{\circ}\text{C}$  for 5 min, ethanol (1 ml, 70%, v/v) was added to pellets and centrifuged at  $14000 \times g$  at  $4\text{ }^{\circ}\text{C}$  for 5 min. Resulting pellets were dissolved in PCR grade water ( $40\text{ }\mu\text{l}$ ) and stored at  $-20\text{ }^{\circ}\text{C}$ . Extracted genomic DNA ( $5\text{ }\mu\text{l}$ ) was mixed with  $2\text{ }\mu\text{l}$  gel loading dye and run at 60 V for 15 min for qualitative and quantitative analysis (Gel documentation system BIO RAD, UK).

Polymerase Chain Reaction was performed in DNA Engine Tetrad 2 thermal cycler (BIO-RAD, UK) at initial denaturation (95 °C, 5 min) followed by 35 denaturation cycles at 95 °C, 30 sec, annealing at 55 °C, 30 sec, elongation at 72 °C, 1 min and final elongation was done at 72 °C, 10 min. For PCR, universal primers 27F (5'AGAGTTTGGATCCTGGCTCAG3') and 1492R (5'GGTTACCTTGTTACGACTT3') were used. PCR products were purified using multiscreen PCR filter plate (Millipore, USA). 16S ribosomal RNA gene of the purified DNA was sequenced using primers 518 F (5'CCAGCAGCCGCGGTAATACG 3') and 800R (5'TACCAGGGTATCTAATCC 3') at Macrogen, South Korea. Sequence alignment was done using Bioedit sequence alignment editor 7.0.2 (Ibis therapeutics, Carlsbad, CA). Database search for homologous sequences performed using Basic Local Alignment Tool (National Center for Biotechnology Information, USA). Sequences with an identity of 99% or higher to those in databases were assigned to the same species [20]. Partial sequences of 16S rRNA gene of the isolates were deposited at the Genbank of NCBI, USA. Phylogenetic relationship among the isolate was predicted using MEGA7 as per the neighbor-joining [21]. Fungal isolate was identified by amplification of

fungal rRNA genes [22]. Sequence alignment and sequence deposition was done as per the method given for bacterial isolates.

### 3. RESULTS AND DISCUSSION

#### 3.1 General Observations

*Jaadi* is prepared by alternatively placing layers of gutted and cleaned wholesome fish, fillets or slices with a mixture of dry salt crystals: *Goraka* (at a ratio of 3:1) interspersed between the layers, stored in large clay/ceramic pots (*Jaadi barani*) and sealed with airtight lids (*Jaadi muudi*) (Fig. 1). Over the years, with the increase in production capacity, the processors have opted to replace the air-tight clay pots with cement tanks or plastic barrels, and covered it with cloth. The choice for type of fish used in *Jaadi* processing, was dependent on the fishing season and cost of raw materials. Seven varieties of fish, namely, Sail fish (*Istiophorus platypterus*), Frigate tuna (*Auxis thazard*), Mulletts (*Liza sp.*), Spanish mackerel (*Scomberomorus commersoni*), Trevally (*Caranx ignobilis*), Ribbon fish (*Lepturacanthus savalaa*) and White sardinella (*Sardinella albella*) are predominantly used for *Jaadi* processing in the Southern and Western coastal belt.

