Inorganic salts and the functional properties of fresh and frozen muscle proteins

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Changes in protein solubility /PS/ and in hydration /WHC/ were followed in minced cod meat washed with distd. water and contg. added NaCl, KCl, $MgCl_2$, $CaCl_2$, and phosphates. Water extd. fresh mince has a high WHC, ca.21g H_2O/g myofibrillar proteins. At 1-5 mM of added salts per 100g, in the pH range 5,5-9,5 WHC drops markedly. In minces stored 24 hr at 4°C and pH 5,4-7,5 the PS decreases with increasing acidity. At pH 5,4 the drop is higher in presence of chlorides in low concns. Larger addns. of salts decrease the PS also at higher pH. In water extd. and frozen minces after 2 weeks at -20°C at pH 7,2 and 6,4 the PS is by 25% and 50% lower, resp., than before freezing. Low amts. of NaCl and $MgCl_2$ /μ=0,15/ added to the mince prior to freezing have no effect on PS at pH 6,0-7,2, while KCl and $CaCl_2$ at pH 7,2 decrease the PS, by 25% and 35%, resp. At $\mu > 0{,}25$ KCl, phosphates, and KCl+$CaCl_2$ have during 2 weeks a protective effect on PS. In water extd. mince PS decreases after 1 day at -20°C by 15%, which corresponds to a drop in PS in unextd. samples at pH 6,9 only after 3 weeks. A mixt. of salts added to the water extd. mince, corresponding in compn. and μ to those in cod flesh brings about after prolonged frozen storage a drop in PS much higher than that in extd. samples.

Der Einfluss von inorganischen Salzen auf die funktionellen Eigenschaften der Muskelproteine

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Proteinlöslichkeit /PS/ und Hydrat^{ta}ion /WHC/ wurden im zerkleinerten Kabeljaufleisch /Farce/ das mit destilliertem Wasser extrahiert und mit NaCl, KCl, $MgCl_2$, $CaCl_2$ und Phosphaten versetzt worden war, gemessen. Frische extrahierte Farce hat ein hohes WHC, rund 21g H_2O/g myofibrillären Proteine, während Zugabe von 1-5 mM Salze pro 100g beim pH 5,5-9,5 die Hydratation wesentlich herabsetzt. In Farcen nach 24 St. bei 4°C und pH 5,4-7,5 ist die PS kleiner bei niedrigerem pH, insbesondere bei Salzzugabe. In extrahierten und gefrorenen Farcen ist die PS nach 2 Wochen bei -20°C und pH 7,2 oder 6,4 um entsprechend 25% und 50% niedriger als in ungeforenen Proben. Kleine Zugaben von NaCl und $MgCl_2$ /μ=0,15/ vor dem Gefrieren haben keinen Einfluss auf die PS bei pH 6,0-7,2, aber KCl und $CaCl_2$ bei pH 7,2 setzen die PS entsprechend um 25% und 35% herab. Bei $\mu > 0{,}25$ stabilisieren KCl, Phosphate und KCl+$CaCl_2$ die PS während 2 Wochen bei -20°C. In extrahierten Farcen fällt die PS bei -20°C um 15% nach 1 Tage und in nichtextrahierten Proben bei pH 6,9 erst nach 3 Wochen. In extrahierten Farcen, die mit einer Salzmischung, die qualitativ und quantitativ etwa der im Ausgangsmaterial entspricht, ist nach einer längeren Gefrierlagerung die PS wesentlich kleiner als in den extrahierten Kontrollproben.

4.4

Sels minéraux et les proprietés fonctiornelles des protéines musculaires frais et congélés

