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THE EFFECT OF DIFFERENT TREATMENTS ON THE SHELF LIFE AND BACTERIAL FLORA OF VACUUM PACKED TRENCHED SARDINES *AMBLYGASTER SIRM*

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The shelflife of vacuum packed trenched sardines (*Amblygaster sirm*) subjected to a potassium sorbate dip, irradiation and salting was determined. Fish packs treated with a 2% potassium sorbate dip had a shelflife of 50 days with a 29 day lag period in bacterial growth while untreated fish had a shelf life of 20 days with no lag period. Irradiated fish (200 krad) had a shelf life of 26 days, with Micrococci being unaffected by irradiation. Similarly *Pseudomonas* spp. were dominant in sorbate treated fish. The spoilage flora in all packs were dominant in Streptococci.

INTRODUCTION

In Sri Lanka, as in most developing countries, little attention is directed towards post-harvest aspects in the fishery trade. A majority of the catch is transported and retailed inadequetly iced or un-iced. Thus the fish finally reaches the consumer in a fairly poor condition.

Packaging protects food products from external contamination and contributes towards extending the shelf life of the product. The use of various pretreatments along with packaging could aid in improving the keeping quality of fish.

Vacuum packaging is commonly used for the packaging of various food products. Huss (1977) describes the advantages of vacuum packaging with the ultimate objective of obtaining a washed, cleaned, gutted, 'kitchen ready', safe product. Hardy & Hobbs (1968) described vacuum packaging as a means of improving the keeping quality of fish at chilled and frozen temperatures. Gorczyca (1983) has found that pretreatment with potassium sorbate extended the shelf life of vacuum packed fish fillets stored at 4°C.

The present study was carried out to evaluate the effect of several pretreatments on the shelf life of vacuum packed trenched sardines stored at 4°C.

MATERIALS & METHODS

Handling of Fish

Trenched sardines (*Amblygaster sirm*, Hurulla) were caught by gill netting off the coast of Negombo, Sri Lanka. Fish samples were obtained from the landing site. The fish were transported iced in insulated boxes to the laboratory. On arrival the fish were deheaded, enviserated, washed and dipped in saturated brine solution for 7 minutes.

Pretreatment of Fish

The dressed fish were divided into 4 batches and each batch was individually treated as listed below.

One batch was dipped in a 2% potassium sorbate solution for 15 minutes and drained well.

The second batch was vacuum packed, stored in ice and irradiated by a Co^{60} irradiator resulting in a dose of 200 krad.

The third batch was allowed to remain in a saturated salt solution for 24 hours, washed lightly, drained and vacuum packed.

The fourth batch was vacuum packed in an untreated condition.

Fish Packaging and Storage

Fish (200g) were packed in nylon/poly propylene transparent laminates with O_2 permeabilities of $0.03g/m^{-2}$ 24 h^{-1} atm^{-1} (20°C). Sealing was carried out in Bibun (Bibun Company, Japan) vacuum sealer.

The packs were stored at 4°C in an incubator where a constant temperature was maintained throughout the study. Microbiological, chemical and visual analysis was carried out at suitable regular intervals.

Microbiological analysis

Total bacterial counts (5°C, 30°C Aerobic and 35°C Anaerobic incubation) were estimated by plating (on nutrient agar, NA) ten fold serial dilutions of fish samples prepared by homogenizing 50g of fish obtained through the body region in 450 ml of sterile peptone (0.1%) diluent. Surface and pour plate techniques were employed in order to obtain total viable counts. The number of major representative colonies obtained at 30°C (triplicate sampling) were recorded, Colonies of each type were restreaked on NA to check for purity and then stocked on NA slants and stored at 4°C. Isolates from salt saturated samples were plated and stocked on seawater agar.

Biochemical characterization of isolates were carried out according to the schemes of Shewan, Hobbs and Hodgkiss (1960); Cowan and Steel (1974) and Lee and Pfeifer (1975). All isolates were identified upto generic level.

Anaerobic counts were obtained from plates incubated in anaerobic jars, using BBL gas generating kits.

Chemical Analysis

Fish fillets (50g) were minced to a smooth paste of which 25g was removed for determination of total volatile nitrogen (TVN Anon 1977) and Trimethylamine (TMA, Anon 1977). pH was measured from fish/distilled water (ratio 1 : 3) mascerates by using a Radiometer 26 pH.

