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AN ATTEMPT AT OPTIMALIZATION OF FECAL STREPTOCOCCI ENUMERATION IN COMMINUTED FROZEN FISH

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Key words: fecal streptococci, frozen fish.

Among the various methods used for fecal streptococci enumeration in frozen minced fish it is recommended to use the method which provides for a to 2 hours resuscitation of defrosted material in 1% buffered peptone water prior to inoculation onto the KF selective medium. The twenty four hours deep-freeze storage of minced fish resulted in a smaller reduction of the initial number of *Str. faecium* at -10°C, while for *Str. faecalis* the reduction was smaller at -30°C. Extented deep-freeze storage gave a higher survival of the tested fecal streptococci strains at -30°C.

Fecal streptococci are a frequent component of frozen food microflora, including food of marine origin. They were isolated from frozen cod, haddock and marine perch fillets [9, 20], salmon and halibut [19], marine crustaceans [4, 9, 19] and fish sticks and bars [9, 19]. The number of fecal streptococci in frozen food of marine origin ranged from 0 to 1.9×10^4 CFU*)/g [4, 9, 19, 20].

The recovery of fecal streptococci from frozen food depends on several factors such as temperature and speed of freezing and defrosting, suspending medium, time of deep-freeze storage, etc. [1, 10, 14, 23]. Equally essential is the method of enumeration including the diluent used, time of resuscitation within the recovery medium and the isolation medium applied [2, 3, 11, 13, 14, 16, 18, 22].

The aim of this study was to determine the effect of deepfreeze storage at -10°C and -30°C, the influence of resuscitation time within various recovery media and the isolation media applied on the recovery of fecal streptococci from comminuted frozen fish.

MATERIAL AND METHODS

The raw material used was comminuted fresh herring from the North Sea, prepared under laboratory conditions.

*) CFU — colony forming units.

The strains used were: *Str. faecalis* NCIB 775, *Str. faecium* NCIB 8274 and *Str. faecium* NCIB 11181 obtained from the National Collection of Industrial Bacteria at Torry Research Station, Aberdeen, Scotland.

The actively growing on the BHI (Oxoid) medium, 24 h culture of the tested strain was centrifuged and suspended in syntetic sea-water (pH — 7.0). Cell suspension was then introduced into comminuted herring meat so as to reach an initial inoculum of 10^6 CFU/g. When thoroughly mixed, comminuted fish meat was divided into 100 g portions, packed into plastic bags and subjected to slow freezing at the temperature of -10°C and -30°C.

Microbiological analysis of amples was carried out after 0, 1, 7 and 28 days of deep-freeze storage at the above temperature. This analysis was proceded by a 1 hour thawing of the samples at room temperature.

Decimal dilutions of the samples were prepared within the three resuscitation media, that is: 0.1% buffered peptone water, 1% buffered peptone water and 1% buffered tryptone soya peptone broth (TSB). Resuscitation of the damaged fecal streptococci cells within the above given media was carried out for 0, 2 and 4 hours. The number of fecal streptococci was estimated by means of direct surface plating of serial dilutions onto nonselective PCA medium and three selective media: BαS, KF and AZ. The composition of the isolation media was as follows: PCA — peptone 5 g, Lab Lemco beef extract 1 g, yeast extract 2 g, NaCl 5 g, agar 20 g /l (pH — 7.4); BαS — tryptose 20 g, yeast extract 5 g sodium azide 0.4 g, dextrose 2 g, $Na_2HPO_4 \times 2\ H_2O$ 4 g, TTC 0.1 g, agar 10 g /l (pH — 7.2); KF — polypeptone peptone 10 g, yeast extract 10 g, sodium azide 0.4 g, maltose 20 g, lactose 1 g, NaCl 5 g, sodium glicerophosphate 10 g, agar 20 g, phenol red 0.018 g, TTC 0.1 g/l (pH — 7.2); AZ — tryptose 10 g, Lab-Lemco beef extract 3 g, sodium azide 0.2 g, NaCl 5 g, agar 12 g/l (pH — 7.2).