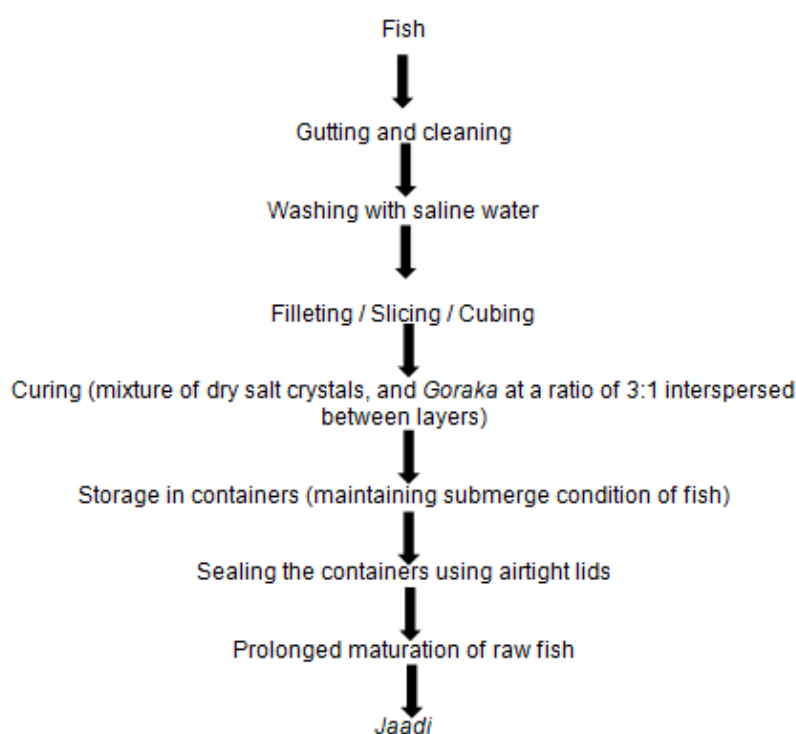


Fig. 1. Traditional *Jaadi* Manufacturing Process

In the Southern coastal belt, only five medium scale processing facilities, with a production capacity of 1-2 MT/batches per day, were found to be well established and regular, in processing, hence was selected for the study. The choice for the types of fish used in processing included; sail fish, mullets, Spanish mackerel, ribbon fish, trevally, frigate tuna and mackerel tuna. Common salt and *G. cambogia* were the only two ingredients used in processing. Curing of fish was done in either concrete tanks (1m<sup>3</sup>) or plastic barrels (200 L), with a time period of 12 weeks. The end point of curing process or maturity was determined by visual examination, for the growth of fungal mycelium covering the liquid surface in the tank/barrel.

In comparison with the Southern coastal belt, in the Western coast, only backyard micro-scale business operations were in place. Out of twelve, only two organized and regularly operating production facilities were found, hence identified for the study. Their choice for types of fish included trevally, white sardinella and Indian mackerel. In their processing technique, two additional ingredients; turmeric (*Curcuma longa*) and curry leaves (*Murraya koenigii*) were added along with common salt and *G. cambogia*. Their curing capacity was limited to 10-20L clay pots/day and was covered air –tight with lid.

Extremely unsatisfactory food safety and sanitary practices including poor personal hygiene were observed in all production facilities, in both areas. All production facilities had deviated from traditional practices i.e., use of fresh fish, reuse of cleaned clay/ceramic jars, and length curing periods and smaller batch operations. Sea water collected from nearby polluted shallow sea was used for cleaning purposes as well as in the manufacturing process, leading to potential risk of chemical and microbiological contamination. In some instances, poorly handled and improperly stored deteriorating fish was also used for processing *Jaadi*. Curing containers were reused without appropriate cleaning. None of the critical quality parameters i.e., pH, water activity, salt content was monitored during processing, resulting in considerable variability in product characteristics, especially texture, observed even within the same batch. The degree of maturity was based on visual examination, whereas duration of the curing process was cut-down in some occasions, to meet the market demand. The finished product had not been tested for its product quality.

### 3.1.1 Physicochemical characteristics and microbiological quality

The pH, water activity and salt content of the *Jaadi* samples collected from the processing facilities in Southern and Western coast given in Table 1. In the final product, the salt content ranged between 28.4 % to 40.5 % and the water content ranged between 0.73 and 0.82. Even though it is thought that salting reduces the water activity, thereby controlling the enzymatic and microbial growth, in both coast belt processing facilities, the conditions were found favorable for growth and survival of microorganisms. The pH of the products had decreased to a range between 3.60 and 5.85. Although high salt content is maintained in the traditional salting process [23], high salt may affect the sensory quality of the product, negatively. This was clearly observed when sensory factors (appearance, odour, flavor and texture) of *jaadi* were assessed using 9-point hedonic scale test.

In *Jaadi* samples obtained from Southern coast, was also found to be infested with maggots and moulds. The presence of Coliforms in all processing facilities indicating lack of GMP, while S2 and S4 was detected with *E. coli* indicating a potential food borne outbreak associated with consumption of the product.

