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Effects of primary processing and pre-salting of Baltic herring on contribution of digestive proteases in marinating process

Patryk Kamiński[1*], Mariusz Szymczak[1], Barbara Szymczak[2]

[1] Department of Toxicology, Dairy Technology and Food Storage, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology in Szczecin, 71-459 Szczecin, Papieza Pawla VI 3, Poland.
[2] Department of Microbiology, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology in Szczecin, 71-459 Szczecin, Papieza Pawla VI 3, Poland.
* Patryk Kamiński, Department of Toxicology, Dairy Technology and Food Storage, West Pomeranian University of Technology in Szczecin, 71-459 Szczecin, Papieza Pawla VI 3, Poland. Tel.: +48 91 449 65 01, e-mail: patryk-kaminski@zut.edu.pl

Abstract: The low-technological quality of herring caught during the feeding season makes it impossible to achieve full ripeness of the meat of marinades. One solution may be to assist ripening using herring digestive tract proteases. Therefore, whole herring, headed herring and fillets were marinated for 2-14 days using the German (direct) and Danish (pre-salted) methods. The results showed that the mass of marinades from fillets was lowest than from herring with intestines and correlated strongly with salt concentration in the Danish method, in contrast to the German method. Marinades from whole and headed herring had significantly higher trypsin, chymotrypsin, carboxypeptidase-A and cathepsin activities than marinated fillets. The herring marinated with viscera had 2-3 times higher non-protein nitrogen, peptide and amino acids fractions, as well as ripened 3 days faster than the marinated fillets. After 2 weeks of marinating, the fillets did not achieve full ripeness of the meat, unlike marinades made from whole and headed herring. The pre-salting stage in the Danish method significantly reduced cathepsin D activity by the 10th day of marinating, which was compensated by digestive proteases only in the case of whole or headed herring. The digestive proteases activity in the fillets was too low to achieve the same effect. Sensory evaluation of texture and hardness-TPA correlated strongly with several proteases in whole herring marinades, in contrast to a weak correlation with only one protease when marinating fillets. Marinating with intestines makes it possible to produce marinades faster, more efficiently and with higher sensory quality from herring of low-technological quality.

Keywords: herring; primary processing; marinades; intestines proteases; cathepsins; pre-salting,

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1. Introduction

The high degree of ripeness of the meat of herring marinades promotes high sensory quality and nutritional value, and facilitates digestion, especially in the elderly or those with certain gastrointestinal diseases. Cold ripened marinades are produced mainly from herring, the technological quality of which varies seasonally [1]. Due to the high price of the raw material, the industry tries to marinate cheaper herring caught at the beginning of the feeding season, resulting in low ripeness of marinades. The ripening of marinated herring is mainly related to cathepsins D, L and B activity [2, 3]. Therefore, herrings with low cathepsins activity (caught during heavy feeding) are worst suited for the marinades production. High cathepsins activity promotes better meat texture and the formation of a high content of protein hydrolysis products (PHP), which provides sensory and nutritional characteristics to marinades. Peptides released from marinated herring proteins also show biological activity [4, 5].

Kamiński et al. [6] showed that cold storage of herring prior to marinating allows the use of digestive proteases to improve the low technological quality of the raw material. During the herring's 7 days of cold storage, trypsin, chymotrypsin and carboxypeptidases diffused from the viscera into the muscle, which started proteolysis of the meat and increased cathepsin activity during marinating. The result was an increase in the sensory evaluation of marinades for taste, aroma and texture. The disadvantages of this method are the requirement of additional time before marinating and a lower yield of marinade mass. Currently Baltic herring after primary processing (heading, filleting) is marinated in 2 ways. The longest known method called ~~Germany~~ or classic or direct method consist of marinating of fillets in acid-salty brine for 7 days. This method has a 75-85% efficiency of the marinating process [7]. The second method, called the Danish method, has a 90-100% efficiency and is based on wet salting of fillets for 2 days, followed by marinating in an acid-salty brine [8]. Despite the fact that the Danish method has been known in the industry for many years, there is a lack of research results about the effect of pre-salting on proteases activity in the process of marinating herring.

Currently, cold marinades are mainly made from herring fillets, but marinated sprat in northern and central Europe and marinated sardine in southern Europe and western Asia are also available on the market. Baltic herring caught at the beginning of the feeding season is characterized by a small size similar to that of a large sprat [9]. Large sprats are subjected to nobbing before marinating, which involves separating the head along with the digestive tract, without opening the abdominal cavity, without removing the pyloric caceae and gonads [10]. The pyloric caceae contain very active proteases [6, 11]. Practice shows that mechanical nobbing is not always effective and some of the sprat is marinated with whole intestines. There are no results in the literature for the marinating method of whole or nobbed herring, especially in combination with the Danish marinating method, in which pre-salting may change the activation of digestive proteases, as occurs during traditional salting of herring [12, 13].

Proteases activity is crucial in the ripening process of marinated herring meat, but so far proteases activity has only been studied during the classic marination of fillets [14, 2]. The effect of pre-salting on proteases activity in marinades is unknown, while the contribution of digestive proteases during marination has been described in only one publication [6]. Therefore, the aim of this study is to determine the effect of primary processing of Baltic herring (whole, headed, fillets) with and without pre-salting stage on contribution of digestive and cathepsins proteases during ripening of marinades using Danish and Germany methods.