The results presented in this work are the geometric average of three repetitions.

RESULTS AND DISCUSSION

Surveys on the survival of fecal streptococci subjected to freezing and deep-freeze storage in various environments showed essential differences in their recovery depending on the suspending medium and the method of enumeration chosen.

Recovery of *Str. faecalis* from various suspending media after for example 1 month storage at -20°C, ranged from 0 to 79% of its initial population [11, 23]. The hundred percent recovery stated by Larkin et al. [7, 8] both on frozen green peas and in orange concentrate, for all the experimental temperatures and times of deep-freeze storage, were probably due to the MPN method used for enumeration.

Survival of *Str. faecalis* NCIB 775 and *Str. faecium* NCIB 8274 in comminuted herring stored for 28 days at -10°C was 56 and 60% and at -30°C — 80 and 88%, respectively.

In both freezing temperatures, somewhat higher recovery was noted for *Str. faecium*. Higher survival, within the frozen material of such fecal streptococci as *Str. faecalis* var. liquefaciens and *Str. faecium*, compared to survival of *Str. faecalis* was observed also by Kereluk and Gunderson [5] and Hall et al. cited by Olson [14].

The effect of the temperature of -10°C and -30°C on the number of *Str. faecalis* NCIB 775 and *Str. faecium* NCIB 8274 recovered from comminuted fish samples varied according to the time of storage (Fig. 1).

