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Fish microbiology

EFFECT OF FROZEN STORAGE AT –30°C ON THE SURVIVAL OF CHOSEN INDICATOR MICROORGANISMS IN MINCED FISH

WPŁYW SKŁADOWANIA ZAMRAŻALNICZEGO W –30°C NA PRZEŻYWALNOŚĆ WYBRANYCH DROBNOUSTROJÓW WSKAŹNIKOWYCH W ŚRODOWISKU FARSZU RYBNEGO

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The paper presents changes in number of *E.coli*, *Str. faecalis*, psychrophilic, psychrotrophic and proteolytic bacteria surviving frozen storage at –30°C in minced blue whiting without and with additives.

INTRODUCTION

Differences in qualitative and quantitative composition of the frozen food microflora and various susceptibility of microorganisms to freezing temperatures in various environments make selection of a proper criterion for the microbiological quality evaluation to be very essential.

Ussualy microbiological quality estimation of frozen food of marine origin is directed on pathogenic microorganisms, spore-forming anaerobic bacteria, total viable count of aerobic bacteria (7), sometimes proteolytic bacteria (8) and fecal coli as indicator organism (7, 8).

According to the available references, however, fecal streptococci have been suggested to be better indicator of microbial quality of frozen food than fecal coli

(1,2,3,4,11,14,15). Fecal streptococci have been isolated more often and in greater numbers than *E.coli* from the frozen food samples. Yet easier was their identification.

The aim of this work was to estimate an effect of frozen storage at –30°C both on the survival of *E.coliNCIB 86* and *Str.faecalis NCIB 775* as well as on the changes in the total viable count (TVC) of psychrophilic, psychrotrophic and proteolytic bacteria in fish mince.

MATERIAL AND METHODS

The raw material used was both the minced blue whiting prepared from frozen fish fillets and frozen minced blue whiting with additives used in production of "the Warsaw fillets"*.

The test strains used were *Str. faecalis* NCIB 775 and *E.coli* 86 from the National Collection of Industrial Bacteria, Aberdeen, Scotland.

Actively growing on the BHI medium (6) 24h cultures of the tested strains, centrifuged, washed and suspended in a syntetic marine water were introduced into minces as to reach an initial inoculum of 10^4 CFU/g.

The 100g portions of the minced fish, placed within the sterile Petri dishes were stored at –30°C for 1,7,28 and 61(62) days. Each time three 100 g portions were collected for the analysis.

Prior to the microbiological analysis, samples were thawed for 8h at 0 to +4°C, homogenized in 0.1% buffered peptone water for 2′ and then resuscitated for 1h at room temperature.

The microbiological analysis included:

1. Enumeration of the fecal streptococci by means of a surface spreading of the decimal dilutions on B α S medium (37°C/48h).
2. Enumeration of the most probable number (MPN) of *E.coli* on the BGB medium, followed by confirmatory tests (37°C/24–48h).
3. Enumeration of TVC of psychrophilic and psychrothrophic bacteria by spreading the decimal dilutions onto Frazier's medium (20°C/5 days).
4. Enumeration of the TVC of proteolytic bacteria on Frazier's medium.

Presented data are geometric average of six repetitions.

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* ingredients of the "Warsaw fillets" (in kg):

blue whiting scraps	–	93.0
powdered milk	–	1.0
salt	–	1.6
peper	–	0.2
dried onion	–	0.9
water	–	3.3

RESULTS AND DISCUSSION

A preliminary microbiological analysis of minced blue whiting stored frozen under industrial conditions showed visible differences in a total contamination level, due to the type of mince (Table 1). With maximum total viable count (TVC) being equal to 10^5 CFU/g (16), none of the tested batches of minced blue whiting with additives have met the requirements. Fecal streptococci and *E.coli*, although present in all the tested mince batches, except for the batch 4, have not exceed 10^1 CFU/g.

Differences in contamination level of a frozen raw material of marine origin noted by Silvermann et. al (13) were much higher and ranged from 0 to 1.9×10^2 CFU/g for *E.coli*.

Results of the analysis of the effect of frozen storage at −30°C on survival of *Str.faecalis* and *E.coli* in the minced blue whiting confirmed higher survival of the latter to freezing remperatures and frozen storage. 24 hours storage at −30°C survived 80% of the initial population of *Str.faecalis* and only 5−10% of *E.coli* population (Fig. 1a and b). After 62 days of frozen storage at −30°C number of *E.coli* cells, in both types of minces, was within the limits detectable by the applied method. Reduction in *Str.faecalis* cells number, in minces without and with additives, after that time was equal to 40 and 26%, respectively.