2. Materials and Methods

2.1. Baltic herring and marinating methods

Baltic herring (Clupea harengus membras) was caught in December (FAO 27IIId), transported in polystyrene ice boxes to the laboratory within 24h and Quality Index Method (QIM) and morphometry of whole herring was assessed [6, 15]. The herring was in the feeding season, as the gonads had a mass of 11.3 ±4.0g and maturity stage III on the Maier scale, while the weight of the digestive tract was 6.5 ±1.8g. The herring was 22.3 ±0.8cm long and weighed 113.0 ±28.2g, with meat containing 11.7 ±0.1% lipids, 71.9 ±0.1% water and 15.9 ±0.3% protein.

The herring was divided into 3 raw material groups: whole herring, headed herring and fillets (control sample), which were marinated by the (G-) German or (D-) Danish method obtaining marinated ~~fillets~~ samples: G-whole, G-headed, G-fillets, and D-whole, D-headed, D-fillets. The German method was performed marinating each group of herring separately in 5% acetic acid and 6% table salt solution for 14 days [2]. The Danish method was performed using salting for 2 days in 10% brine, followed by marinating in 5% acetic acid and 1.8% table salt solution for 12 days [8]. All herring samples were

marinated at 7°C, fish to brine ratio was 1:1 (w:w). After 2, 4, 7, 10 and 14 days of marinating, the fish were transferred to a sieve and herrings were weighed.

2.2. Meat moisture, salt and pH analysis

The marinated herrings were filleted, skinned and minced. The pH value in meat with distilled water homogenate (1:10, w:v) was determined using a digital pH-meter. Moisture, salt contents and total acidity were determined using standard AOAC analytical techniques.

2.3. Extractions and activity assay of proteases

Digestive proteases extracts were made from fresh (raw material) and marinated herring meat using water (1:10, v:w) and TrisHCl 150 mM buffer at pH 7.8 (to buffer acetic acid in marinades), respectively [6]. Cathepsins from fresh and marinated meat were extracted with water. Samples were homogenised for 30 seconds at 35000 rpm and after 30 min centrifuged (9000 g, 10 min, 4°C). The resulting supernatant was centrifuged at 21000 g (10 min, 4°C) to obtain the crude proteases extract, where digestive proteases and cathepsins activity were determined. Amidase trypsin (Am-Tr) was measured at pH 8,2 against N-benzoyl-DL-arginine (BApNA) [16]; Esterase trypsin (Es-Tr) at pH 8.2 against Nα-p-Tosyl-L arginine methyl ester hydrochloride (TAME) and Chymotrypsin at pH 7.8 against N-Benzoyl-L-tyrosine ethyl ester (BTEE) [17]; Carboxypeptidase-A (Cp-A) at pH 7.5 against 4-Methoxyphenylazoformyl-Phe (AAFP) [18]. The activity of cathepsin B against Z-RR-MCA, cathepsin D against Mca-GKPILFFRLK(Dnp)-r-NH2 with and without pepstatin-A, and cathepsin L against Z-FR-MCA were determined according to [2]. All proteases analyses were performed at 37°C using continuous spectrophotometric rate determination, and units of enzyme activity were converted per 1 g of tissue. Chemicals from PeptaNova (Concord, CA, USA) and ultrapure water were used for analysis.

2.4. Non-protein nitrogen fraction analysis

Determinations of (a) non-protein nitrogen by Kjeldahl's method (AOAC no. 940.25), (b) peptides [PHP(R)] and amino acids [PHP(A)] fractions by Lowry's methods as modified by [19], were carried out in 50 g·kg−1 trichloroacetic acid (TCA) extract.

2.5. Texture profile analysis (TPA)

TPA-hardness was determined in 4 fillets from each sample with a TA-XTplus Texture Analyzer (Stable Micro Systems, Godalming, UK). Hardness tests included twice compression of sample in the same spot with a cylindrical probe P10 (10 mm) up to 50% of fillet height at the speed of 5 mm·s-1, and were conducted in 3 replications for each fillet separately (12 replications in each sample), only the dorsal muscle ~~in~~ area from 2/10 to 6/10 fillets length measured from the head side. Their course ~~was~~ recorded as curves representing changes of force in time.

2.6. Sensory profiling

Marinated meat was analyzed by sensory profiling performed by a trained sensory panel, using a five-point unstructured scale with 0.5-point accuracy anchored at their extremes with minimum and maximum degrees of acceptance [20]. Briefly, four skinned fillets from each sample were served in porcelain trays. The assessors used water and flat bread to clean their palate between samples. The sensory attributes were: texture, flavor, odour, and appearance. The sum of individual scores gave a total score that represented the overall sensory evaluation of the marinades.

2.7. Microbiological analysis

Three fillets from marinated herring were taken for analysis using disinfected (peracetic acid, 30% H_2O_2) knives and plastic boards. The fillets were held with sterile

tweezers and rinsed 1-2 sec with cold tap water to remove any remaining viscera and blood. The preparation of the test samples was performed according to the ISO standard [21], in which the total number of psychrophilic bacteria [22], mesophilic bacteria [23], yeasts and moulds [24] and *Enterobacteriaceae* [25] were determined.

2.8. Statistical analysis

Results were analyzed statistically using one-way analysis of variance (ANOVA) with Statistica 13.3 software (Statsoft, Tulsa, OK, USA). The ANOVA ~~P value~~ was set at 0.05, and the differences between treatments were examined using the post hoc Tukey's test of honestly significant differences (P<0.05) [26]. Principal Component Analyses (PCA) was used to investigate correlations between proteases activities, marinating time, proteolysis indicators and sensory quality separately for each marinating method and raw materials [27, 28]. All measurements were taken in triplicate and results are presented as mean and standard deviation.

