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Survival of *Salmonella* in Shrimp and Froglegs

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Survival and viability of ten serotypes of *Salmonella* isolated from seafoods at refrigerated and frozen storage temperatures and at different pH values were studied. *Salmonella* sertoypes were inoculated in two concentrations (low, $> 10^3$/g and higher, $> 10^5$/g populations) in shrimp, shrimp homogenates, froglegs and frogleg homogenates. All the serotypes of *Salmonella* survived during refrigerated storage (2 to 5°C). Duration of survival was proportional to the initial concentration of inoculation. Heavily inoculated samples showed the presence of *Salmonella* upto five months in the muscle portions of prawns and froglegs, while survival in respective homogenates were up to three months. Lower populations of *Salmonella* types were destroyed at 60°C by one min heating of the homogenates. With the larger populations, *S. anatum, S. cubana, S. enteritidis* and *S. senftenberg* survived at 60°C by one min heating. Above 80°C none survived by five min heating. All the serotypes tested were sensitive to pH below 5.0 and above 7.5.

Salmonellae are causative agents for food borne diseases and their aetiology is well known. The public health significance of *Salmonellae*, their isolation and enumeration are well documented. (Bowmer, 1965; Dack, 1955; Edwards & Ewing, 1972; Harvey & Phillips, 1961; Jadin *et al.*, 1956; Galton *et al.*, 1964; Gulasekaram *et al.*, 1956; Liston *et al.*, 1971; Marth, 1969; Ray *et al.*, 1971; Shewan & Liston, 1955; Shewan, 1971; Thatcher, 1963). But little information is available on the survival of *Salmonellae* in various environmental conditions of temperature and pH. Such a study would help to elucidate its potential role in foodborne illness associated with the consumption of seafood products. The object of this study is therefore, to collect information on the conditions of survival of the various salmonella serotype under low temperature and varying pH.

Materials and Methods

Ten species of *Salmonella*, namely, *S. typhimurium, S. typhi, S. anatum, S. heidelberg, S. roan, S. weltevreden S. senftenberg, S. saintpaul, S. cubana* and *S. beriely* used in this study were isolated previously from various seafoods (Table 1). Identification of these serotypes to the genus level is carried out with the help of National Salmonella and Escherichia Centre, Kausauli. These stock cultures were maintained in brain heart infusion agar (BHI) or in nutrient agar (NA) slants and preserved at 4°C.

Survival studies were carried out in headless peeled and deveined shrimps *(P. indicus)* and processed froglegs *(Rana hexadactyla)* and their respective homogenates were prepared by blending equal amounts of shrimps or froglegs muscles with sterile isotonic saline solution Before blending, the shrimp and froglegs were treated with 20 ppm chlorine water to eliminate other native flora.

Shrimp, froglegs and their respective homogenates were inoculated with appropriate dilutions of 24 h grown cultures of *Salmonella* to give approximately 2×10^2 and 2×10^5 cells per g. In the case of shrimp and froglegs, inoculations were carried out by injecting the organisms in the required concentrations. Inoculated samples were stored at two temperatures, namely, 2–6°C and-18°C.

Table 1. *Salmonellae isolated from different fish and fishery products*

Species	Source
S. typhimurium	Frozen seer fish, froglegs, shrimps
S. typhi	Frozen froglegs, shrimps
S. saintpaul	Frozen froglegs, shrimps
S. cubana	Frozen shrimps, froglegs, fishmeal
S. roan	Frozen froglegs, prawns
S. heidelberg	Frozen prawns
S. senftenberg	Prawnshell, fishmeal, raw shrimp
S. weltevreden	Frozen cuttle fish, raw shrimp
S. beriely	Prawnshell waste
S. anatum	Frozen froglegs

The initial levels of the organisms were determined by using a non-inhibitory medium of tryptone glucose yeast extract (TGE) agar with appropriate dilution techniques. Further isolation and enumeration were carried out by pre-encirchment in either lactose broth or peptone broth. These broths were incubated for 24 h at 37°C, and further selectively enriched in selinite cystine and tetrathionate broths for 24 h at 37°C.