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Les changements de la solubilité /PS/ et de l'hydratation /WHC/ des protéines étaient examinés dans la chair de merlu concassée qui était lavée dans l'eau distilleé, et à qui on ajoutait des NaCl, $MgCl_2$, $CaCl_2$ et de phosphates. Lorsqu'on ajoute 1-5 mM de sel par 100g à pH 5,5-9,5, la WHC diminue considérablement. Dans la chair conservée à 4°C et à pH 5,4-7,5, la PS diminue au cours de l'augmentation de l'acidité. La diminution est plus considérable à pH de 5,4 en présence des chlorides à basse concentration. L'addition accrue de sel fait diminuer la PS également à pH plus élevés. Pour la chair extractée de l'eau et congelée pendant 2 semaines à -20°C et à pH de 7,2 et 6,4 la PS est resp. 25% et 50% inférieuere par rapport à sa valeur précedente. Les basses quantités de NaCl et $MgCl_2$ μ=0,15 ajoutées à la chair avant la congèlation n'ont pas d'éffets sur la PS dans pH de 6,0-7,2. Cependant, KCl et $CaCl_2$ à pH 7,2 font diminuer la PS resp. de 25% et 35% environ. A μ > 0,25 le KCl, les phosphates et les KCl+$CaCl_2$ exercent un éffet protéctif sur la PS. Dans la chair extractée de l'eau la PS diminue de 15% après un jour à -20°C. Un mélange de sels ajouté à la chair traitée par l'eau correspondant à la composition et à la force ionique de celuí de la chair de merlu provoque après une plus longue conservation sous forme congelée une diminution de la PS beaucoup plus considerable que dans les échantillons traitées par l'eau.

Неорганические соли и функциональные свойства свежих и мороженных мышечных белков

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Изменения растворимости белков /Р.Б./ и гидратации исследовались в раздробленном мясе трески /фарш вымытый водой/ содержащем добавки **KCl, NaCl, $MgCl_2$, $CaCl_2$** и фосфатов. Добавление 1-5 **mM** соли на 100**гр.** мяса при **pH** 5,5-9,5 вызывает значительное уменьшение гидратации. В фарше после 24 часов при 4°C и **pH** 5,4-7,5 РБ уменьшается с понижением **pH**. При **pH** 5,4 спад РБ выше в присутствии хлоридов в низких концентрациях. Высшая концентрация солей снижает РБ также при высших **pH**. В вымытом водой и замороженном фарше после 2 недель при -20°C при **pH** 7,2 и 6,4 РБ соответственно падает на 25% и 50%. Добавление в фарш перед замораживанием **NaCl** и $MgCl_2$ до μ = 0,15 не влияет на РБ при **pH** 6,0-7,2 по сравнению с фаршем замороженном без соли. Зато **KCl** и $CaCl_2$ при **pH** 7.2 снижает РБ соответственно на 25% и 35%. При μ > 0,25 **KCl**, фосфаты и **KCl + $CaCl_2$** оказывают защитный эффект на РБ в течение 2 недель хранения при -20°C. В фарше вымытом водой РБ падает на 15% спустя день хранения при -20°C. Соли добавленные в фарш вымытой водой в композиции и концентрации в которой выступают в свежем мясе трески вызывают снижение РБ ниже чем в невымытых пробах.

4.4

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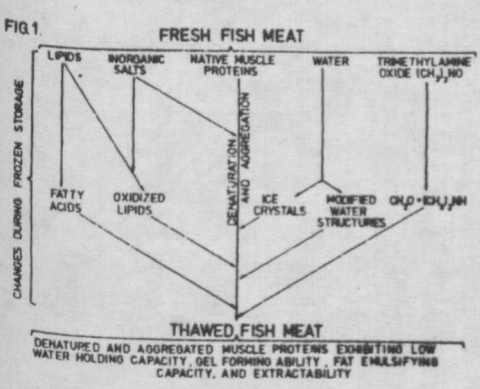
Introduction

The technological value of frozen muscle foods decreases during long-term storage due to deterioration of the water and fat binding capacities and the ability to form gels after heating. These undesirable changes, especially pronounced in fish minces, are caused by alterations in proteins which comprise both partial deconformation of the native molecules and creation of new bonds leading to the formation of different protein-protein and protein-lipid aggregates. These adverse changes in muscle proteins have been investigated in model systems as well as in different frozen meat and fish products. The current concept is that they are caused by concurrent action of several factors inherent in different degrees in various raw materials /Fig.1/. There is considerably large pool of experimental evidence regarding the effects induced in frozen systems by lipids with their oxidation products and formaldehyde produced by decomposition of trimethylamine oxide in fish, as well as on the protective action executed by many highly hydrophilic additives, antioxidants, and neutral lipids. The deteriorative changes in frozen protein systems are considered also to be caused by disintegration of tissue compartmentization, the action of ice crystals, alterations in water structures, and the influence of inorganic salts which undergoe concentration due to freezing-out of water. The complex interactions of tissue salts with muscle proteins and other meat components participating in the freezing changes have been, however, less thoroughly investigated, mainly in protein solutions as model systems /Snow 1950, Buttkus 1970/ and are rather only generally treated in recent reviews /Sikorski, Olley and Kostuch, 1976, Partmann 1977/. This study was undertaken to furnish more evidence on the factors influencing the participation of inorganic salts in the deteriorative changes in muscle proteins during frozen storage.