3. Results

3.1. Mass yield, pH and salt concentration

The mass of herring marinated by the German method (G-) decreased by day 14 (Fig. 1A), which was caused by a decrease in meat pH close to the isoelectric point and in result loss of water (Fig. 1B). The mass of German marinades correlated significantly with marinating time, most strongly for G-whole r = -0,987 (Fig. 2A). In the case of the Danish (D-) method, the yield increased after 2 days due to protein salting and moisture increase, but when acetic acid was added, fish mass and moisture significantly decreased (Fig. 1AB). The mass of Danish marinates also correlated significantly with marinating time, but the correlation was lower than in German marinates. After 4-7 days of marinating, the mass yield of G-fillets marinades averaged 78.7%, while G-hedead and G-whole marinades had higher yields of 4.1 and 15.0 percentage points, respectively (Fig. 1A). At the same time, D-fillets marinades had an average mass yield of 83.3%, while D-headed and D-whole marinades were 4.7 and 9.1 percentage points higher, respectively. Thus, the advantage higher mass yield of the Danish method over the German method when marinating fillets [8] was not present in the case of marinating whole herring.

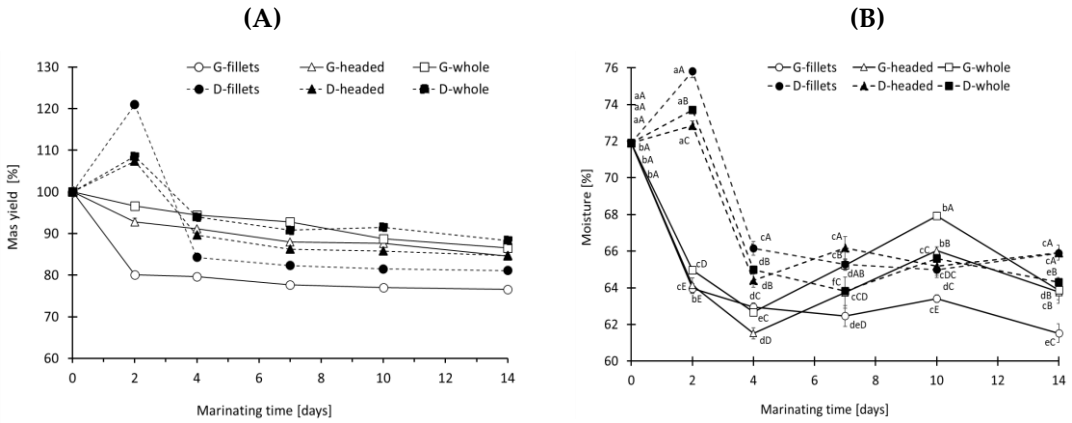


Figure 1. Effect of marinating method and degree of primary processing of Baltic herring (whole, headed, fillets) on **(A)** mass yield and **(B)** moisture during marinating. G- – German method, D- – ~~Denmark~~ method. ^{abc}Results marked with the same lower case letter do not differ statistically by the influence of the refrigerated storage time; ^{ABC}Results marked with the same capital letter do not differ statistically by the influence of the marinating method.