In the Southern coast, Total Plate Count and Yeasts and Mould count of the samples varied between  $4.4 \pm 0.2 \times 10^9$  CFU to  $5.3 \pm 0.1 \times 10^{11}$  CFU and  $1.1 \pm 0.1 \times 10^1$  CFU to  $1.3 \pm 0.4 \times 10^3$  CFU, respectively. In the Western coast, the Total Plate Count and Yeasts and Mould counts varied between  $3.6 \pm 0.1 \times 10^5$  CFU to  $8.3 \pm 0.1 \times 10^7$  CFU and  $0.1 \pm 0.1 \times 10^1$  CFU to  $0.4 \pm 0.0 \times 10^1$  CFU, respectively (Table 2) clearly indicating less contamination in the West coast.

### 3.1.2 Isolation and identification of contaminating microorganisms

Eleven bacterial strains; *Bacillus cereus* strain I (MN726935.1), *Bacillus cereus* strain II (MN901259.1), *Bacillus haikouensis* strain I (MN901262.1), *Bacillus pumilus* strain I (MN901264.1), *Bacillus pumilus* strain II (MN901263.1), *Bacillus thuringiensis* strain I (MN901165.1), *Bacillus thuringiensis* strain II (MN901257.1), *Bacillus paralicheniformis* (MN901167.1), *Acinetobacter baumannii* (MN901499.1), *Staphylococcus saprophyticus* (MN901156.1), *Bacillus licheniformis* (MN726987.1) and a fungal strain *Trichoderma*

*longibrachiatum* (MN907169.1) were isolated from *Jaadi* samples obtained from Southern coast (Table 3). All five processing centers in Southern coast were observed for the presence of fungus having conidiophores in the slight aerial mycelium with cottony pustules [24] identified morphologically and confirmed as *T. longibrachiatum*.

Two bacterial strains; *Bacillus cereus* strain III (MN901161.1) and *Bacillus haikouensis* strain II (MN726976.1) were isolated from *Jaadi* samples obtained from Western coast (W2) (Table 3). The absence of *E. coli* and maggots in *Jaadi* samples of Western coast may be attributed to the addition of ingredients turmeric and curry leaves [25,26]. Results of evolutionary relationships of taxa of the bacterial pathogens isolated from *Jaadi* samples collected from Southern and Western coastal belt are illustrated in Fig. 2.

Among the isolated microorganisms, majority (eleven strains) belong to Genus *Bacillus*. This includes enterotoxin-producing *Bacillus cereus*, a similar association of *B. cereus* with seafood has been earlier reported as vehicle for foodborne illness [27,28]. *B. licheniformis* is progressively recognized as a human pathogen causing serious infections including food poisoning [29]. Pumilacidins synthesizing *B. pumilus* have also reported to associate with food poisoning [30]. *B. cereus* like enterotoxin producing *B. thuringiensis* have been reported to be detected in ready-to-eat food demonstrating the possibility to be the real causative agent of earlier identified certain

*B. cereus* induced foodborne outbreaks [31]. *Bacillus paralicheniformis* reported to associate with food spoilage and occurrences of foodborne gastroenteritis [32]. However, no descriptive information is available on novel facultative anaerobic halotolerant bacterium *Bacillus haikouensis* [33]. The isolate *A. baumannii* is known as a multidrug-resistant biofilm-forming and is also identified as an emerging foodborne pathogen in the community [34]. The isolate, *S. saprophyticus* is a commensal with relatively high salt tolerating organism and one of the frequently detected organisms associated with community-acquired urinary tract infections which has not been reported as causative agent of neither food spoilage nor food borne infections [35].

The evolutionary history was inferred using the Neighbor-Joining method [21]. The optimal tree with the sum of branch length = 0.29495978 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches [36]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [37] and are in the units of the number of base substitutions per site. This analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were total of 659 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [38].