Table 2. *Effect of refrigerated storage (2–6°C) on Salmonella strains in shrimp and froglegs*

	Shrimp		Days								Froglegs		Days							
			1		3		7		10				1		3		7		10	
	S	Sh	S	Sh	S	Sh	S	Sh	S	Sh	F	Fh	F	Fh	F	Fh	F	Fh	F	Fh
S. typhi	4.2×10^3	3.9×10^3	+	+	+	+	+	+	+	+	1.9×10^8	4.1×10^3	+	+	+	+	+	+	+	+
	1.9×10^6	1.1×10^6	+	+	+	+	+	+	+	+	1.7×10^6	3.4×10^6	+	+	+	+	+	+	+	+
S. typhimurium	3.7×10^3	2.4×10^3	+	+	+	+	+	+	+	+	2.1×10^3	4.1×10^3	+	+	+	+	+	+	+	+
	2.7×10^6	3.6×10^6	+	+	+	+	+	+	+	+	4.2×10^6	2.5×10^6	+	+	+	+	+	+	+	+
S. anatum	5.2×10^3	2.4×10^3	+	+	+	+	+	+	+	+	2.1×10^3	4.2×10^3	+	+	+	+	+	+	+	+
	5.1×10^6	2.1×10^6	+	+	+	+	+	+	+	+	3.1×10^6	1.1×10^6	+	+	+	+	+	+	+	+
S. saintpaul	1.6×10^3	3.4×10^3	+	+	+	+	+	+	+	+	4.6×10^3	2.4×10^3	+	+	+	+	+	+	+	+
	5.2×10^6	2.4×10^6	+	+	+	+	+	+	+	+	3.6×10^6	4.2×10^6	+	+	+	+	+	+	+	+
S. cubana	3.2×10^3	2.4×10^3	+	+	+	+	+	+	+	+	1.6×10^3	5.1×10^3	+	+	+	+	+	+	+	+
	2.3×10^6	3.2×10^6	+	+	+	+	+	+	+	+	5.2×10^6	3.5×10^6	+	+	+	+	+	+	+	+
S. roan	2.4×10^3	5.2×10^3	+	+	+	+	+	+	+	+	2.8×10^3	4.2×10^3	+	+	+	+	+	+	+	+
	1.6×10^6	2.7×10^6	+	+	+	+	+	+	+	+	1.8×10^6	3.9×10^6	+	+	+	+	+	+	+	+
S. heidelberg	4.1×10^3	5.3×10^3	+	+	+	+	+	+	+	+	2.9×10^3	5.8×10^3	+	+	+	+	+	+	+	+
	5.7×10^6	3.6×10^6	+	+	+	+	+	+	+	+	4.1×10^6	2.7×10^6	+	+	+	+	+	+	+	+
S. senftenberg	3.2×10^3	5.2×10^3	+	+	+	+	+	+	+	+	5.3×10^3	3.3×10^3	+	+	+	+	+	+	+	+
	4.1×10^6	3.9×10^6	+	+	+	+	+	+	+	+	3.8×10^6	2.9×10^6	+	+	+	+	+	+	+	+
S. weltevreden	$21. \times 10^3$	4.1×10^3	+	+	+	+	+	+	+	+	1.9×10^3	4.2×10^3	+	+	+	+	+	+	+	+
	4.1×10^6	3.2×10^6	+	+	+	+	+	+	+	+	3.8×10^6	4.8×10^6	+	+	+	+	+	+	+	+
S. berlely	3.1×10^6	3.7×10^6	+	+	+	+	+	+	+	+	4.1×10^6	3.2×10^6	+	+	+	+	+	+	+	+

+ presence of salmonella; – absence of salmonella; S – shrimp; Sh – shrimp homogenate; F– froglegs; Fh – frogleg homogenate

Table 3. *Effect of freezing and cold storage at - 18°C on Salmonella serotypes in shrimp and froglegs*