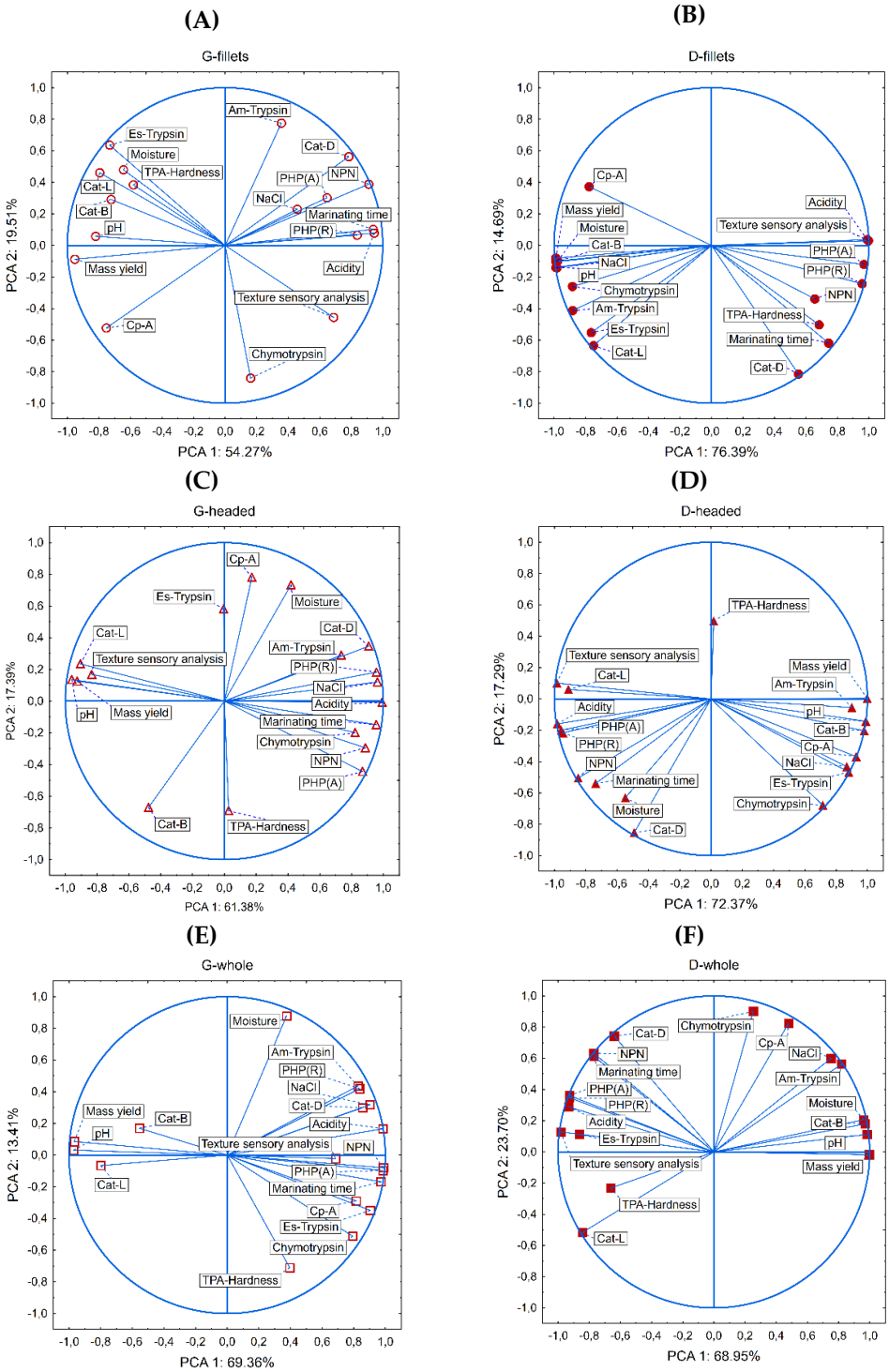


Figure 2. PCA biplot for correlations between proteases activity, physicochemical parameters and sensory analyses of marinades depending on marinating methods: **(A, C, E)** German method (G- –), **(B, D, F)** ~~Denmark~~ method (D- –) and depending on degree of primary processing of Baltic herring: **(A, B)**, fillets of herring **(C, D)** headed herring and **(E, F)** whole herring.

G-fillets marinades after 4-7 days of marinating had an average meat pH value of 3.91, while G-headed and G-whole had 4.16 and 4.26, respectively. (Fig. 3A). The pH value correlated significantly with the mass of German marinades, decreasingly: G-whole (0.973), G-fillets (0.891) and G-headed (0.871) (Fig. 2ACE). The D-fillets marinades had a meat pH of 3.88, while the D-headed and D-whole marinades had a pH of 4.22 and 4.38, respectively. The pH value of D-fillets, D-headed and D-whole marinades correlated

significantly with mass yield of 0.991, 0.877 and 0.750, respectively (Fig. 2BDF). The content of table salt after 4-7 days of marinating in the meat of G-fillets marinades was 2.96% and was 0.07 - 0.17% higher than in G-whole and G-headed marinades (Fig. 3B). For marinating using the Danish method, the differences in salt content between D-fillets marinades (2.80%) and D-headed (2.38%) and D-whole (2.16%) marinades were higher than for the German method (Fig. 3B). PCA analysis showed that the higher the ~~degree~~ primary processing of herring (whole < headed < fillets), the salt concentration correlated less strongly with the mass yield of marinades performed by the German method (G-whole -0.820, G-headed -0.801, G-fillets -0.469) (Fig. 2ACE), in contrast to ~~Denmark~~ method (D-whole 0.750, D-headed 0.877 i D-fillets 0.991) (Fig. 2BDF). Therefore, in marinades made of whole herring the skin reduced the loss of water and protein from the meat [29], and also reduced the diffusion of acetic acid and salt into the meat (Fig. 3AB), which synergistically reduce the water holding capacity of marinated meat [30].

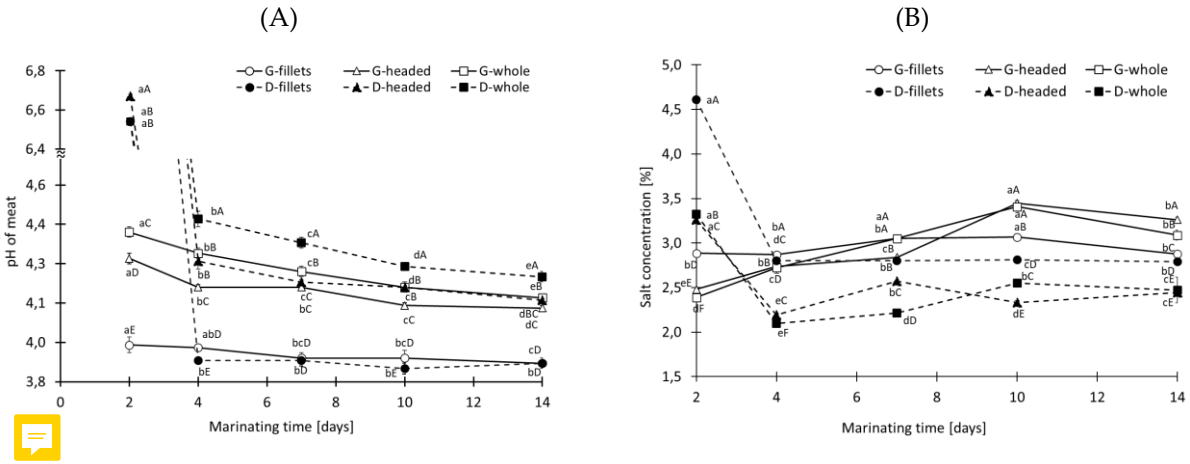


Figure 3. Effect of marinating method (G- – German, D- – Denmark) and degree of primary processing of Baltic herring (whole, headed, fillets) on **(A)** mass yield and **(B)** moisture during marinating. [abc]Results marked with the same lower case letter do not differ statistically by the influence of the refrigerated storage time; [ABC]Results marked with the same capital letter do not differ statistically by the influence of the marinating method