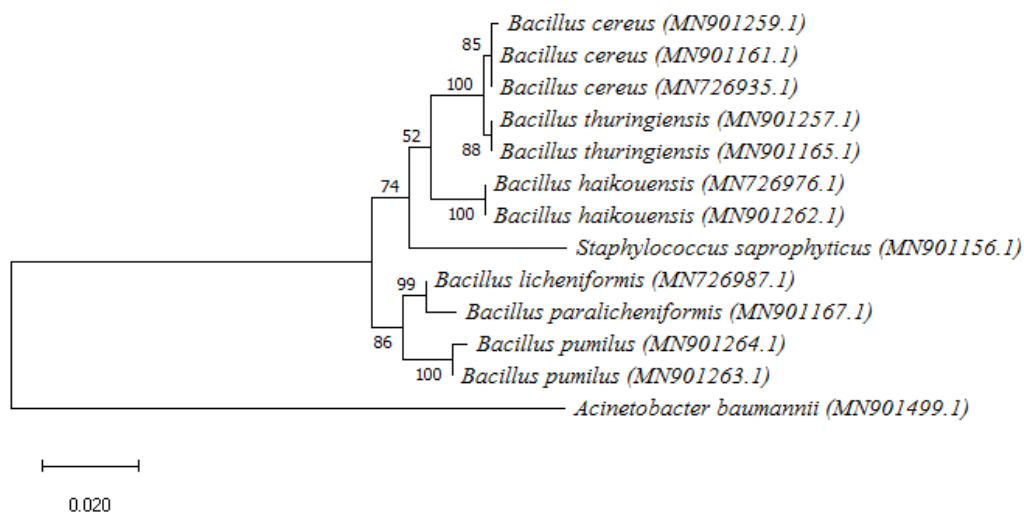


Fig. 2. Evolutionary relationships of taxa

**Table 1. Type of fish used, physicochemical quality and hygienic indications of Jaadi samples collected from processing centers in Western and Southern Coastal Belt**

Sample code	Type of fish used	pH	a <sub>w</sub>	Salt content (%)	Maggot infestation
S1	Mixed Jaadi*	5.75 ± 0.04	0.73 ± 0.0	28.35 ± 0.07	+
S2	Sail fish ( <i>Istiophorus platypterus</i> )	5.54 ± 0.09	0.75 ± 0.0	27.83 ± 0.77	+
S3(I)	Frigate tuna ( <i>Auxis thazard</i> )	4.70 ± 0.06	0.76 ± 0.0	27.43 ± 0.62	+
S3(II)	Mulletts ( <i>Liza sp.</i> )	5.47 ± 0.17	0.75 ± 0.0	28.06 ± 0.22	+
S3(III)	Spanish mackerel ( <i>Scomberomorus commersoni</i> )	4.26 ± 0.01	0.82 ± 0.0	30.81 ± 1.01	+
S4(I)	Frigate tuna ( <i>Auxis thazard</i> )	5.56 ± 0.03	0.75 ± 0.0	24.82 ± 1.29	+
S4(II)	Trevally ( <i>Caranx ignobilis</i> )	5.02 ± 0.02	0.76 ± 0.0	39.78 ± 0.18	+
S5	Ribbon fish ( <i>Lepturacanthus savalaa</i> )	3.60 ± 0.02	0.75 ± 0.0	35.87 ± 0.58	+
W1(I)	Trevally ( <i>Caranx ignobilis</i> )	5.85 ± 0.02	0.74 ± 0.0	40.35 ± 0.95	-
W1(II)	White sardinella ( <i>Sardinella albella</i> )	5.41 ± 0.01	0.73 ± 0.0	36.07 ± 1.79	-
W2(I)	Indian mackerel ( <i>Rastrelliger kanagurta</i> )	4.79 ± 0.02	0.75 ± 0.0	38.17 ± 0.07	-
W2(II)	White sardinella ( <i>Sardinella albella</i> )	4.72 ± 0.03	0.74 ± 0.0	40.47 ± 0.14	-

Data is expressed as mean ± SD, n=6 S (Southern coast) W (Western coast) The letters S1, S2, S3, S4, S5, W1 and W2 refers to Jaadi samples collected from different processing centers in Southern and Western Coastal Belt of Sri Lanka. I, II, III refers to Jaadi prepared using different varieties of fish. + (Present) - (Absent) \* Jaadi prepared using the combination of *I. platypterus*, *A. thazard*, *Liza sp.*, *S. commersoni*, *C. ignobilis* and *L. savalaa*

