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PREPARATION, COMPOSITION AND PROPERTIES OF FISH SILAGE PRODUCED WITH POST-COAGULATION SLUDGE

Post-coagulation sludge was added to fish hydrolyzates (silage). Silage was prepared using whole sprats (*Spratus spratus*). Technology of the process has been modified and sulphuric acid was replaced with phosphoric acid. The objective was to evaluate the quality of produced silage during storage, particularly the changes of peroxide value and amount of free fatty acids in silage fat.

1. INTRODUCTION

The world fisheries and aquaculture supplied about 142 million tons of fish in 2008, of which 115 million tons was used as human food, providing around 17 kg (live weight equivalent) per capita/per person. Aquaculture accounted for 46% of total food fish supply, a slightly lower proportion with respect to that reported in the past. Huge amount of fish and other marine organisms were eliminated as under-utilized species which in most cases were returned into water (FAO, 2010). Total global by -catch is difficult to quantify because of incomplete information and because of the fact that various states define it differently. Nevertheless, the latest published estimate of global discards from fishing is of about 7 million tons [1].

The constant development of the fish industry generates a large quantity of waste, which means that not only considerable quantities of good quality protein are discarded annually but also environmental pollution is increased. Considering 45% of the live weight to be the waste, it can be estimated that nearly 63.6 million tons of waste is generated globally [1]. The major non-edible by-products arising out of fish processing include viscera, skin, scales and bones. In many countries, the fishery sector is unorganized and has problem of disposing fish waste generated as a consequence of

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processing fish for the consumption. This waste is collected and dumped in waste sites which sometimes are left without any form of control. These fish wastes are an important source of proteins and lipids and therefore special efforts are being made to recover these valuable substances [2–5].

Another problem is that in fish processing plants a huge amount of wastewater is generated. The quality of wastewater discharged from fish processing plants depends on the water management system exploited in the plants. One of the indicators which allow estimating the correctness of the management of water and wastewater in the plant is water consumption for particular processing operations and fish products. Saving water while maintaining the technological and sanitary standards may cause lower volume of wastewater with higher pollutant parameters. There is always some water used in such technological operations as fish pretreatment, curing or sterilization while the rest of it gets utilized to maintain the sanitary conditions of the plant as well as freezing [6]. For example, both thawing with water and sterilization of cans discharge lower contamination load than steaming or marinating of fish.

Wastewater from the fish processing industry contains large amounts of fish parts, fatty substances, soluble proteins, non-protein nitrogen compounds, minerals, acetic acid, etc. In wastewater, the concentration of protein and fat can reach 2.6% and 4%, respectively. Such tremendous losses of valuable substances should be avoided at all costs. Wastewater with high content of organic substances has direct influence on sewage collection system and municipal sewer system. However, the worst influence is observed in the work of the municipal wastewater treatment plant. Organic components of food industry wastewater cause putrefaction of sewage sludge, mainly due to presence of protein and make the efficiency of activated sludge much worse (especially fat is responsible for this).

One of the possibilities is to recover them during wastewater treatment [7, 8]. Wastewater from fish processing can be treated in various ways: mechanically, chemically or biologically [9]. Chemical treatment is a coagulation process in which added chemicals (coagulants) form flocks to which dispersed pollutants easily attach [10]. This material is separated from wastewater by sedimentation with aluminum or iron salts usually used as coagulants. Post-coagulation sediment from fish processing wastewater consists of coagulant (in a form of aluminum or ferric hydroxide) and separated pollutants, e.g. proteins, fats and other colloids. However the main component in this sludge is water. Post-coagulation sludge containing nutritious substances can be used as an additive to animal feed production.

One of the promising alternative uses in animal feeding is fish silage which has various industrial applications. It is friendly to the environment, safer, technologically simpler, and more economical than the manufacture of fishmeal [11, 12]. Today silage technology is recognized as being the most useful for solving the waste problem in the fish industry. Fish silage concentrate is also a highly digested protein hydrolyzate which is convenient as a protein supply.

The advantage of fish silage production is that the proteolytic enzymes present in the fish hydrolyze protein and fat and this autolysis, accelerated by weak or strong acids, reaches the highest activity at pH between 2 and 4. In addition at these pH, proliferation of microorganisms is avoided. The production of fish silage on a commercial scale is limited because its high water content makes it difficult to transport the material for long distances. The resultant liquid can then be dried or mixed with cereals, or other carbohydrate sources, and then dried. The dried mixture is easier to manage in the preparation of diets for animal feeding [13]. However, in the areas close to the fish processing plants, silage could be used instead of more expensive animal feeds. Although acid guarantees the stability of the product, pH and storage temperature affect the protein and lipid.

There exists abundant information concerning chemical characterization of silages, and their nutritional evaluation has been carried out on poultry, pigs, fish, and ruminants [14–17]. The nutritional value of balanced diet is determined mainly by essential amino acid composition and ratio. Vegetable protein sources are often deficient in some essential amino acids. However, the composition of these ingredients may be improved by adding protein rich products such as fishmeal or silage [18, 19].