3.2. Activity of digestive proteases and cathepsins

During marinating fillets, mainly muscle proteases - cathepsins - were active, while during marinating whole and headed herring, digestive proteases such as trypsin, chymotrypsin and carboxypeptidases also diffused into the meat. The results showed that digestive proteases were already active in fresh herring meat (0 day of marinating) and their activity decreased during marinating (Fig. 4), mainly due to a decrease in pH [6] After 4 and 10 days of marinating, trypsin esterase activity was higher ~~in~~ marinades made using the German method than the Danish method, and G-whole marinades had the highest activity after 4-14 days of marinating (11.95-20.12U) (Fig. 4A). Chymotrypsin activity was lower in marinades from fillets than from whole or headed herring, with the exception of day 4 (Fig. 4C). Chymotrypsin activity decreased the most after 2 days of marinating using the German method or after 4 days of marinating using the Danish method. On subsequent days, chymotrypsin activity increased, especially in marinades made from whole and headed herring and more so in Danish than German marinades. Also, carboxypeptidase-A activity decreased by 2-4 days of marinating, except for fillets where activity decreased by 10-14 days (Fig. 4D). Between 4 and 14 days of marinating, an increase in carboxypeptidase-A activity was noted in marinades made by both methods from whole herring. D-whole and G-whole marinades from days 7 to 14 had significantly the highest carboxypeptidase-A activity, 9.22-9.54U and 6.89-8.95U, respectively. Marinating time correlated significantly positively with trypsin esterase activity in G-whole (0.956) and D-

whole (0.726) (Fig. 2EF). There was also a significant positive correlation between marinating time and all digestive proteases in G-whole marinades (Fig. 2E), except for Cp-A activity, which correlated negatively with marinating time in fillets (G-fillets -0.842, D-fillets -0.739) (Fig. 2EF). Salt concentration and pH value in marinades significantly correlated with all proteases in G-whole and D-whole marinades (Fig. 2EF) and in D-fillets and D-headed marinades (Fig. 2BD). It can be noted that the marinating of whole herring with intestines contributed to greater digestive proteases activity in meat than the marinating of fillets obtained after cold stored whole herring [6].

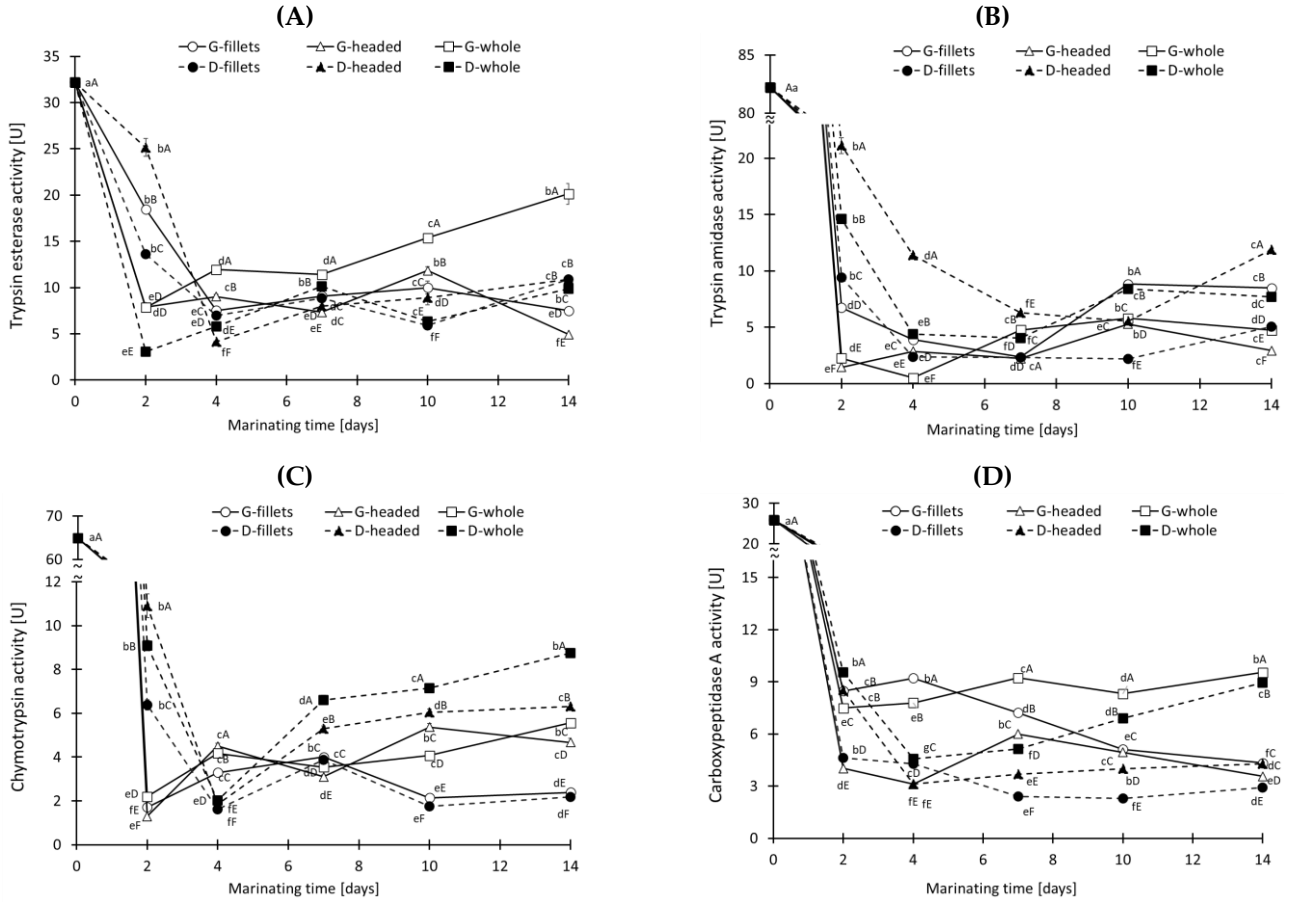
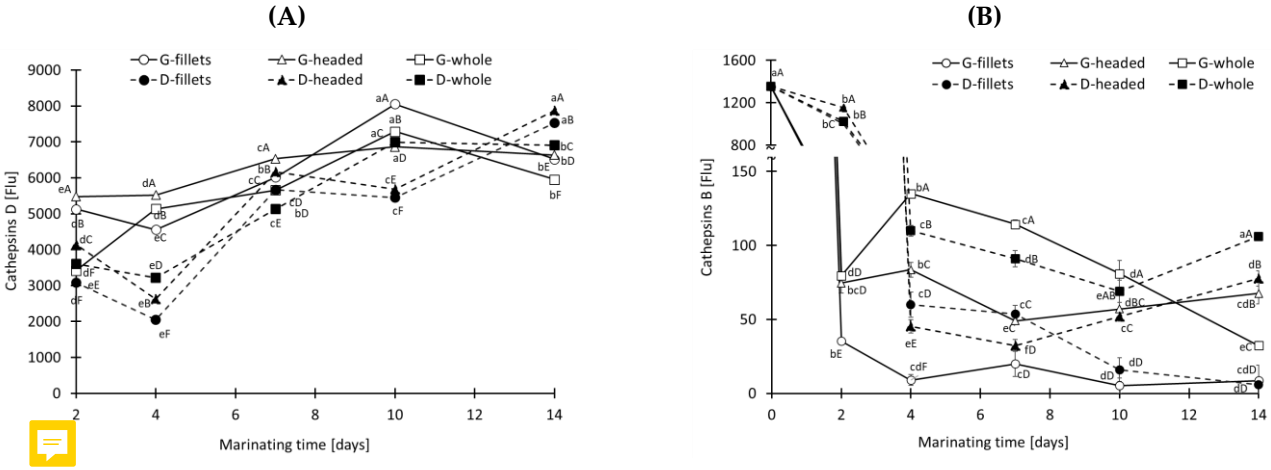