**Table 2. Microbiological quality of Jaadi samples collected from processing centers in Western and Southern Coastal Belt**

Sample code	TPC log CFU/g	Y&M log CFU/g	Coliform	<i>E. coli</i>
S1	4.5 × 10 <sup>10</sup>	1.3 × 10 <sup>3</sup>	Detected	ND
S2	5.3 × 10 <sup>11</sup>	1.6 × 10 <sup>2</sup>	Detected	Detected
S3(I)	2.9 × 10 <sup>11</sup>	1.2 × 10 <sup>3</sup>	Detected	ND
S3(II)	4.4 × 10 <sup>9</sup>	0.7 × 10 <sup>2</sup>	Detected	ND
S3(III)	6.4 × 10 <sup>10</sup>	1.1 × 10 <sup>2</sup>	Detected	ND
S4(I)	5.1 × 10 <sup>10</sup>	0.6 × 10 <sup>1</sup>	Detected	Detected
S4(II)	4.5 × 10 <sup>11</sup>	0.8 × 10 <sup>1</sup>	Detected	Detected
S5	6.1 × 10 <sup>10</sup>	1.1 × 10 <sup>1</sup>	Detected	ND
W1(I)	8.3 × 10 <sup>7</sup>	0.4 × 10 <sup>1</sup>	Detected	ND
W1(II)	7.9 × 10 <sup>8</sup>	0.1 × 10 <sup>1</sup>	Detected	ND
W2(I)	5.5 × 10 <sup>9</sup>	0.3 × 10 <sup>1</sup>	Detected	ND
W2(II)	3.6 × 10 <sup>5</sup>	0.1 × 10 <sup>1</sup>	Detected	ND

Data is expressed as mean of 6 parallel experiments. S (Southern coast) W (Western coast) The letters S1, S2, S3, S4, S5, W1 and W2 refers to Jaadi samples collected from different processing centers in Southern and Western Coastal Belt of Sri Lanka. I, II, III refers to Jaadi prepared using different varieties of fish. TPC (Total Plate Count), Y&M (Yeasts and Moulds), CFU (Colony Forming Units) ND (Not Detected)

**Table 3 Identification of microorganisms associated with Jaadi**

Sample code	Genus /Species identification	NCBI Genbank accession number
S1	<i>Bacillus cereus</i> strain I	MN726935.1
	<i>Bacillus thuringiensis</i> strain I	MN901165.1
S2	<i>Bacillus paralicheniformis</i> <i>Trichoderma longibrachiatum</i>	MN901167.1
		MN907169.1
S3(I)	<i>Acinetobacter baumannii</i>	MN901499.1
	<i>Staphylococcus saprophyticus</i>	MN901156.1
S3(II)	<i>Bacillus pumilus</i> strain I	MN901264.1
S3(III)	<i>Bacillus haikouensis</i> strain I	MN901262.1
S4(I)	<i>Bacillus cereus</i> strain II	MN901259.1
	<i>Bacillus thuringiensis</i> strain II	MN901257.1
S4(II)	<i>Bacillus licheniformis</i>	MN726987.1
S5	<i>Bacillus pumilus</i> strain II	MN901263.1
W1(I)	-	-
W1(II)	-	-
W2(I)	<i>Bacillus cereus</i> strain III	MN901161.1
W2(II)	<i>Bacillus haikouensis</i> strain II	MN726976.1

S (Southern coast) W (Western coast) The letters S1, S2, S3, S4, S5, W1 and W2 refers to Jaadi samples collected from different processing centers in Southern and Western Coastal Belt of Sri Lanka. I, II, III refers to Jaadi prepared using different varieties of fish.