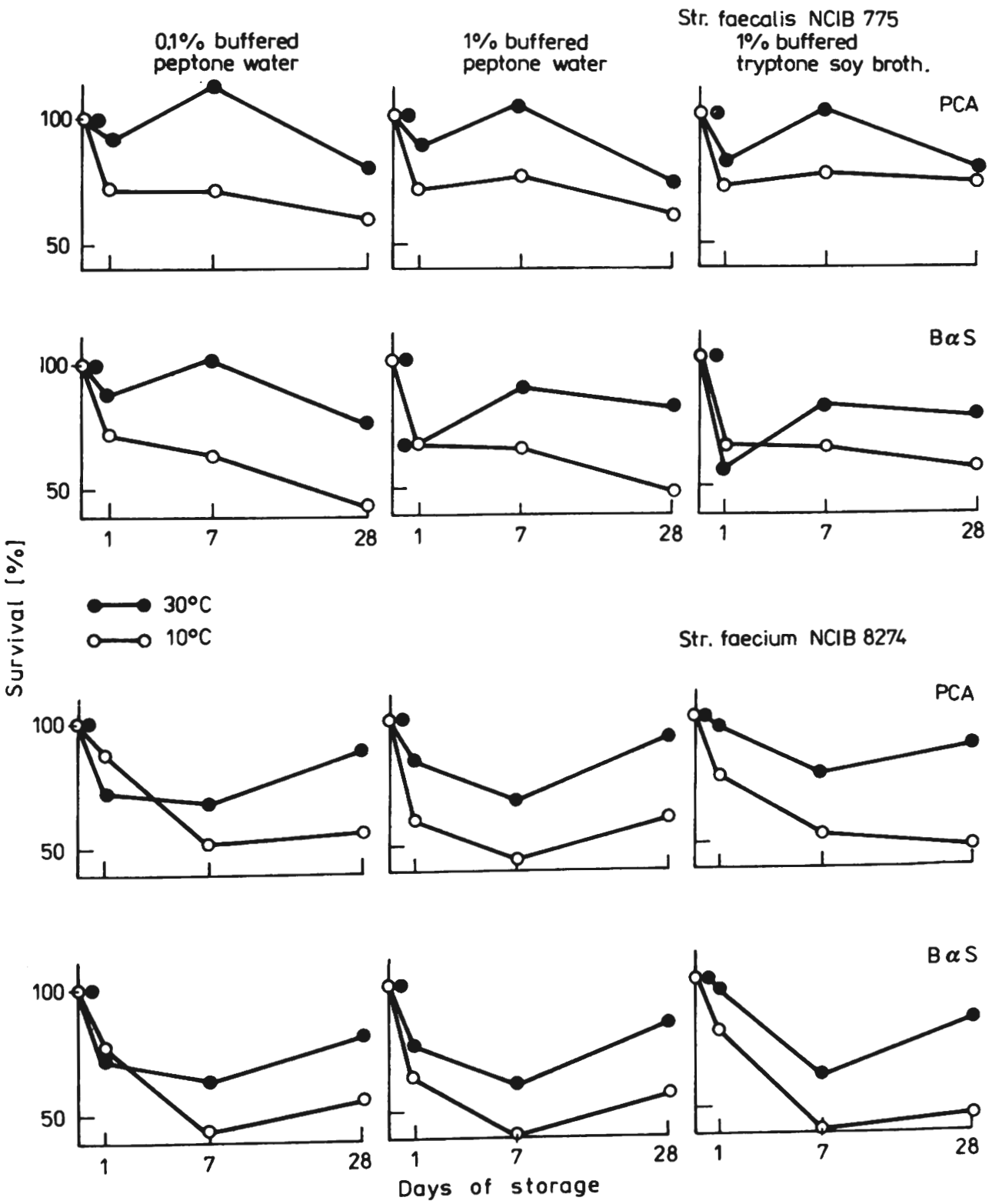

Fig. 1. Effect of diluents and isolation media on the recovery of fecal streptococci from comminuted frozen herring stored at -10°C and -30°C (no resuscitation)

For prolonged deep-freeze storage (7 to 28 days) higher recovery of the two strains tested was noted for samples stored at -30°C. In case of short-time storage (24 h) at -10°C and -30°C, its effect on the recovery was less univocal and depended both on the strain tested and the diluent applied. After 24 h storage, and when using 0.1% buffered peptone water as a diluent, survival of *Str. faecium* was higher at -10°C. With richer diluent higher recovery of *Str. faecium* was noted for samples stored at -30°C.

Woodburn and Hussemann-Strong [23], regardless of the time of deep-freeze storage noted the smallest reduction in number of *Str. faecalis* R 26, within simplified food substrates, at -30°C and the highest one at -11°C. According to Hall et al. cited by Olson [14] survival of *Str. faecalis* on frozen green peas was also higher at -29°C than at -18°C. However, recovery of *Str. faecium* under the same conditions was higher at -18°C [14].

Several authors have pointed to the role of peptides within the recovery media [3, 12, 13, 22]. In general practice however one of the most frequently used diluents is 0.1% peptone water [3, 13, 17, 21].

The effect of the three diluents used on the recovery of the fecal streptococci from cominuted frozen fish is illustrated on Fig. 1. According to the data presented in Fig. 1, the richer was the diluent the lower the recovery of *Str. faecalis* 775 from samples stored 24 h at -30°C. A reverse dependence was noted for *Str. faecium* 8274 under analogous conditions. When deep-freeze storage was extended to 28 days, higher survival, as a rule, was noted for the richer diluents applied. This dependence was characteristic practically for all the tested strains on the majority of isolation media.

Various species of fecal streptococci usually present in frozen food and the differences in survival of the tested strains have led to the conclusion that 1% buffered peptone water is the optimum recovery medium for the enumeration fecal streptococci in frozen comminuted fish.

Figher recovery of *Str. faecalis* from minced fish stored 24 h at -30°C with 0.1% buffered peptone water used as a diluent or with richer diluents in case of extended storage or storage at -10°C points out to differences in the type of cell damage under the above conditions.

Besides the diluent, the number of fecal streptococci recovered depends on the time of resuscitation within the diluent. When enumerating *Str. faecalis* surviving 24 h storage at -10°C and 30°C, 0 to 2 hours resuscitation at room temperature 0.1% buffered peptone water was chosen as the most convenient (Fig. 2). The same diluent used for samples stored 28 days at -30°C gave the highest recovery with direct plating procedure. When using richer diluents, resuscitation time can be extended up to 2 hours. For samples stored at -10°C, 0 to 4 hours resuscitation within the two richer diluents gave approximate results.

An inadequate choice of the diluent and resuscitation time may lead either to a further reduction of the number of living cells or their proliferation in the medium.