Figure 4. Effect of marinating method (G- – German, D- – Denmark) and degree of primary processing of Baltic herring (whole, headed, fillets) on activity of **(A)** trypsin esterase, **(B)** trypsin amidase, **(C)** chymotrypsin and **(D)** carboxypeptidase-A during marinating. [abc]Results marked with the same lower case letter do not differ statistically by the influence of the refrigerated storage time; [ABC]Results marked with the same capital letter do not differ statistically by the influence of the marinating method

Cathepsin D activity in marinades made by the German method increased 3-4 times after 4-7 days of marinating, while in marinades made by the Danish method it increased 2 to 3 times (Fig. 5A). Therefore, cathepsin D activity significantly positively correlated with marinating time in all samples (Fig. 2). German marinades had maximum cathepsin D activity (6987 - 8051U) after 10 days, while in Danish marinades its activity increased until the 14th day, reaching 6913-7867U. Low pH along with the lowest possible salt concentration are required to activate cathepsin D [2]. In G-headed and G-whole samples, cathepsin D activity increased with decreasing pH, as confirmed by a significant correlation -0.793 and -0.861, respectively. In Danish marinades after 2 days, the salt concentration was about 1.5 times higher than in German marinades, while the pH was close to

neutral compared to strongly acidic in German marinades. Therefore, it is likely that the lowest cathepsin D activity in Danish marinades was due to the inhibitory effect of salt and the lack of activating acetic acid during the first two days of brining [31]. Cathepsin D activity is important for the ripening of marinated meat, because this endoprotease prepares substrates for the other cathepsins [32]. Cathepsin L activity after 2 days of marinating the fillets increased twice to 612-683U, after which it decreased to 170-380U (Fig. 5B). In marinades from headed herring, cathepsin L activity increased 6.5 times after 2 days in the German method (1679U) or after 7 days in the Danish method (1692U). In marinades from whole herring, cathepsin L activity increased as much as 8 and 9 times after 4 days in the Danish (2000U) and German (2260U) methods, respectively, and then decreased, reaching 1400-1499U after 14 days of marinating. Cathepsin L, like cathepsin D, is also an acidic endopeptidase, but requires the presence of salt and a higher pH for optimal activity [2]. Therefore, cathepsin L activity correlated positively with the pH value only in G-fillets (0.593), G-headed (0.964) and G-whole (0.736) marinades. In turn, salt concentration correlated significantly with cathepsin L activity for all marinades except G-fillets. In the case of cathepsin B, the activity of this endopeptidase decreased 20-40 times after 2-7 days of marinating, the fastest in G-fillets marinades and the slowest in D-whole marinades (Fig. 5B). The cause was the strong inactivation of cathepsin B by salt and acetic acid [2]. Thus, the results showed that the activity of digestive proteases and cathepsins was higher in marinades made from whole and headed herring than from fillets, especially after 2-7 days of marinating. The higher cathepsin activity in marinades from ungutted herring was possibly related to (i) the release of cathepsins from lysosomes by digestive proteases [6, 33], (ii) the reduction of cathepsin loss from meat to brine by the herring skin [2], (iii) the slower diffusion of acetic acid into meat [31], which inhibits the activity of alkaline digestive proteases. Also, higher cathepsin D activity may have promoted the release of intracellular proteases [34].