Organoleptic Evaluation

Vacuum packs were examined visually for gas and overall appearance, and on opening for odour (fresh, seaweedy, neutral, ammoniacal, putrid, sulphidy, fruity), texture, (firm, soft) and other characteristics (digestion and slime) by a trained panel (2-4 members).

RESULTS

A shelflife of 50 days was obtained for fish treated with a 2% potassium sorbate dip, while irradiated and untreated fish were acceptable for upto 26 & 22 days respectively. Salt saturated packs did not show signs of spoilage for upto 57 for days, (Table 1).

Sorbate treated fish were rejected due to the development of ammoniacal odours. An extended lag (29 days) was also observed from the 21st to the 50th day, (Figure 2) during which time a shiny appearance and a neutral odour was retained. TVN values ranged from a minimum of 13.6 to 74.4 mg at rejection, (Table 2).

TABLE 1.

SHELF LIFE OF VACUUM PACKED *Amblygaster sirm* STORED AT 4°C

Treatment	*Shelf life (days)*	*Lag period (days)*	* *Criteria for rejection*		
			Nature/Pack	*Odour*	*Texture*
Untreated	22	None	No gas	Acidic	Softening
Potassium sorbate dip (2%)	50	29	No gas	Slight NH_3	Soft
Irradiated (200 krad)	26	14	No gas	Fruity	Soft/ No digestion
Salted (5% Nacl)	57 * *	None * * *	—	—	—

* Evaluated by 2 – 4 panelists * * Not rejected * * * Reduction in total counts.

Total aerobic bacterial counts (30°C and 5°C incubation) and total anaerobic bacterial counts in treated and untreated vacuum packs were more or less in the same range throughout the study, (Figrues 1, 2, 3 & 4). The similarity in counts of the 30°C aerobic and 35°C anaerobic counts may indicate the probable facultative nature of the flora in the packs. Initial bacterial counts ranged from 10^2-10^4 cfu/g in all treatments while at rejection levels they were around 10^6 cfu/g, excepting irradiated packs, which were rejected at 10^7 cfu/g. Salt saturated packs did not develop any off odours, and had very low bacterial counts.

Irradiated packs were rejected due to the development of fruity odours. TVN at rejection was 40.8 mg, (Table 2). A lag (14 days) in bacterial counts was observed from the 12th day to the 26th day, (Figure 3). Unlike in other treatments the overall appearance was shiny and firm upto the point of rejection, (Table 1).

Untreated samples were acceptable upto 22 days with TVN values at rejection being 45.8 mg, (Table 2). However no lag in total bacterial counts was observed as in the other treatments. The development of acidic odours were the main criteria for rejection.

TABLE 2.

CHANGE IN TOTAL VOLATILE NITROGEN (TVN) TRIMETHYLAMINE (TMA) AND pH DURING STORAGE AT 4°C

Treatment		*TVN** *mg/100g flesh*	*TMA** *mg/100g flesh*	*pH**
Untreated	1st day	17.6	8.2	6.8
	15th day	25.7	6.0	6.2
	22nd day	45.8	5.1	6.2
Potassium sorbate (2%) dip	1st day	13.4	2.8	6.3
	22nd day	45.1	2.9	6.4
	50th day	74.4	—	6.4
Irradiation (200 krad)	1st day	18.6	2.1	6.0
	12th day	36.7	2.3	6.7
	26th day	40.8	5.5	6.7
Salted	1st day	20.3	7.0	6.2
	22nd day	24.2	2.9	6.1
	50th day	35.5	—	6.0

* Average of duplicate samples.

Composition of initial bacterial flora isolated from untreated fish packs stored at 4°C consisted of a mixture of Enterobacteriaceae (*Enterobacter* species identified on API), *Bacillus* species and *Pseudomonas* species. The flora of sorbate treated packs were dominated by *Pseudomonas* spp. (89%), while flora of vacuum packed irradiated fish was entirely Micrococci (100%), (Table 3). Salt saturated packs were dominated by *Vibrio* species. On spoilage or rejection, flora of irradiated, untreated and sorbate treated fish were dominant in Streptococci, while salted packs had a dominant flora of Micrococci and Streptococci, (Table 3).

TABLE 3.