Presence of such organisms in *Jaadi* clearly proclaims poor hygienic practices and corresponding food safety risk associated with the product. Further, use of non-chlorinated water in the cleaning and production of *Jaadi* as well as use of ingredients that are contaminated with halophilic bacteria and mould i.e., salt might contribute to spoilage [23]. Interestingly, *Salmonella*; frequently detected human pathogen detected in salted fish with water activity ranging 0.94 to 0.99 [39] was not isolated from any of the samples, may indicate that water activity of the *Jaadi* (<0.73), had controlled its growth. Similarly, *Pseudomonas* spp. was not detected in any of the samples, even though reported that *Pseudomonas* strains that could survive in 20% salt marine waters [40], high salt content of *Jaadi* samples might have contributed to inhibiting their growth.

The fungus isolated during this study, *Trichoderma longibrachiatum*, is well-thought-out as an emerging fungal pathogen and is the most recurrent human pathogen within the genus [41]. With this background, experts suggest either ignoring or taking special care during the handling of plant growth promoting *T. longibrachiatum* in biotechnology and agriculture [42].

No added organic acid is used for curing of fish. Acidity (pH > 3.6) of all samples of *Jaadi*, indicate that no sufficient acid concentrations available for action of enzymes in breaking down proteins into soluble particles. This is further confirmed by the non-liquefying of raw material

even after achieving maturity in three months. When cultured in selective media, Lactic Acid Bacteria was not detected in *Jaadi* samples hence did not show any evidence on availability of fermentation inducing microorganisms. These observations and results thereby contradict the hitherto explanation of *Jaadi* as a fermented fish product [5] that contains partially hydrolyzed flesh and organs [9]. We observed *Jaadi* as a naturally cured fish, preserved through osmotic dehydration. However further studies need to be to carry out to confirm this observation.

This study has identified the potential health risks in consuming *Jaadi* with reference to the food borne pathogens and food spoilage causing microorganisms associated with the product. The common belief spread among consumers, that *Jaadi* processing is incomplete without the observation of maggots, is also detrimental. Improper and unhygienic current processing practices also reported to limit the shelf-life of final product [10]. *Jaadi* processing facilities of both Southern and Western coastal belt of Sri Lanka require scientific and technical knowledge to upgrade their processing in order to assure food safety and product quality.

#### 4. CONCLUSION

*Jaadi* is an osmotically dehydrated product and not a fermented product as discussed in literature. The product is contaminated with pathogenic as well as spoilage causing microorganisms therefore unfit for human consumption. Lack of knowledge and awareness



on crucial food safety practices in the *Jaadi* processing facilities of Southern and Western coastal belt required immediate attention. The study recommends implementing systematic food safety programme such as GMP/HACCP to assure product safety and quality. Further, use of correct combination of food additives will upsurge the shelf-life and sensory properties of the final product.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- Northern Province Sustainable Fisheries Development Project: Point Pedro. Ministry of Fisheries and Aquatic Resource Development and Rural Economic Affairs Sri Lanka;2018. Accessed 10 May 2021. Available:Harbor[https://www.adb.org/sites/default/files/project-documents/49325/49325-002-iee-en\\_12.pdf](https://www.adb.org/sites/default/files/project-documents/49325/49325-002-iee-en_12.pdf).
- FAO Fishery Country Profile - Sri Lanka;2006. Accessed 10 May 2021. Available:<http://www.fao.org/fi/oldsite/FCP/en/LKA/profile.htm>.
- National Aquatic Research and Development Agency. Sri Lanka fisheries industry outlook;2018. Accessed 10 May 2021. Available: <http://www.nara.ac.lk/wp-content/uploads/2017/09/fisheries-industry-outlook-2018-converted-Copy.pdf>.
- Export Development Board Sri Lanka. Seafood export performance. 2021. Accessed 10 May 2021 Available:<https://www.srilankabusiness.com/sea-food/seafood-export-performance.html>.
- Mihiranie S, Jayasinghe JK, Jayasinghe CVL, Wanasundara JPD. Indigenous and traditional foods of Sri Lanka. *J. Ethn. Food*;2020. Available:<https://doi.org/10.1186/s42779-020-00075-z>.
- Kose S. Evaluation of Seafood Safety Health Hazards for Traditional Fish Products: Preventive Measures and Monitoring Issues. *Turkish J. Fish.Aquat.Sci.* 2010;10:139-160.
- Mehmetoglu AC. Food safety challenges associated with traditional foods of Turkey. *Food Sci. Technol.* 2018;38:1-12. Available:<http://10.1590/1678-457x.36916>.
- Huss H, Ababouch L, Gram L. Assessment and Management of Seafood Safety and Quality. Food and Agriculture Organization of the United Nations;2003. Accessed 10 January 2020 Available:<http://www.fao.org/3/y4743e/y4743e00.htm>.
- Anuruddi HIGK, Sharma UNS, Lakshman PLN. A study on different production and processing methods of “Jaadi” in southern Sri Lanka. Proceedings of the 7th International Conference of Sabaragamuwa University of Sri Lanka. 2019;211.
- Jayasinghe PS, Bamunuarachchi A, Fonseka TSG. Survey on the Quality of Jaadi Available Sri Lankan Market. *J.Natl.Aquat.Resour.Res.Dev.Agency.* 2000;36:26-34.
- Goonaratne J, Divisekera DMWD, Madage SSK, Indunil RKPN. Isolation of microorganisms associated with Jaadi produced in western coastal belt of Sri Lanka. Proceedings of the 68<sup>th</sup> annual sessions of the Sri Lanka Association for the Advancement of Science. 2012;97.
- AOAC. Official Methods of Analysis. 18th Edition, Association of Official Analytical chemists, Gaithersburg;2007.
- ISO 4833-1. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique;2013.