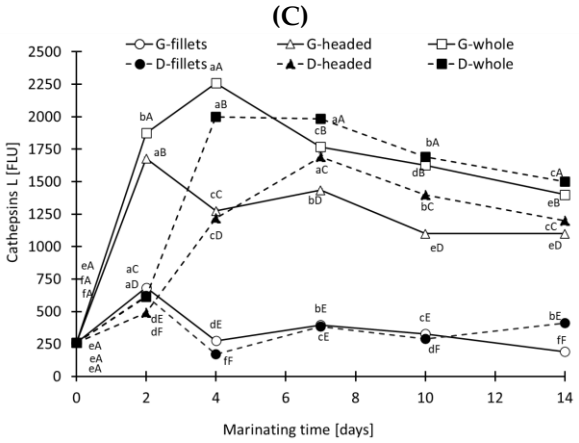


Figure 6. Effect of marinating method (G- – German, D- – Denmark) and degree of primary processing of Baltic herring (whole, headed, fillet) on activity of **(A)** cathepsin L, **(B)** cathepsin D and **(C)** cathepsin B during marinating. [abc]Results marked with the same lower case letter do not differ statistically by the influence of the refrigerated storage time; [ABC]Results marked with the same capital letter do not differ statistically by the influence of the marinating method

3.3. Protein hydrolysis products (PHP)

The process of enzymatic ripening of herring meat leads to the formation of TCA-soluble proteins, peptides and amino acids, the quantitative composition of which allows to assess the dynamics of the ripening process of marinades (36, 3]. The average NPN content of fillets marinated 2-14 days was significantly lower by 66-91% than in marinades from whole or headed herring (Fig. 6A). NPN content in fillets after 2 days decreased to 156 and 117 mg in the German and Danish methods, respectively. The reason for the decrease in NPN content in the German method was the diffusion of NPN from the meat into the marinating brine [29], while in the Danish method there was an additional increase in moisture during salting stage. As a result, fillets marinated 2 days contained 16-18% less NPN than the raw material. This phenomenon did not occur in whole or headed herring, where diffusion was limited by the skin, causing these marinades to contain 344-356 mg of NPN after 2 weeks, 60-65% more than the raw material (Fig. 6A). The higher NPN content was also due to higher proteases activity in marinades form whole and headed herring.

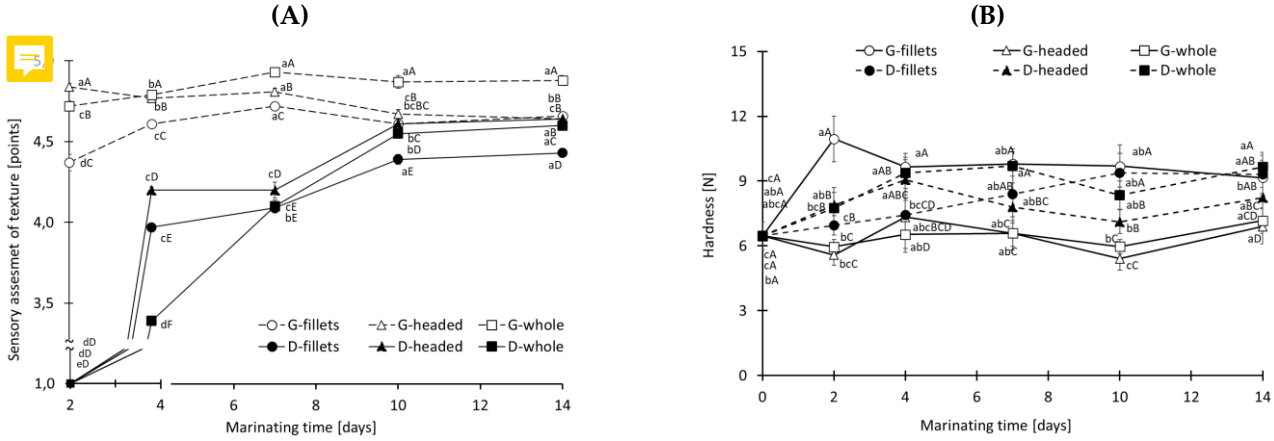


Figure 7. Effect of marinating method (G- – German, D- – Denmark) and degree of primary processing of Baltic herring (whole, headed, fillets) on **(A)** texture sensory evaluated and **(B)** hardness evaluated by TPA. [abc]Results marked with the same lower case letter do not differ statistically by the influence of the refrigerated storage time; [ABC]Results marked with the same capital letter do not differ statistically by the influence of the marinating method

The peptides fraction content of G-fillets marinades increased significantly only up to the 4th day reaching 92-103 mg (Fig. 6B), which is characteristic for German method [31]. On the other hand, during marinating of whole and headed herring, the peptides content increased up to the 10th day reaching 232-234 mg PHP(R). In the case of the Danish method, the peptides content decreased by a few mg after 2 days due to an increase in the moisture of the salted meat, after which it increased sharply to 7 days in fillets and headed herring or to 14 days in whole herring. After 7-10 days, marinades from fillets and headed herring obtained by the Danish method contained more peptides compared to the German method (Fig. 6B). Peptides content was not significantly different after 7-10 days for whole herring marinated by both methods. This shows that the inhibition of cathepsin D by salt and the lower dynamics of mainly peptides formation in the Danish method than in the German method were balanced by high digestive proteases activity. Also, the content of the amino acids fraction changed during marinating similarly to the content of NPN and peptides. After 7 days of marinating, G-fillets marinades contained the least PHP(A) (55.8mg), followed by D-fillets (73.7mg), G-whole (81.6mg), G-headed (90.3mg), D-whole (96.9mg), and the most D-headed 106.1 mg (Fig. 6C). For the amino acids fraction, the increase in content was characteristic up to 7-10 days in fillets, while up to 14 days in marinades from ungutted herrings.