FIG.1



Experimental

Various amounts of inorganic salts were added to washed or unwashed Baltic cod mince of

FIG.2

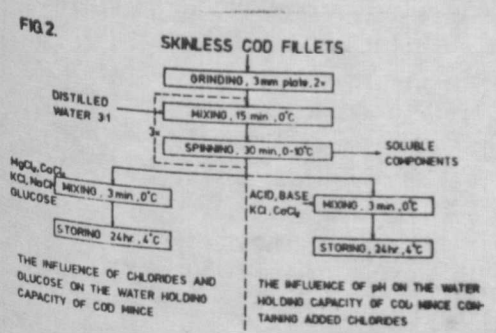


FIG.3 THE INFLUENCE OF IONIC STRENGTH, pH, AND TIME OF STORAGE ON PROTEIN EXTRACTABILITY IN COD FLESH

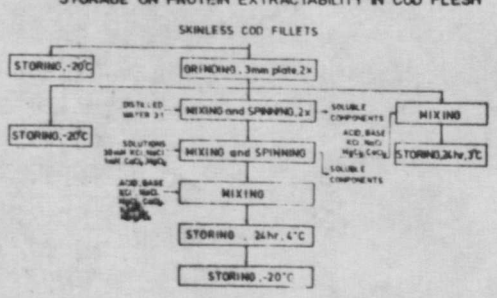


FIG.4. THE INFLUENCE OF CHLORIDES AND GLUCOSE ON THE WATER HOLDING CAPACITY OF WASHED COD MINCE

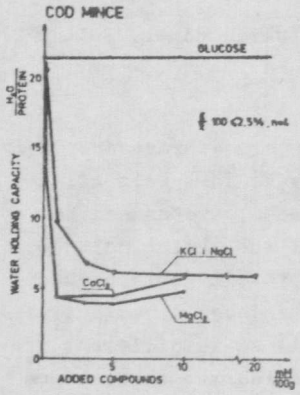
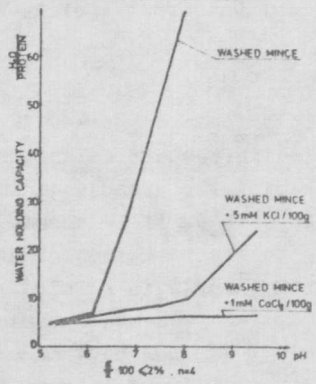


FIG.5. THE INFLUENCE OF pH ON THE WATER HOLDING CAPACITY OF WASHED COD MINCE CONTAINING LOW ADDITIONS OF CHLORIDES



different pH and their influence on protein solubility /PS/ and water holding capacity /WHC/ was determined in the fresh state or after frozen storage. The PS was assayed in homogenized samples at constant pH 7.5 and ionic strength 0.9. WHC was determined by centrifuging the meat:water slurries /9:1/. The flow sheets of the experimental procedures are given in fig.2 and 3. All results presented below are given as mean values of at least 4 determinations in samples taken from 1 portion of mince.