The idea of the work was to supply a certain amount of post-coagulation sludge for silage production. There is lack of data concerning production of fish silage with post-coagulation sludge. There are some literature reports about fish silage production with addition fish waste like: intestines, heads, wastes from fish meal production. The results showed the possibility of production of good quality fish hydrolysates with organic wastes.

Little is known about the chemical changes occurring during the liquefaction and storage of the product, especially with the addition of post-coagulation sludge. Lipid oxidation is an important factor that lowers the quality of fish products, particularly during storage. The purpose of the present study was to determine the changes in the nutritional quality and chemical composition that occur during silage processing when whole fish is used and with addition of post-coagulation sludge.

2. EXPERIMENTAL PROCEDURES

Silage preparation. To produce fish silage, the sprat (*Spratus spratus*) from the Baltic Sea (Gulf of Gdańsk) and the viscera from trout (*Oncorhynchus mykiss*) were used. The sprat and trout viscera was passed through a meat grinder with disc holes of 5 mm. The silage was acidified to pH 2 with sulfuric or phosphoric acid (1 wt. %). As a preservation agent, sodium metabisulfite was used (1.3 wt. %). The hydrolysis was carried out at 15 and 32 °C during 30 days. The temperature of 32 °C was chosen because of the highest activity of enzymes in fish at this particular temperature. The

temperature 15 °C was the ambient temperature. For some groups of silage, the post-coagulation, 20% of sludge was added, the amount which resulted in the best quality of fish silage.

In our experiments, the coagulation of fish processing wastewater was performed with the use of ferric sulfate. When the post-coagulation sludge is added to hydrolyzates, iron hydroxide is converted into ferric sulphate, which is well soluble in water. Too high content of soluble iron compounds being easily absorbable should be avoided, as the excessive amount of the iron in the diet is harmful. Therefore in selected silages, sulphuric acid was replaced with phosphoric acid to make iron non-absorbable. In Table 1, the composition of raw materials used to silage preparation and for further investigation is presented.

Table 1

Composition [%] of raw materials used to silage preparation

Component	Fish	Sludge	Fish + sludge 20%
Water	75.2	90.2	77.4
Fat	6.5	5.5	6.3
Protein	14.05	2.9	13.02
Ash	2.6	1.2	2.5

Post-coagulation sludge was more hydrated than raw fish (water content 90.2%). Comparing the composition of fish material and post-coagulation sludge, it can be observed that the fish contained about twice more protein than in the post-coagulation sludge (in sludge approximately 33%, in fish 60%), while the sludge contains much more fat than raw fish material (sludge 60.5%, fish about 28%). The composition of silages is presented in Table 2.

Table 2

Composition, designation and temperature of produced silages

Sample	Designation	Composition	Temperature of incubation [°C]
1	HSA32	ground raw fish + H_2SO_4	32
2	HPA32	ground raw fish + H_3PO_4	32
3	HSA15	ground raw fish + H_2SO_4	15
4	HPA15	ground raw fish + H_3PO_4	15
5	HSS32	ground raw fish + H_2SO_4 + 20% sludge	32
6	HPS32	ground raw fish + H_3PO_4 + 20% sludge	32
7	HSS15	ground raw fish + H_2SO_4 + 20% sludge	15
8	HPS15	ground raw fish + H_3PO_4 + 20% sludge	15

Chemical analysis. The proximate analysis: moisture, crude fat, protein and ash was carried out on the raw material and silage according to the standard methods. The protein hydrolysis value was measured using 20 vol. % of trichloroacetic acid [20]. The protein hydrolysis value is reported as the proportion of non-protein nitrogen to total nitrogen. Dry weight was determined after drying at 105 °C to constant weight. After drying, the ash was determined after heating at 580 °C. Crude protein was estimated by multiplying total nitrogen by the factor of 6.25. Total nitrogen content was determined by the Kjeldahl procedure. Crude fat after Sohxlet extraction of dry samples with diethyl ether was determined. Free fatty acids (FFA) content was determined by titration with KOH. To determine the peroxide value liberated iodine was treated with sodium thiosulfate.

3. RESULTS AND DISCUSSION

In the first series of experiments, the hydrolyzates were produced only from fish, without sludge. In the next series, the hydrolyzates were produced from fish with the addition of 20% of post-coagulation sludge. The characteristic of hydrolyzates is presented in Table 3. The sludge addition decreases protein content in silage by about 2–3%. Other parameters have not changed significantly.