DOMINANT MICROFLORA ISOLATED FROM VACUUM PACKED *Amblygaster sirm* AT INITIAL AND FINAL STAGES OF STORAGE (4°C)

Treatment	*Distribution of Microflora (6 days)*	(%)	*Distribution of Microflora (at spoilage)*	(%)
* Untreated	Enterobacteriaceae	67.0	Streptococci	99.5
	Bacillus		*Pseudomonas*	0.5
	Pseudomonas	33.0		
* Potassium sorbate 2% dip	*Pseudomonas*	89.4	Streptococci	96.0
	Bacillus	9.7	Staphylococci	
	Enterobacteriaceae	1.0	*Pseudomonas*	4.0
* Irradiation 200 krad	Micrococci	100	Streptococci	87.8
			Pseudomonas	12.2
			Vibrionaceae	
* * Salted	Vibrionaceae	100	Micrococci	76.0
			Streptococci	24.0

* Obtained by triplicate sampling and averaged major representative colonies on NA plates incubated at 30°C.

* * Similarly obtained by plating on seawater agar plates.

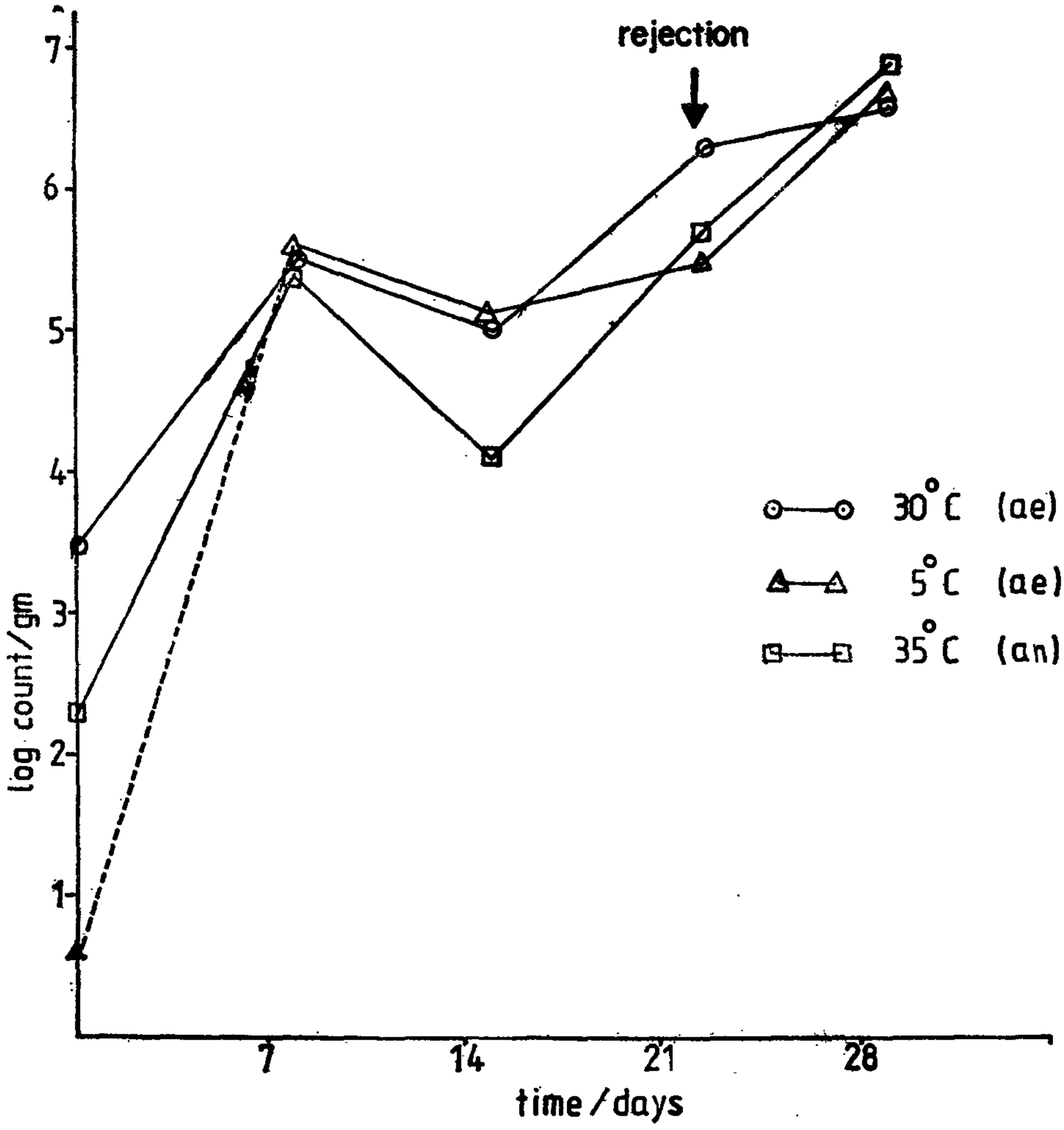

Fig. 1 : Change in total aerobic (ae) and anaerobic (an) counts at incubation temperatures of 30°C. 5°C. (ae) and 35°C (an) obtained from vacuum packed *Amblygaster sirm* sotred at 4°C.