Results and discussion

Water extracted fresh mince has a high WHC, about 21g H_2O/g proteins. The decrease of WHC observed in samples containing added salts /Fig.4/ apparently does not depend upon the osmotic pressure, as addition of up to 20 mM glucose/100g of mince has no significant influence on the measured values. Lower WHC in samples containing added salts, as compared to those in washed control /Fig.5/ may result in the alkaline range of pH by neutralization of ionized groups in the side chains of proteins. The observed very low hydration of the meat in presence of $CaCl_2$, however, is probably caused additionally by crosslinking via chelate formation with carboxyl and imidazole groups of proteins /Marguerie, Chagniel, and Suscillon, 1977/. In minces stored 24 hr at 4°C PS decreases with the change in pH from 7.2 to 5.5 /Fig.6/. The same is also true in samples containing 0.2M of added NaCl or KCl/100 g, while in the presence of $MgCl_2$ and $CaCl_2$ in the same concentration PS is very significantly lower than in the control and does not depend so much upon acidity of the mince /Fig.7/. The loss in solubility in the low range of pH is probably caused by interference of the added salts with water structures, while in the alkaline range it is induced additionally by the binding of divalent cations to ionized and polar groups in proteins /Hippel and Schleich, 1969, Kołodziejska and Sikorski, 1976/. The observed larger effect caused by KCl than by NaCl /Fig.8/ may result from the fact that Na, being a smaller cation, can less effectively change the native protein-water structure /Lewin, 1974/. A similar relationship was found also in minces containing divalent cations - Mg^{2+} exhibits a lower effect than Ca^{2+} at the same concentration. In water extracted and frozen minces after 2 weeks at -20°C at pH 7.2 and 6.4 the PS is by 25% and 50% lower, respectively, than before freezing /Fig.9/. Apparently by ionization of the side chain groups in the higher pH range and increased binding of water by proteins the deteriorative freezing changes are more effectively retarded than they are enhanced

FIG.6. THE INFLUENCE OF pH ON PROTEIN EXTRACTABILITY IN COD MINCE KEPT 24 hr AT 4°C

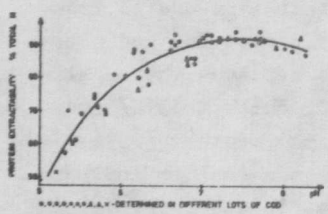


FIG.7. THE INFLUENCE OF pH ON PROTEIN EXTRACTABILITY IN COD MINCE CONTAINING ADDED CHLORIDES, AFTER 24 hr AT 4°C

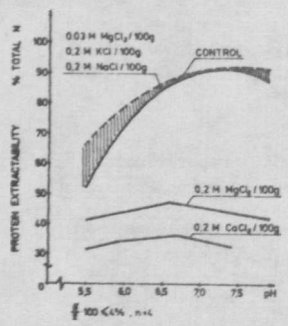
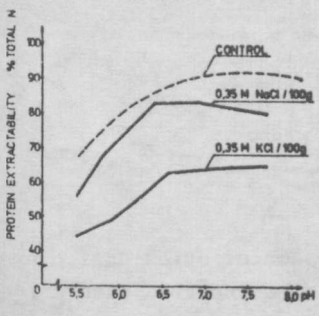
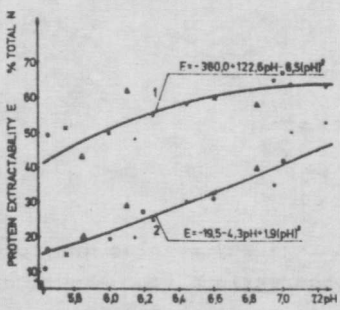


FIG 8. THE INFLUENCE OF pH ON PROTEIN EXTRACTABILITY IN COD MINCE CONTAINING ADDED SALTS, AFTER 24hr AT 4°C



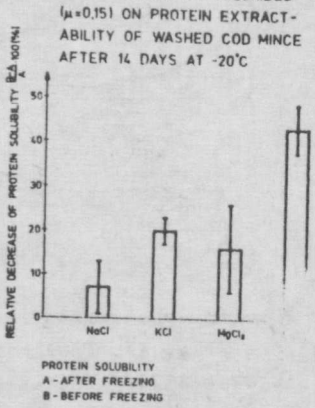
$\bar{x}$ 100 ⩽ 3,5% , n=4

FIG 9. THE INFLUENCE OF pH ON PROTEIN EXTRACTABILITY IN WASHED COD MINCE KEPT 14 DAYS AT -20°C



- DETERMINED IN DIFFERENT LOTS OF COD
1 - PRIOR TO FREEZING
2 - AFTER FROZEN STORAGE

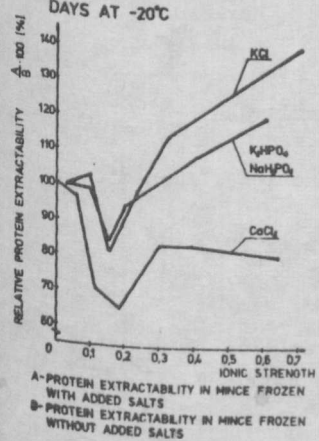
FIG 10. THE INFLUENCE OF CHLORIDES (μ=0,15) ON PROTEIN EXTRACTABILITY OF WASHED COD MINCE AFTER 14 DAYS AT -20°C