Statistical analysis showed that NPN, peptides and amino acids content correlated in G-fillets marinades mainly with cathepsin D (0.637-0.931) and Am-Tp (0.633) activity, while in G-headed additionally with chymotrypsin activity (0.610-0.705), and in G-whole marinades additionally with Es-Tp and Cp-A activity (0.580-0.946) (Fig. 2ACE). For the Danish method, PHP content correlated in D-fillets marinades also mainly with cathepsin D activity (0.565-0.746), in D-headed additionally with cathepsin L (0.704-0.935), and in D-whole additionally with Es-Tp (0.725-0.815) (Fig. 2BDF). Thus, the lower degree of preliminary processing of herring promoted more significant correlations of digestive proteases with PHP and with higher cathepsin D activity. The results showed a synergistic effect of digestive and muscle enzymes in the proteolysis of marinated herring proteins (Fig. 2), confirming the results of the Kamiński et al. [6].

3.4. Hardness-TPA and sensory assessment of marinated meat

~~For~~ two weeks of marinating, the highest texture rating in sensory analysis ~~was~~ given to marinades made using the German method, with the exception of the 14th day in the case of marinades made from headed herring (Fig. 7A). G-whole marinades scored highest in texture, with scores ranging from 4.79 to 4.93 after 4-14 days of marinating. D-fillet received the lowest marks of 4.20-4.43 for texture. German marinades needed only 4-7 days to achieve maximum marks for texture, while Danish marinades needed at least twice as long. Texture profile analysis (TPA) showed that the hardness-TPA of the meat of G-whole and G-headed marinades averaged 6.5 N, while the hardness of G-fillets marinades was significantly higher at 9.6 N (Fig. 7B). On the other hand, in the case of the Danish method, the hardness-TPA of the marinades increased to 4-10 days obtaining higher values than the German G-headed and G-whole marinades. This could have been due to the lower activity of cathepsins L and D in Danish than German mariners. Cathepsin L hydrolyzes collagen, which has a greater impact on meat hardness than muscle proteins [35], which are mainly hydrolyzed by cathepsin D [37]. It was observed that cathepsin L activity correlated positively with sensory evaluation of texture in marinades from herring with intestines (G-headed 0.847, D-headed 0.861, D-whole 0.779), contrary to a negative correlation in marinades from fillets (G-fillets -0.709, D-fillets -0.772). Although both marinating methods had a positive effect of marinating with digestive proteases on improving meat texture, significant hardness-TPA correlations with texture in sensory analysis were only for G-whole (0.620) and D-whole (0.568) marinades (Figure 2EF).

The results of sensory analysis additionally showed that a bitter taste appeared in the D-whole and D-headed marinades after 4 days of ripening, the intensity of which increased during marinating (data not shown). The bitter taste was not present in D-fillets

marinades, indicating that it was caused by the intestines of the fish. In the case of the German method, the bitter taste also appeared, but only after 10 days of marinating in G-whole and G-headed marinades. The bitter taste could have come from blood from the gastrointestinal tract or from bitter peptides, which are mainly released by chymotrypsin [38]. Chymotrypsin activity after 10-14 days of marinating was highest just in D-whole and D-headed marinades, then significantly lower in G-whole and G-headed marinades, and lowest in fillets marinades (Fig. 4C). In turn, carboxypeptidase-A, which is known to reduce bitter taste [39], was most active in G-whole marinades (Fig. 4D), which likely protected these marinades from the rapid appearance of bitter taste.

3.5. ~~Microflora~~ of herring

Contamination of fresh herring meat with psychrophilic bacteria was 6.60 log cfu/g, mesophilic bacteria <0.1 ~~log~~, moulds and yeasts <0.1 ~~log~~ and bacteria Enterobacteriaceae 3,00 log cfu/g (data not shown). After marinating, microbiological contamination of the four groups of bacteria was below 0.1 log, except for psychrophile contamination after 2 days of Danish marinades: D-fillets 5.46, D-headed 6.30 i D-whole 5.28 log cfu/g. Microbiological analysis showed that the addition of acetic acid completely inhibited the proliferation of microorganisms in marinated meat, including Enterobacteriaceae from the digestive tract of the herring. Acetic acid among other organic acids used in marinades has the strongest bacteriostatic effect [40]. Also Kamiński et al. [6] showed that Enterobacteriaceae bacteria were the most resistant to marinating, but did not pose a threat to the microbiological quality of the marinades.

4. Conclusions

Nowadays, marinating is used not only to preserve herring, but also allows to create unique sensory and health-promoting characteristics of fish products. The development of new marinating technologies will make it possible to meet the new needs of consumers and processors. Studies have shown that marinating whole herring using the German method increased the mass yield of marinated fillets by several percent, which was previously only possible using the Danish method, which has several disadvantages: it increases the salt content of the diet, inhibits the ripening of the meat and promotes a bitter taste. Marinating of whole herring reduces the occurrence of these defects. In addition, marinating of ungutted herring promoted a longer and greater increase in the content of protein hydrolysis products (PHP), which give marinades their characteristic flavor and exhibit biological activity. The higher PHP content was due to two phenomena: higher proteolytic activity in the meat and reduced PHP loss from the meat to the brine. Probably, the greater variety of hydrolytic specificity of proteases in marinades from ungutted fish increased sensory evaluation, compared to marinated fillets. The high activity of digestive proteases made it possible to reduce the marinating time to 4 days compared to 7 days for classic German method marinating fillets to achieve similar meat texture and flavor quality.

The results showed that the contribution of the most important cathepsin D to meat ripening can be supplemented or even largely replaced by digestive proteases, but the right conditions for their activity must be applied. This is crucial knowledge in the case of marinating herring of low technological quality or non-herring fish resistant to marinating, whose meat does not reach full ripeness.

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