Further reduction in number of *Str. faecalis* was noted, e.g. for samples stored 28 days at -30°C, after their 2 h resuscitation in 0.1% buffered peptone water. A similar dependence was noted for 2 h resuscitation of samples, stored 24 h at -30°C richer diluents. According to Postgate and Hunter [18], further reduction in the number of bacteria within a resuscitation medium can be explained by an ex-

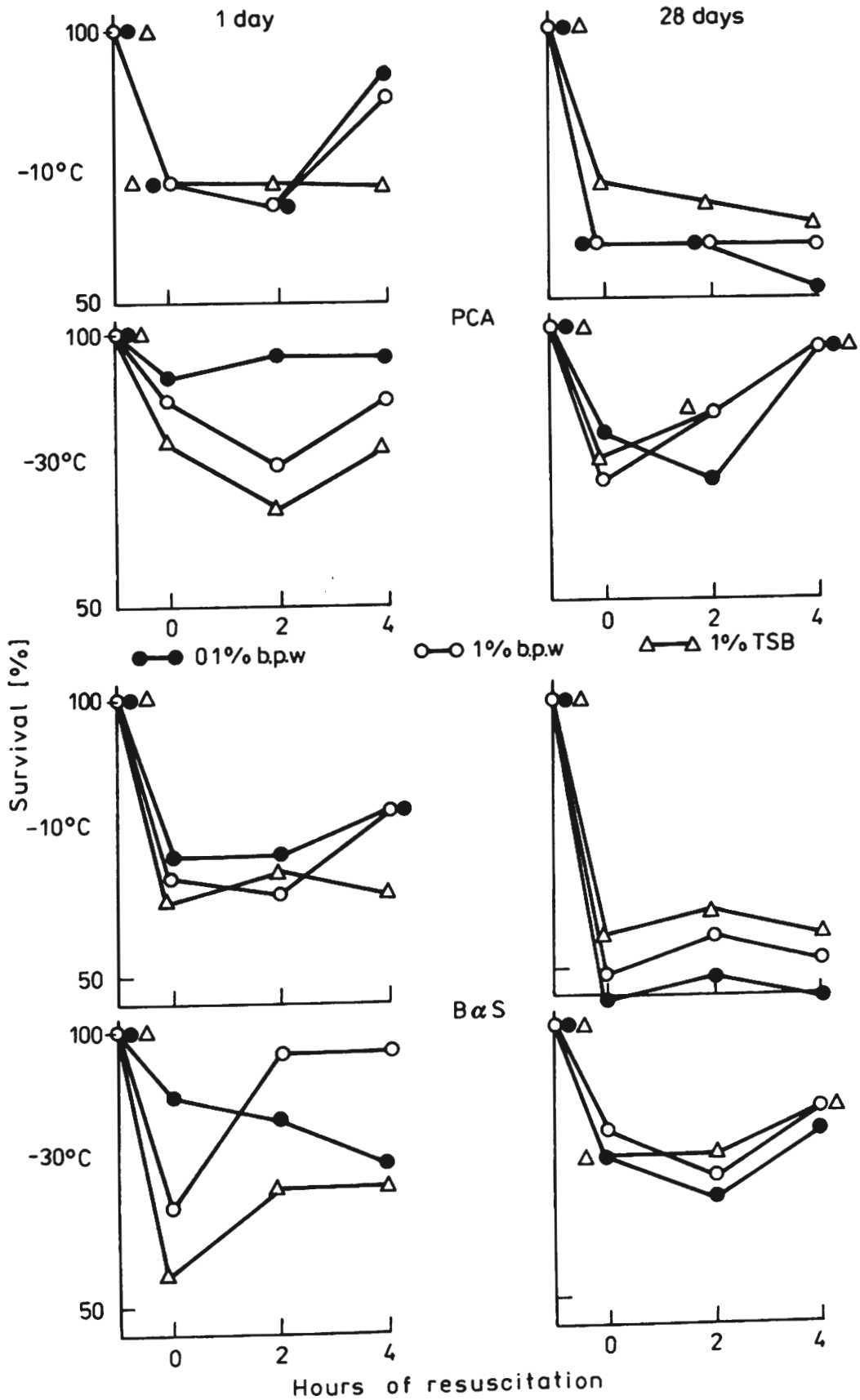

Fig. 2. Effect of resuscitation time in 3 resuscitation media on the recovery of *Str. faecalis* NCIB 775 from comminuted frozen herring stored at -10°C and -30°C (PCA and BαS)

tended lag-phase of cells surviving the freeze-thawing process, where further reduction occurs.