A smaller reduction of the initial number of both tested indicator microorganisms was noted in samples of minced fish with additives, which may point out to a protective role of the applied additives towards tested microbes.

A simillar relation was noted for changes in total number of psychrophilic and psychrothrophic bacteria in stored frozen minces (Fig. 1c).

Character of changes in number of proteolytic bacteria, in stored frozen minces, differed from the ones mentioned above. With the freeze-thawing method applied, increase in number of proteolytic bacteria, in minced fish samples with additives stored 24h at −30°C, was noted (Fig. 1d).

Increase in number of psychrotrophic bacteria in fish, while frozen, was noted by Koser et. al. cited by Zaleski (16). An explanation to it was their possible multiplication during slow freezing. A multiplication during slow freezing could have been just the cause of increse in number of proteolytic bacteria noted. Yet both; the initially higher number of proteolytic bacteria in minced fish with additives and possible protective effect of the additives could have supported it.

It is worth to be mentioned, that for many psychrophilic, proteolytic rods representing *Pseudomonas* genus, the minimal growth temperature is below 0°C and time of one generation at 0°C ranges from 10 to 30 hours (12).

Repeated increase in numbers of proteolytic bacteria, noted in both types of minces after their prolonged frozen storage, could have been caused either by their multiplication while thawing the samples or by activisation of proteolytic enzymes, of the other bacteria present, by frooze-thawing process (Fig. 1d).

Table 1

Number of chosen bacteria in comminuted blue whiting, when stored frozen under industrial conditions (−30°C)

Type of sample	Batch	Days of storage	TVC* of psychrophiles and psychrotrophs CFU/g **	TVC of proteolytic bacteria CFU/g	TVC of proteolytic bacteria %	TVC of fecal streptococci CFU/g	NPL of E. coli CFU/g
comminuted	1	28	5.8×10^4	5.5×10^3	9.5	5.2	1.4
blue	2	28	3.6×10^4	4.3×10^3	12	0.9	0.3
whiting flesh	3	28	2.0×10^4	2.9×10^3	14.5	0.9	2.5
comminuted	4	1	9.7×10^5	2.9×10^5	30	1.1×10^2	1.5
blue whiting	5	7	1.3×10^7	1.7×10^6	13	0.9	1.2
flesh	6	7	1.0×10^7	2.5×10^6	25	0.9	0.8
with	7	7	1.2×10^7	3.2×10^6	27	0.9	0.5
additives***	8	7	1.8×10^7	5.7×10^6	32	0.9	0.8

* – Total viable count; ** – colony forming units;

*** – ingredients (in kg) –

blue whiting scraps	– 93
powdered milk	– 1
salt	– 1.6
peper	– 0.2
dried onion	– 0.9
water	– 3.3