	Shrimp		Days										Froglegs		Days							
			30		60		90		120		150				30		90		120		150	
	S	Sh	S	Sh	S	Sh	S	Sh	S	Sh	S	Sh	F	Ff	F	Fh	F	Fh	F	Fh	F	Fh
S. tiphi	2.1×10^3	3.7×10^3	+	+	+	+	+	+	+	−	+	−	2.2×10^3	4.7×10^3	+	+	+	+	+	−	+	−
	3.2×10^6	5.1×10^6	+	+	+	+	+	+	+	+	+	−	3.7×10^6	2.9×10^6	+	+	+	+	+	+	+	−
S. typhimurium	4.2×10^3	3.4×10^3	+	+	+	+	+	+	+	−	−	−	3.9×10^3	3.6×10^3	+	+	+	+	+	−	−	−
	5.1×10^6	2.4×10^6	+	+	+	+	−	+	−	−	+	−	4.5×10^6	2.7×10^6	+	+	+	+	+	+	−	−
S. anatum	4.2×10^3	4.5×10^3	+	+	+	+	+	+	+	−	−	−	2.9×10^3	5.1×10^3	+	+	+	+	+	−	+	−
	3.8×10^6	2.6×10^6	+	+	+	+	+	−	−	−	+	−	4.1×10^6	2.6×10^6	+	+	+	+	+	−	+	−
S. saintpaul	2.8×10^3	4.3×10^3	+	+	+	+	+	−	−	−	−	−	5.2×10^3	2.8×10^3	+	+	+	+	+	−	−	−
	4.1×10^6	2.9×10^6	+	+	+	+	+	−	−	−	−	−	3.4×10^6	4.1×10^6	+	+	+	+	−	−	−	−
S. cubana	2.9×10^3	6.1×10^3	+	+	+	+	−	−	−	−	−	−	5.2×10^3	7.1×10^3	+	+	+	+	−	−	−	−
	4.3×10^6	4.6×10^6	+	+	+	+	+	+	−	−	−	−	6.1×10^6	2.9×10^6	+	+	+	+	+	−	−	−
S. roan	5.9×10^3	6.1×10^3	+	+	+	+	−	−	−	−	−	−	8.3×10^3	3.9×10^3	+	+	+	+	+	−	−	−
	2.8×10^6	4.6×10^6	+	+	+	+	+	+	−	−	−	−	5.8×10^6	7.2×10^6	+	+	+	+	−	−	−	−
S. heidelberg	4.1×10^3	6.1×10^3	+	+	+	+	+	+	+	+	−	−	6.1×10^3	6.9×10^3	+	+	+	+	−	−	−	−
	7.1×10^6	3.9×10^6	+	+	+	+	+	+	−	−	−	−	6.3×10^6	8.1×10^6	+	+	+	+	−	−	+	−
S. senftenberg	3.2×10^3	5.1×10^3	+	+	+	+	+	+	−	−	−	−	3.1×10^3	5.8×10^3	+	+	−	−	−	−	−	−
	4.1×10^6	3.2×10^6	+	+	+	+	−	−	+	−	−	−	8.1×10^6	6.2×10^6	+	+	+	+	−	−	−	−
S weltevreden	3.8×10^3	6.2×10^3	+	+	+	+	+	−	−	−	−	−	4.3×10^3	7.2×10^3	+	+	+	+	+	−	−	−
	8.1×10^6	3.8×10^6	+	+	+	+	+	+	−	−	−	−	3.5×10^6	6.2×10^6	+	+	+	+	−	−	−	−
S. berlely	3.9×10^3	8.4×10^3	+	+	+	+	−	−	+	−	−	−	7.1×10^3	2.9×10^3	+	+	+	+	−	−	−	−
	3.8×10^6	2.9×10^6	+	+	+	+	+	+	−	−	+	−	8.2×10^6	3.9×10^6	+	+	+	+	−	−	−	−

+ presence of *Salmonella*; − absence of *Salmonella*; S - shrimp; Sh – shrimp homogenates; F – froglegs; Ff – frogleg homogenate