14. ISO 21527-2. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0,95;2013.
15. ISO 4831. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique;2006.
16. ISO 7251. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *Escherichia coli* — Most probable number technique;2005.
17. Collee JC, Fraser AG, Marmion BP, Simmons A. Mackie and MaCartney Practical Medical Microbiology, 14<sup>th</sup> edition, Church hill Livingstone, London, UK;2006.
18. Leck A. Preparation of lactophenol cotton blue slide mounts. Community Eye Health. 1999.  
Accessed 10 May 2021  
Available:<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1706009/>.
19. Divisekera DMWD, Samarasekera JKRR, Hettiarachchi C, Gooneratne J, Choudhary MI, Gopalakrishnan S and Wahab A. Lactic acid bacteria isolated from fermented flour of finger millet, its probiotic attributes and bioactive properties. Ann Microbiol. 2019;69:79–92.  
Available: <https://doi.org/10.1007/s13213-018-1399-y>.
20. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J. Mol. Biol. 1990;215:403-410.  
Available: [https://10.1016/S0022-2836\(05\)80360-2](https://10.1016/S0022-2836(05)80360-2).
21. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4: 406–425.  
Available:<https://doi.org/10.1093/oxfordjournals.molbev.a040454>.
22. White TJ, Bruns TD, Lee SB, Taylor, JW. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics;1990.  
Accessed 10 May 2021  
Available:<https://www.researchgate.net/publication/223397588>.
23. Oliveira H, Pedro S, Nunes ML, Costa R, Vaz-Pires P. Processing of Salted Cod (*Gadus* spp.): A Review Compr. Rev. Food Sci. Food Saf;2012.  
Available: <http://doi: 10.1111/j.1541-4337.2012.00202.x>.
24. Samuels GJ, Ismaiel A, Mulaw TB, Szakacs G, Druzhinina IS, Kubicek CP, Jaklitsch WM. The Longibrachiatum Clade of *Trichoderma*: a revision with new species. Fungal Divers. 2012; 55:77–108.  
Available: <https://doi.org/10.1007/s13225-012-0152-2>.
25. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. Biomed Res Int; 2014.  
Available:<https://doi.org/10.1155/2014/186864>.
26. Rajendran MP, Pallaiyan BB, Selvaraj N. Chemical composition, antibacterial and antioxidant profile of essential oil from *Murrayakoenigii* (L.) leaves. *Avicenna J Phytomed*. 2014; 4:200-214.
27. Rahmati T, Labbe R. Levels and toxigenicity of *Bacillus cereus* and *Clostridium perfringens* from retail seafood. J Food Prot. 2008;71:1178-85.  
Available: <http://doi: 10.4315/0362-028x-71.6.1178>. PMID: 18592743.
28. Naas H, Aboubaker G, Salah MA, Hanan E, Gammoudi F, Abolghait S, Moawad A, & Barbieri I, Eldaghayes I, Zurghani M. *Bacillus cereus* an emerging public health concern in Libya: Isolation and antibiogram from food of animal origin;2018.  
Available: <http://doi.org/10.13140/RG.2.2.34889.24161>.
29. Haydushka IA, Markova N, Kirina V, Atanassova M. Recurrent sepsis due to *Bacillus licheniformis*. J Glob Infect Dis. 2012;4:82-83.  
Available: <http://doi:10.4103/0974-777X.93768>.
30. From C, Hormazabal V, Granum PE. Food poisoning associated with pumilacidin-producing *Bacillus pumilus* in rice. Int J Food Microbiol. 2007;115:319-24.  
Available: <http://doi:10.1016/j.ijfoodmicro.2006.11.005>. Epub 2007 Jan 13. PMID: 17275116.
31. EFSA Panel on Biological Hazards. Scientific opinion on the risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs;2016.  
Available:<https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2016.4524>.  
Accessed 10 January 2020