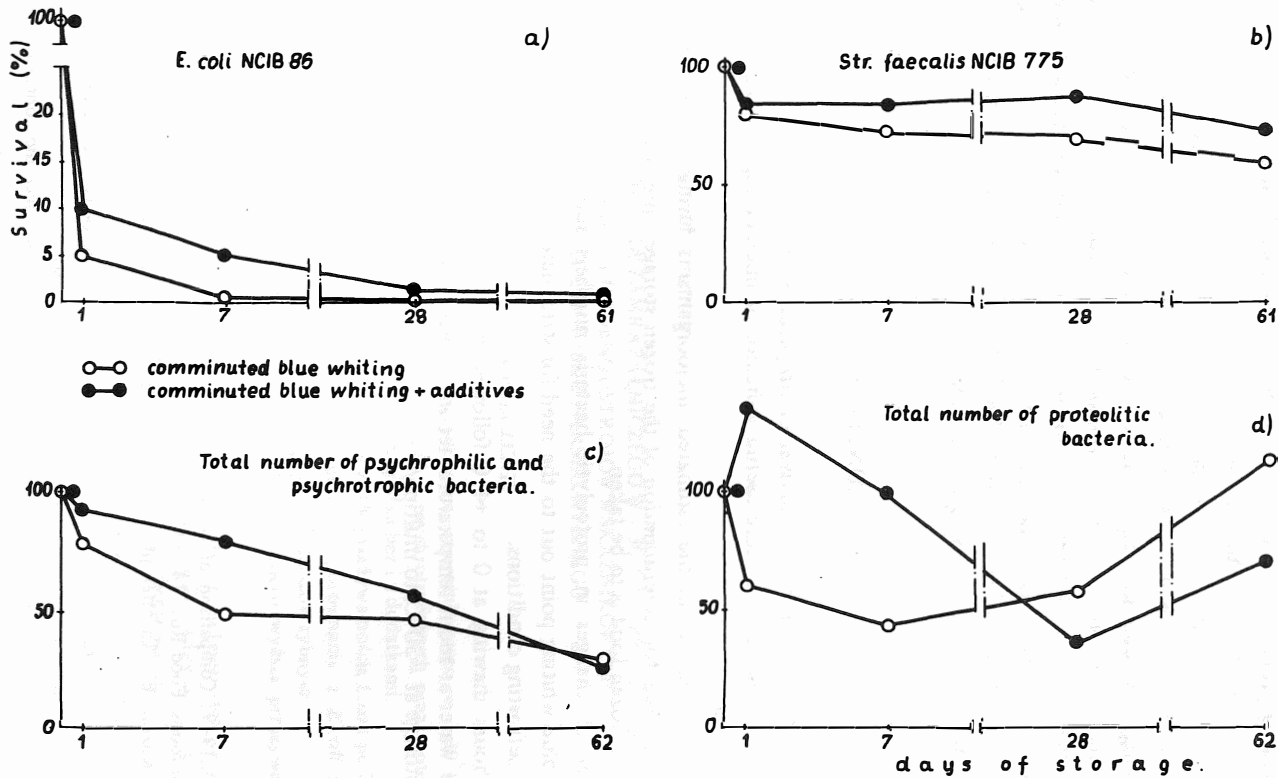

Fig. 1. Effect of frozen storage at −30°C on survival of chosen bacteria in comminuted blue whiting, in %

Okuzumi et. al. (5) pointed out to the possible activisation of bacterial enzymes during a freeze-thawing process. They stated i.g. an increased proteolytic activity of E.coli cells after frozen storage at −20°C and defrosting.

As to evaluate properly the microbial quality of frozen food, equally essential are the criteria of evaluation chosen and ways of samples treatment applied, prior to microbial analysis. Obligatory rules and regulations are not very strict about that (7,8,9,10). They permit to analyse the samples when frozen or after defrosting (8,9,10). Samples can be defrosted either at 10 to 20° C (9) or at 0 to +4° C (8) for the time not precisely stated.

Various thawing methods applied in practice and various time of frozen storage after which microbiological analysis of frozen food, based on various evaluation criteria, is carried out let to draw the following conclussions:

CONCLUSIONS

1. Much higher, then for *E.coli*, survival of *Str.faecalis* in frozen minced blue whiting, proves *Str.faecalis* to be a more usefull indicator of microbiological quality of frozen fish minces then *E.coli*.
2. Variability in number of the estimated microorganisms during frozen storage points out to the need for determination of the frozen storage time, after which microbial analysis of frozen food is to be taken.
3. Character of changes in proteolytic bacteria numbers surviving frozen storage in minced blue whiting point out to the need for strict determination of the minced fish samples defrosting conditions.
4. The 8 hours thawing at 0 to +4° C followed by 1h resuscitation in 0.1% buffered peptone water at room temperature, iet the proteolytic bacteria, surviving prolonged frozen storage at −30° C, to multiply.

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STRESZCZENIE

W pracy zajęto się określeniem wpływu składowania zamrażalniczego w –30°C na liczbę *Escherichia coli, Streptococcus faecalis,* psychrofilnych i psychrotrofowych oraz proteolitycznych bakterii przeżywających proces w środowisku farszu z błękitka bez i z dodatkami.

60 do 74% przeżywalność *Str.faecalis*, w porównaniu z 1% przeżywalnością *E.coli* po 2 miesiących składowania zamrażalniczego w –30°C, wykazała większą w porównaniu z *E.coli* przydatność *Str.faecalis* jako wskaźnika jakości mikrobiologicznej mrożonych farszów rybnych.

Spośród dwóch testowanych rodzajów farszów z błękitka, nieco wyższą przeżywalność oznaczanych drobnoustrojów notowano w farszu z dodatkami.

Charakter zmian liczbowych bakterii proteolitycznych w trakcie składowania zamrażalniczego farszów, stwierdzony przy zastosowanej metodzie rozmrażania prób i ożywiania, poprzedzającej analizę mikrobiologiczną, wykazał potrzebę jednoznacznego określenia warunków postępowania z próbą przed jej analizą.

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