Table 4. *Sensitivity of Salmonella organisms exposed to higher temperature*

Organisms	Shrimp homogenates Temperature							Froglegs homogenates Temperature						
		60°C		80°C		90°C			60°C		80°C		90°C	
	Initial counts	1 min	5 min	1 min	5 min	1 min	5 min	Initial counts	1 min	5 min	1 min	5 min	1 min	5 min
S. typhi	3.4×10^2	—	—	—	—	—	—	2.3×10^2	—	—	—	—	—	—
	5.6×10^5	—	—	—	—	—	—	3.8×10^5	—	—	—	—	—	—
S. typhimurium	2.7×10^2	+	—	—	—	—	—	2.4×10^2	—	—	—	—	—	—
	1.9×10^5	+	—	—	—	—	—	4.9×10^5	+	—	—	—	—	—
S. anatum	3.6×10^2	—	—	—	—	—	—	2.5×10^2	—	—	—	—	—	—
	2.9×10^5	+	—	—	—	—	—	4.7×10^5	+	—	—	—	—	—
S. saintpaul	3.1×10^3	—	—	—	—	—	—	2.9×10^2	—	—	—	—	—	—
	3.9×10^5	+	—	—	—	—	—	3.6×10^5	+	—	—	—	—	—
S. cubana	2.1×10^2	—	—	—	—	—	—	2.9×10^2	—	—	—	—	—	—
	4.6×10^5	—	—	—	—	—	—	4.5×10^5	—	—	—	—	—	—
S. roan	1.9×10^2	—	—	—	—	—	—	3.3×10^2	—	—	—	—	—	—
	3.7×10^5	—	—	—	—	—	—	4.2×10^5	—	—	—	—	—	—
S. heidelberg	3.2×10^2	—	—	—	—	—	—	2.6×10^2	+	—	—	—	—	—
	4.9×10^5	+	—	—	—	—	—	3.5×10^2	+	—	—	—	—	—
S. senftenberg	3.0×10^2	+	—	—	—	—	—	1.8×10^2	—	—	—	—	—	—
	4.4×10^5	+	—	—	—	—	—	2.1×10^5	+	—	—	—	—	—
S. weltevreden	1.5×10^2	—	—	—	—	—	—	4.9×10^2	—	—	—	—	—	—
	3.6×10^5	—	—	—	—	—	—	8.6×10^5	+	—	—	—	—	—
S. beriely	4.2×10^2	—	—	—	—	—	—	1.6×10^2	—	—	—	—	—	—
	3.9×10^5	—	—	—	—	—	—	1.9×10^5	—	—	—	—	—	—

+ presence of *Salmonella*; — absence of *Salmonella*

Table 5. *Loss of viability of Salmonella strains at different pH levels in shrimp and frogleg homogenates*

Organisms	Substrates	pH									
		4.0	4.5	5.0	5.5	6.0	7.0	7.5	8.0	8.5	9.0
S. typhi	Sh	—	—	—	+	+	+	—	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. typhimurium	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. anatum	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. saintpaul	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. cubana	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. roan	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. heidelberg	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. senftenberg	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. weltevreden	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—
S. beriely	Sh	—	—	—	+	+	+	+	—	—	—
	Fh	—	—	—	+	+	+	+	—	—	—

+presence of salmonella; —loss of viability; Sh - shrimp homogenates; Fh – frogleg homogenates.
Inoculum level ranged between 10^3 to 10^6 per g of homogenate. pH is adjusted with either HCl or NaOH (1 N)
Incubation at room temperature (28 ± 2°C) for 24 h

Brilliant green agar and bismuth sulphite agar were used as selective media. Tentative identification of *Salmonella* was made by biochemical screening and further confirmed by serological agglutination.

At elevated temperatures like 60, 80 and 90°C the resistance of different *Salmonella* serotypes were studied only in shrimp and frogleg homogenates. The test samples were placed in a water bath maintained at 60, 80 and 90°C. When the equillibrium was reached a known cell concentration of the young culture was added. Isolation of viable salmonellae after heating for one minute and five minutes were enumerated innoninhibitory medium (TGE) and confirmed further by agglutination.