PROTEIN SOLUBILITY
A - AFTER FREEZING
B - BEFORE FREEZING

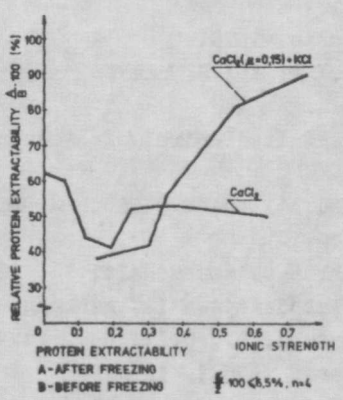
by binding of fatty acids /Hanson and Olley, 1965/. In unwashed samples such results could be also attributed to inhibition of demethylation of TMAO and binding of formaldehyde by proteins /Sikorski, Kostuch, and Kołodziejska, 1975, Kostuch and Sikorski, 1977/. Inorganic salts added prior to freezing to the washed mince in low amounts, corresponding to ionic strength 0.15, induce changes in PS. The decrease in PS depends both on the valence and the radius of the cations. /Fig.10/ and is independent on pH in the range 6.0-7.2. At higher concentrations /μ >0.25/ KCl, phosphates, and a mixture of KCl + $CaCl_2$ have during 2 weeks a protective effect on PS /Fig.11 and 12/. This phenomenon is apperently related to the favourable influence of salts on hydration of proteins as well as the increase in viscosity and retardation of crystallization of water /Fennema, 1973/. A mixture of salts added to the water extracted mince, corresponding in composition and ionic strength to those in cod flesh, caused after prolonged frozen storage a drop in PS much higher than in extracted samples /Fig.13/. Higher decrease in PS in washed mince as compared to that in unwashed samples or fillets may be caused in part by extraction of different inherent cryoprotective substances, including some salts, although washing removes also known denaturing agents, especially TMAO-formaldehyde. The mixture of salts coarsely similar to those originally present in the flesh, added to the washed mince, apparently cannot, however, occupy the original sites in the protein-water structure and thus does not play the same role in the system. Furthermore, the mixture used in our experiments was not identical in composition with that removed during washing. Finally, our results may indicate that in samples containing salts the decre-

FIG 11. THE INFLUENCE OF IONIC STRENGTH ON THE PROTEIN EXTRACTABILITY IN WASHED COD MINCE KEPT 14 DAYS AT -20°C



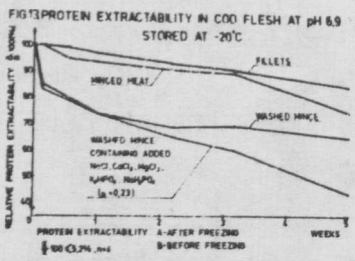
A - PROTEIN EXTRACTABILITY IN MINCE FROZEN WITH ADDED SALTS
B - PROTEIN EXTRACTABILITY IN MINCE FROZEN WITHOUT ADDED SALTS

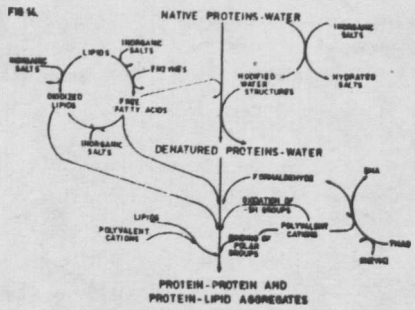
FIG 12. THE INFLUENCE OF IONIC STRENGTH AND $CaCl_2$ ON PROTEIN EXTRACTABILITY IN WASHED COD MINCE KEPT 14 DAYS AT -20°C



PROTEIN EXTRACTABILITY
A - AFTER FREEZING
B - BEFORE FREEZING

$\bar{x}$ 100 ⩽ 3,5%, n=4





ase in PS after prolonged frozen storage may be also due to promotion of autoxidation by the cations /Fig.14/. Preliminary observations from experiments to be reported elsewhere /Kołodziejska, 1979/ indicate, that in samples containing added mixture of salts autoxidation proceeds at a higher rate than in washed controls.

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