Enumeration of the fecal streptococci in food has been carried out on many isolation media, of which 68 are know [15]. The most frequently applied and advised ones comprise B S, KF, SF and AZ media [1, 6, 11, 12, 13, 15, 17, 21].

Among the selective media applied in this work, a slightly higher recovery of *Str. faecalis* and *Str. faecium* from the frozen comminuted fish samples, was noted on KF medium (Fig. 3). The only exception were samples inoculated with *Str. faecalis* 775 stored at -30°C where, with the richer diluents applied, slightly higher recovery was noted on BαS medium.

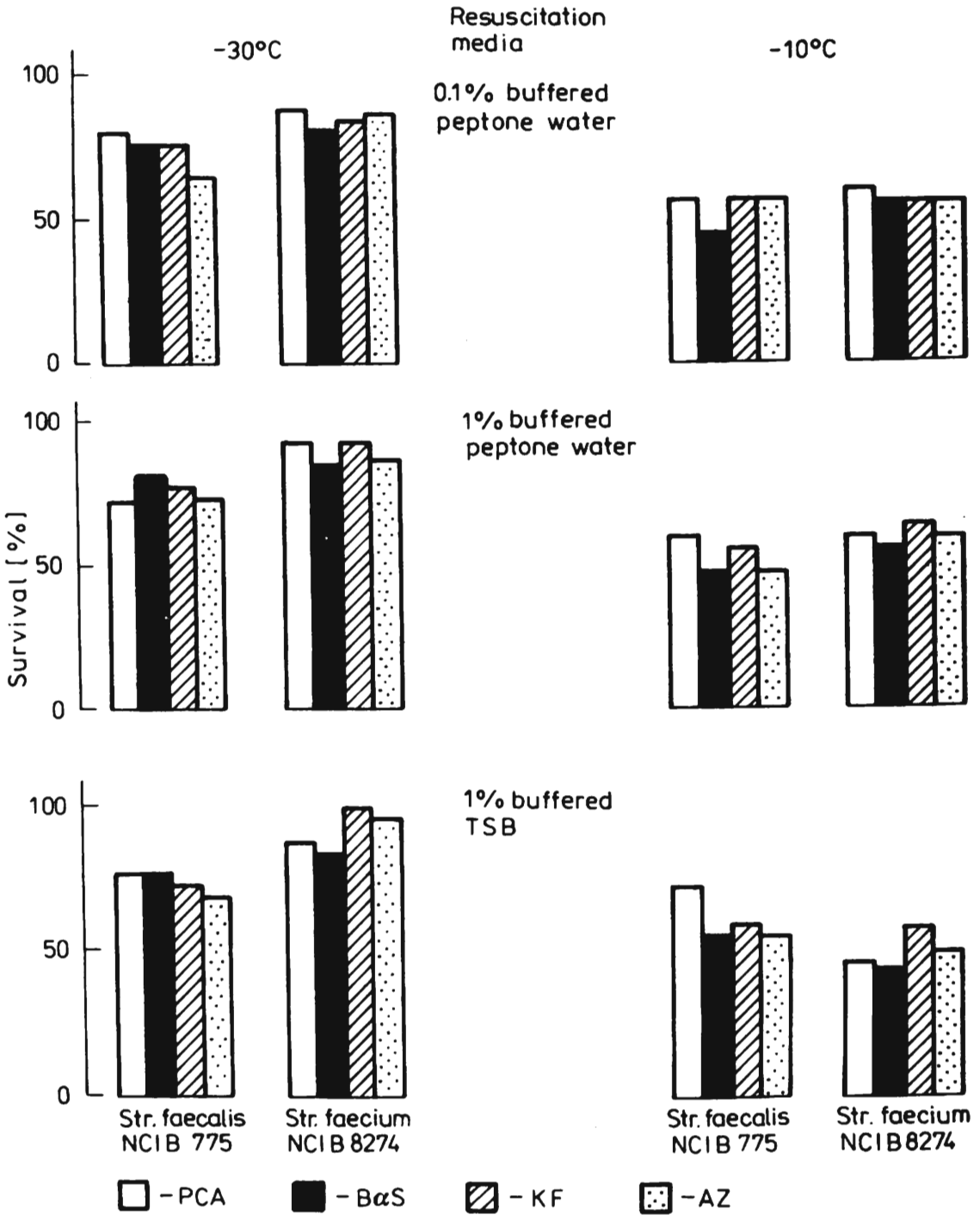

Fig. 3. Effect of the isolation media on the recovery of fecal streptococci from comminuted herring stored at -10°C and -30°C for 28 days

Opinions differ as to the usefulness of various selective media used for the isolation and enumeration of fecal streptococci from frozen material [1, 6, 11, 13, 15]. For example Oblinger [13], while comparing four solid selective media, namely: AZ, Streptosel, KF and m-*Enterococcus* agar, noted the highest recovery of fecal streptococci on the AZ medium. According to Morichi and Yano [11], recovery on KF medium, when compared to 100% on the TYGO medium amounted to $67 \pm 14\%$ only. Although getting the highest recovery on the Thallous acetate agar, Pavlova [15] indicated the KF medium as the best among the tested selective media for fecal streptococci. Also Hall et al. [1] advised the usage of KF medium for isolation and enumeration of fecal streptococci from food subjected to various treatments.

CONCLUSIONS

1. Survival of *Str. faecium* in comminuted fish, kept frozen for 24 hours, is higher at -10°C than at -30°C.
2. Prolonged deep-freeze storage gives a higher survival of *Str. faecalis* and *Str. faecium* at -30°C.