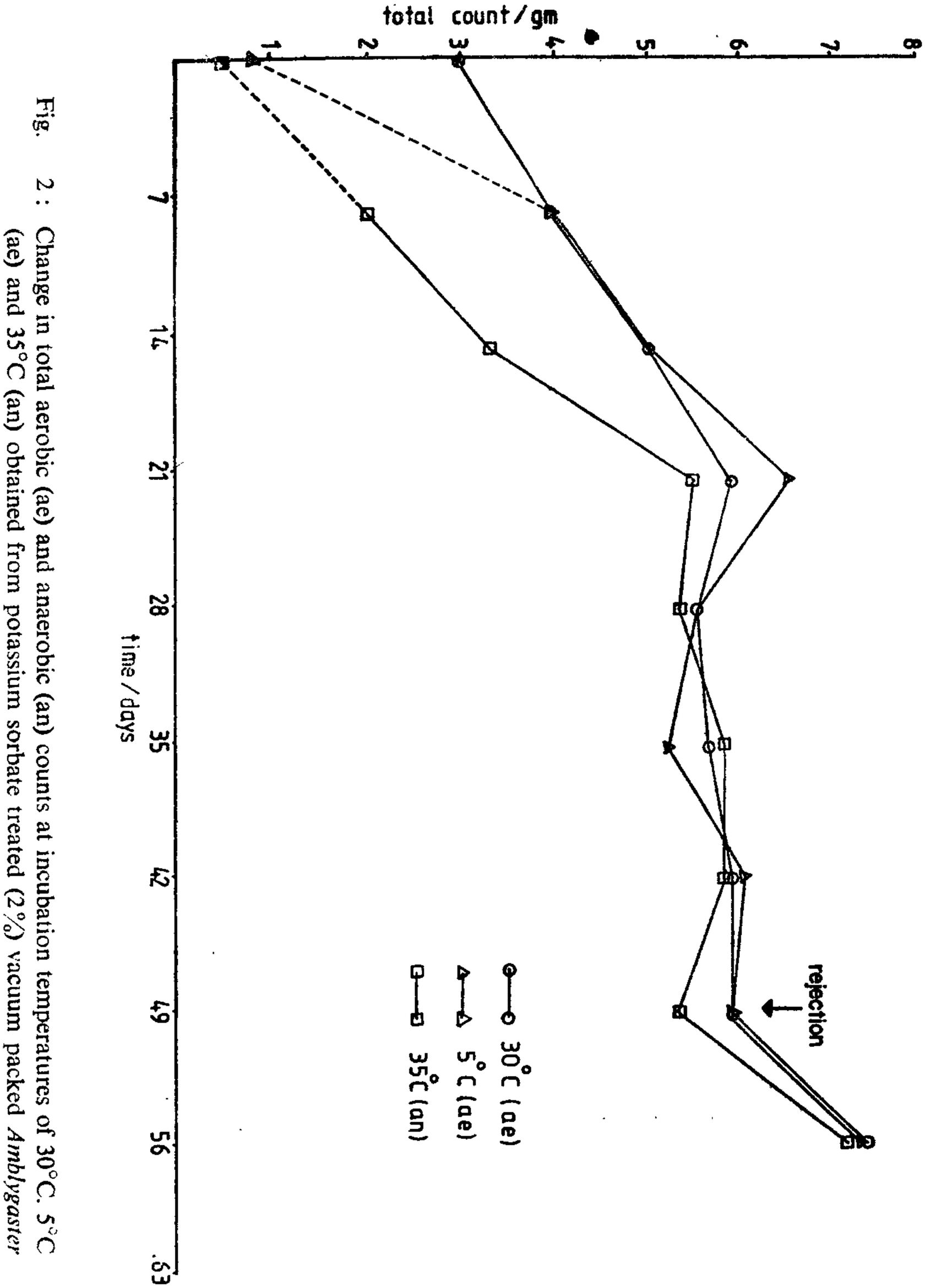

Fig. 2 : Change in total aerobic (ae) and anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) and 35°C (an) obtained from potassium sorbate treated (2%) vacuum packed *Amblygaster sirm* stored at 4°C.