Shrimp and frogleg homogenates were adjusted to various pH ranging from 2 to 9 using either HCl or NaOH (1N). After the stabilization of pH in the sample homogenates different serotypes of *Salmonella* were inoculated to the approximate cell concentration of 2×10^2 and 2×10^5 per g. After incubation for 24 h at room temperature (28 ± 2°C), the homogenates were sampled and enumerated for the survival of *Salmonellae*.

Results and Discussion

Table 2 shows the effect of refrigerated storage on the survival of salmonella serotypes studied. Shrimp, froglegs and their homogenates showed the presence of viable serotypes of *Salmonella* even after ten days storage. Earlier studies of Prescott & Tanner (1938) have indicated the survival of salmonellae even after prolonged storage below 6°C. Angellotti *et al.* (1959) concluded that *Salmonella* can grow and survive at refrigerated temperatures.

The effect of freezing and storage at -18°C on the viability of Salmonellae shows that it is considerably reduced during 4 to 5 months storage (Table 3) in the case of shrimp and in frogleg, survival being more in the homogenates of respective samples. After 150 days' storage at-18°C no viable cell of *Salmonella* was found in shrimp and frogleg and their respective homogenates. In general, the effect of freezing (Luyet&Gehinio, 1940,) temperature and duration of storage (Wiezer & Ostreud, 1945) initial number, types and stages of growth phases of bacteria involved (Hess, 1934; 1950; Record & Taylor, 1953), and physical protection offered by the food itself or its components (Haines, 1938; Hess, 1934; Smith et al., 1951; Squieres & Hartsell, 1955; Woodburn & Strong, 1960) play a vital role. Seafoods protect these organisms against lethal effects of freezing (Raj & Liston, 1961a). Our studies on the survival of *Salmonellae* species strengthen these conclusions.

The difference in the survival period of *Salmonella* strains in shrimp and frogleg may be due to the differences in substrates and temperature of freezing and storage time. Raj & Liston (1961b) studied the survival of *S. typhimurium* in seafoods. He found that the *Salmonella* organisms survived up to 393 days. However, the results of the present investigation showed that the viability of the various serotypes studied was lost after 150 days of storage in shrimp and frogleg as well as in their respective homogenates at -18°C.

Table 4 summarizes the survival of *Salmonella* strains exposed to 60, 80 and 90°C. At 80°C and above the serotypes in all concentrations were killed in one minute heating. 60°C was also shown to be lethel at lower concentrations of organisms. However at the higher concentration of 5×10^8 per g *S. weltevreden, S. heidelberg, S. senftenberg, S. typhimurium, S. saint-paul* and *S. anatum* were found viable after heating for one min. None of the *Salmonellae* species studied, was found viable after 5 min heating.

Generally *Salmonella* species rapidly proliferate at pH 6.5 to 7.5. Table 5 shows that at pH 4.0, 4.5, 5.0, 8.5 and 9.0 the *Salmonella* strains lost their viability in both the homogenates of shrimp and frogleg. The test organisms were sensitive to acidic pH and alkaline pH. Earlier studies by Dernby (1921) confirmed that minimum pH at which *S. typhosa, S. paratyphi* and *S. paratyphi* B grew, were 6.2, 4.5 and 4.5 respectively. Later Stokes & Osborue (1955) stated the inability of *Salmonella* to grow below pH 5.0. Prost & Reimann (1967) demonstrated that pH below 4.5 had a killing effect. In contrast to these observations Chung & Goepfert (1970) confirmed the growth of *S. anatum, S. senftenberg* and *S. tennesse* at pH 4.05. The exact mechanism by which these lower and higher pH exert lethal effects is not understood well, although many investigators are of the opinion that undissociated molecules are responsible for toxicity (Winslow & Lockridge, 1906; Levine & Fellers, 1940; Bergium, 1941). Later Hentages (1967) attributed this to hydrogen ion concentration.

The *Salmonellae* tested in this study did not sruvive under high acidity and alkalinity for a period of 120 min and above. These serotypes evidenced an optimum pH range of 4.5 to 8.0 with regard to survival. Our results are in agreement with the findings of Chung & Goepfert (1970). pH below 4.5 and above 8.0 proved lethal to all the serotypes of *Salmonella* studied.

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