3. In order to enumerate fecal streptococci in comminuted frozen fish it is recommended to use 1% buffered peptone water as diluent.
4. 0 to 2 hours at room temperature was chosen as the optimal resuscitation time for thawed minced fish samples in 1% buffered peptone water.
5. KF medium, when compared to BS and AZ media, gives a slightly higher recovery of fecal streptococci from comminuted frozen fish.

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PRÓBA OPTYMALIZACJI METOD LICZBOWEGO OZNACZANIA PACIORKOWCÓW KAŁOWYCH W MROŻONYM FARSZU RYBNYM

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Streszczenie

Zależność między liczbą paciorkowców kałowych odzyskiwanych z mrożonej żywności, po różnym czasie składowania zamrażalniczego, w różnej temperaturze mrożenia, a obraną metodyką badawczą sprawiła, że w niniejszej pracy podjęto próbę doboru optymalnej metody w celu określenia liczby paciorkowców kałowych w mrożonym farszu rybnym. Spośród zastosowanych metod oznaczania liczby paciorkowców kałowych w mrożonym farszu rybnym za najkorzystniejszy uznano wariant uwzględniający 0 do 2 h ożywianie rozmrożonego materiału w 1% zbuforowanej wodzie peptonowej, poprzedzające posiew na podłoże selektywne KF.

Analiza wpływu składowania zamrażalniczego na przeżywalność paciorkowców kałowych wykazała, że liczba paciorkowców kałowych, przeżywających działanie temp. -30 i -10°C, w środowisku farszu, zależy od czasu składowania zamrażalniczego oraz szczepu testowego. Składowanie zamrażalnicze przez 24 h powodowało mniejszą redukcję wyjściowej liczby *Str. faecium* w temp. -10°C, zaś *Str. faecalis* w -30°C. Przy wydłużonym składowaniu zamrażalniczym wyższą przeżywalność szczepów testowych paciorkowców kałowych notowano w temp. -30°C.