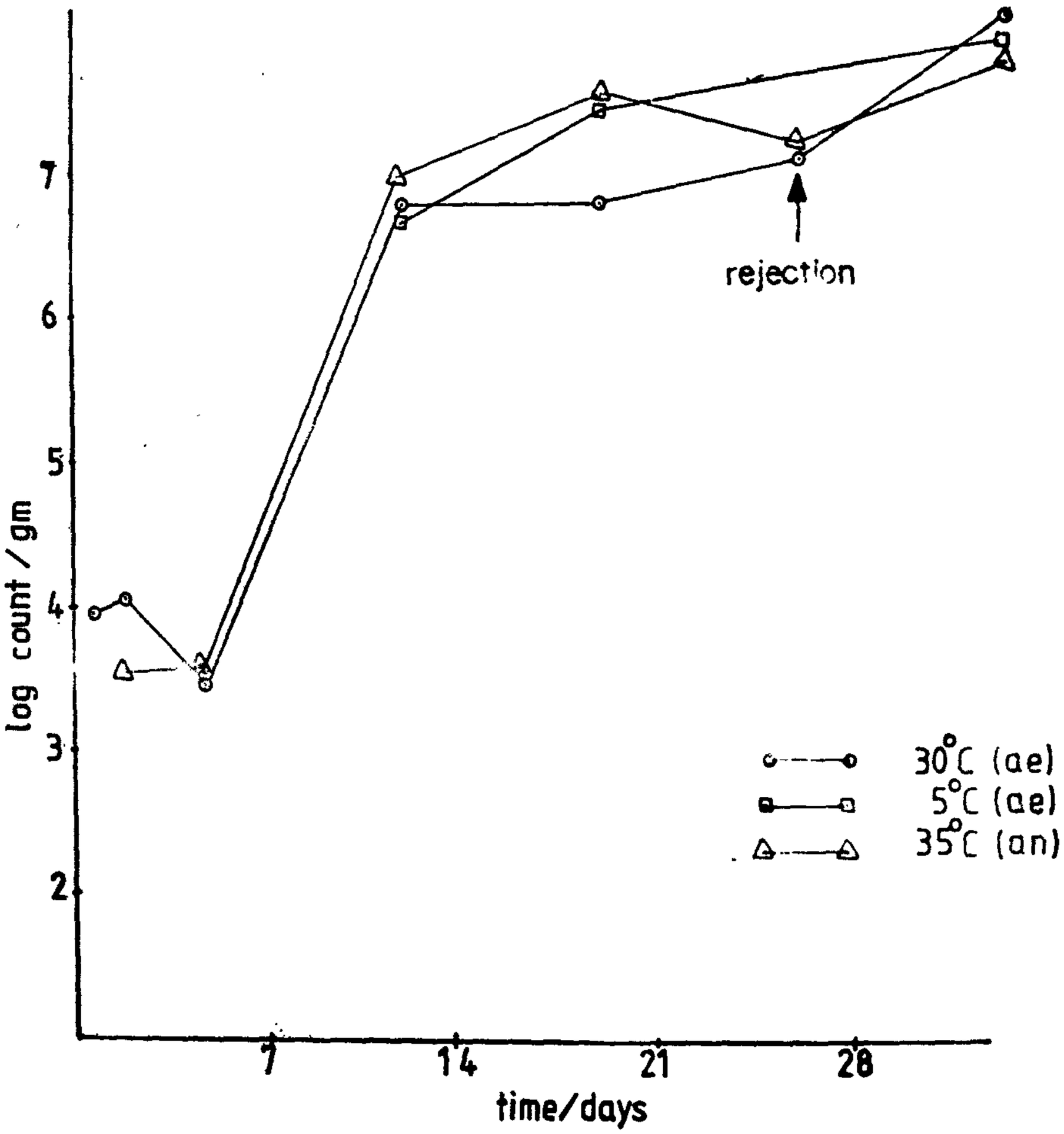

Fig. 3 : Change in total aerobic (ae) and anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) and 35°C (an) obtained from irradiated, vacuum packed *Amblygaster sirm* stoted at 4°C.