32. Salkinoja-Salonen MS, Vuorio R, Andersson MA, Kampfer P, Andersson MC, Honkanen-Buzalski T, Scoging AC. Toxigenic strains of *Bacillus licheniformis* related to food poisoning. *Appl Environ Microbiol.* 1999;65:4637-45. Available:https://10.1128/AEM.65.10.4637-4645.1999. PMID: 10508100; PMCID: PMC91618.
33. Li J, Yang G, Lu Q, Zhao Y, Zhou S. *Bacillus haikouensis* sp. nov., a facultatively anaerobic halotolerant bacterium isolated from a paddy soil. *Antonie Van Leeuwenhoek.* 2014;106:789-794. Available:http://doi: 10.1007/s10482-014-0248-7.
34. Elbehiry A, Marzouk E, Moussa IM, Dawoud TM, Mubarak AS, Al-Sarar D, Alsubki RA, Alhaji JH, Hamada M, Abalkhail A, Hemeg HA, Zahran RN. *Acinetobacterbaumannii* as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic of biofilm formation and phenotypic pattern of antimicrobial resistance. *Saudi J. Biol. Sci.* 2021;28:1158-1166.
35. Ehlers S, Merrill SA. *Staphylococcus Saprophyticus*. StatPearls Publishing. Accessed 10 January 2020 Available:https://www.ncbi.nlm.nih.gov/books/NBK482367.
36. Felsenstein J. Phylogenies and the Comparative Method. *Am. Nat.* 1985;125:1-15
37. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences.* 2004;101:11030-11035. Available:https://doi.org/10.1073/pnas.0404206101.
38. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol.* 2018;35:1547-1549. Available:http://doi:10.1093/molbev/msy096
39. Yoon H, Park BY, Oh MH, Choi KH, Yoon Y. Effect of NaCl on Heat Resistance, Antibiotic Susceptibility, and Caco-2 Cell Invasion of *Salmonella*. *BioMed. Res. Int;*2013. Available:https://doi.org/10.1155/2013/274096.
40. Khan NH, Ahsan M, Taylor WD, Kogure K. Culturability and Survival of Marine, Freshwater and Clinical *Pseudomonas aeruginosa*. *Microbes Environ.* 2010;25:266–274. Available:http://doi:10.1264/jsme2.ME09178.
41. Sandoval-Denis M, Sutton DA, Cano-Lira JF, Gene J, Fothergill AW, Wiederhold NP, Guarro J. Phylogeny of the clinically relevant species of the emerging fungus *Trichoderma* and their antifungal susceptibilities. *J. Clin. Microbiol.* 2014;52:2112–2125. Available:http://doi.org/10.1128/JCM.00429-14.
42. Druzhinina IS, Komon-Zelazowska M, Kredics L, Hatvani L, Antal Z, Belayneh T, Kubicek CP. Alternative reproductive strategies of *Hypocreaorientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable to cause invasive mycoses of humans. *Microbiology.* 2008;54:3447–3459.

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