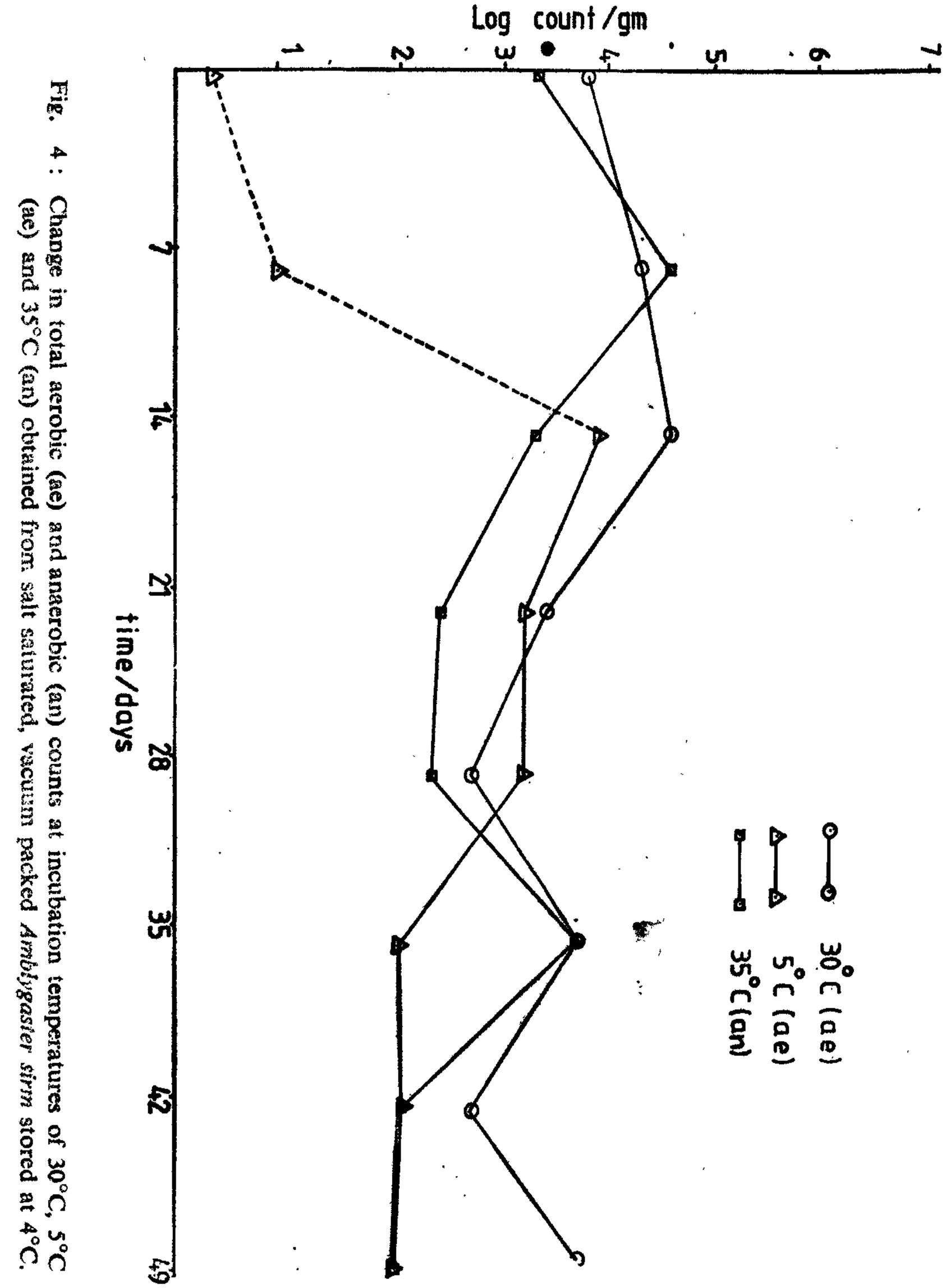

Fig. 4 : Change in total aerobic (ae) and anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) and 35°C (an) obtained from salt saturated, vacuum packed *Amblygaster sirm* stored at 4°C.

DISCUSSION

Vacuum packed trenched sardines had a shelf life ranging from 22 days (if untreated) to 50 days (if dipped in sorbate prior to packaging). An extended lag of 29 days was obtained in sorbate treated fish. Dainty *et al.* (1979) explained this as probably being due to the inhibition of microflora especially *Pseudomonas* spp. Statham *et al.* (1985) found potassium sorbate in combination with vacuum packaging was more effective in extending the shelf life of morwong at 4°C than the use of 100% CO_2 atmosphere. Chung and Lee (1981) showed that 1.0% potassium sorbate extended the lag of English sole to 6 days when stored at 1.1°C, while no inhibition of *Pseudomonas* occurred. In the present study sorbate treated fish was initially dominant in *Pseudomonas* spp. Gorczyca (1983) found that hydrogen sulphide producing bacteria *Alteromonas putrefaciens* a suspected spoiler of chilled fish, was inhibited by potassium sorbate during a storage period of more than 43 days.

In the present study the extended lag observed in sorbate treated samples may probably be due to the combination of vacuum treatment and the use of a sorbate dip.

As in the case of irradiation treatment Liston (1980) states that a selective bacterial flora mainly of *Micrococcus*, *Lactobacillus* and *Coryneforms* is present on low dose (100-200 krad) irradiated fish, indicating that some species are selectively eliminated during pre treatment. In this study the irradiated fish was initially dominant in Micrococci. Liston (1980) also states that the numbers of bacteria necessary to cause spoilage in fish seem to be higher, (10^8 cfu/g). In the present study an increase in shelf life of irradiated fish would have been due to the reduction of numbers of bacteria (mainly spoilers) while counts in the range of 10^7 cfu/g. (higher than the others) were required for spoilage.

The initial flora on salted fish constitute mainly of *Vibrio* species. Salted fish even though not rejected during the trial would not be a suitable substitute for fresh fish due to the high salt content, where the fish would have to be deep fried or desalting of fish prior to consumption would be necessary.

Spoilage flora in all treatments (except salted) were dominant in lactic acid bacteria, (Streptococci). In the present study the presence of lactic acid bacteria may have contributed to the extended shelflives in vacuum packaged fish. Abdel-Bar and Harris (1984) have shown that *Lactobacillus bulgaricus* was able to inhibit psychrotrophic bacteria in refrigerated foods. They also indicated that the hydrogen peroxide and organic acids produced could inhibit the microflora present.

TVN content showed a gradual increase with storage time with values at rejection of untreated and irradiated fish reaching the suggested value of 30-40mg/100g for flesh of cod (Connell 1975), while TMA contents were of little use as an index of spoilage. Poulter *et al.* (1981) observed no correlation of TMA content with taste panel scores for some Sri Lankan fish species.

It is believed that the extended shelf life obtained due to pre treatments such as irradiation and sorbate dips, may favour the growth and toxin production by *C. botulinum*. Ekland and Poyskey (1970) have shown that in packs inoculated with *C. botulinum* and then treated with 100-200 krad irradiation spoilage could be detected prior to the development of toxins. Similarly,

Tomkin *et al.*, (1974) have found potassium sorbate to be effective against *C. botulinum*. Beuchat (1980) showed positive effects of potassium sorbate at concentrations low as 30µg at pH 6.7. Further, Abrahamson *et al.*, (1965) indicated that vacuum packaging only slightly increased the rate of toxin production when compared with aerobic packs.

Thus an extended shelflife of 50 days for sorbate treated and vacuum packed trenched sardines in comparison to 20 days for untreated fish indicate that vacuum packaging alone or in association with pretreatments could give a shelf stable hygienic product. This factor could enhance the distribution and marketability of a dressed and packaged 'ready to cook' product of this nature. Temperature control below 3°C during marketing and distribution is recommended to prevent any possible risk in the event of contamination by pathogens. The use of suitable packaging techniques for tropical fish would enable fishery products to have a more flexible market. Such products would also serve as a wholesome product of quality and convinence to the urban consumers.

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