Table 3

Composition of the hydrolyzates after liquefaction [%]

Sample	Composition	Protein	Fat	Water	Ash
1; 3	ground raw fish + H_2SO_4	14.1	6.2	76.4	2.6
2; 4	ground raw fish + H_3PO_4	14.3	6.3	76.9	2.9
5; 7	ground raw fish + H_2SO_4 + 20% sludge	12.2	6.3	77.4	3.1
6; 8	ground raw fish + H_3PO_4 + 20% sludge	10.9	6.3	78.0	4.2

Protein hydrolysis values are presented in Figs. 1 and 2. Protein solubilisation proceeded during the first five days in all hydrolyzates (independent of the temperature and composition of silage). As expected, the higher temperature resulted in the higher degree of hydrolysis. The degree of hydrolysis in the processes carried out at 32 °C was higher than at 15 °C in the range from 11.6% (for the silage with fish and sulphuric acid) to 21.6% (for the silage with fish and phosphoric acid). The degree of hydrolysis of silage with addition of post-coagulation sludge was about 14% lower at the lower temperature (for both sulphuric and phosphoric acids).

After about 15 days of storage the protein degradation in various hydrolyzates were variable and depended on the type of acid used for hydrolysis and – the first of all – on the temperature of the process (Figs. 1, 2). Only the hydrolyzate produced at 32 °C and with addition of post-coagulation sludge reached the degree of hydrolysis in

close to 45%. The highest value was observed for HPS32 – 48.4%, the lowest for HAS32 – 38%. After storage for the same time at 15 °C, the degree of hydrolysis in all experiments was much lower. The value of protein solubilization does not exceed 33%, and for HPS15 was only 25%.

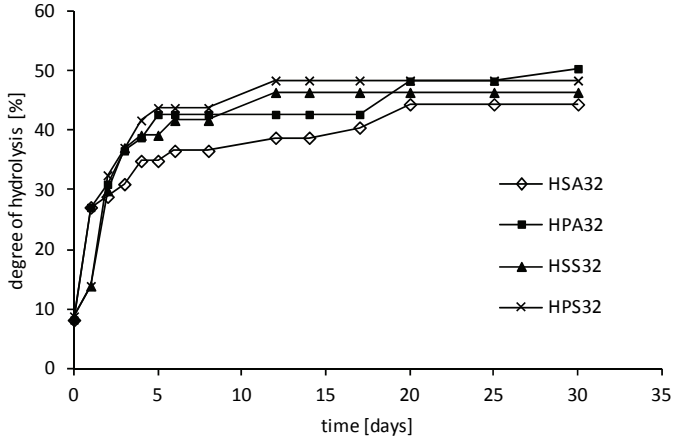

Fig. 1. Time dependences of the degree of hydrolysis in silage stored at 32 °C (cf. Table 2)

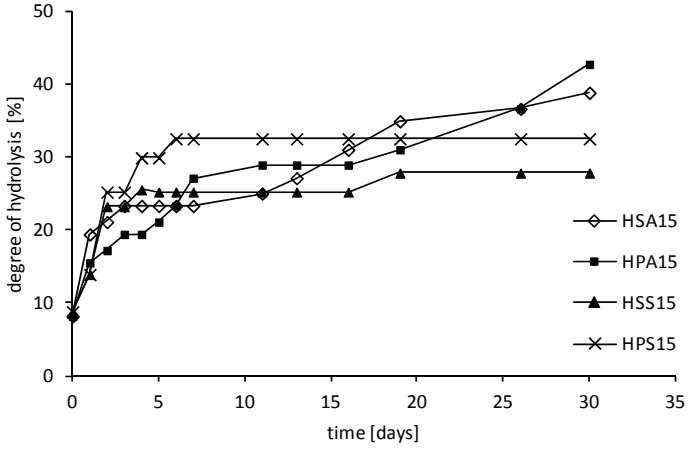

Fig. 2. Time dependences of the degree of hydrolysis in silage stored at 15 °C (cf. Table 2)

After 30 days of storage, only in the hydrolyzate produced with phosphoric acid without post-coagulation sludge at 32 °C over 50% protein has been degraded (50.4% for HPA32). In the other silages produced at higher temperature, the degrees of hydrolysis were lower and ranged from 44.4% (for HSA32) to 48.4% (for HPS31).

In the hydrolyzates produced at 15 °C, only silage with phosphoric acid exceeded 42%.The lowest protein degradation was observed for the mixture with addition of post-coagulation sludge (27.8% for HSS15 and 32.5% for HPS15%). According to our expectations, the higher degree of hydrolysis was obtained for the hydrolyzates at higher temperatures. Regardless of the temperature of the process, a higher degree of hydrolysis finally was obtained in hydrolyzates prepared with phosphoric acid.

Comparing the process of hydrolysis of the raw fish with the process with addition of post-coagulation sludge, it can be stated that in the presence of sludge there is no inhibition of the enzymatic activity (at 32 °C). On the contrary, the final degree of hydrolysis determined as a protein degradation was reached much faster for silage with sludge (after about 12 days) than for only raw fish (25–30 days). It can be suggested that addition of post-coagulation sludge facilitated the hydrolysis process. At 15 °C, negative effect of addition of post-coagulation sludge was observed.

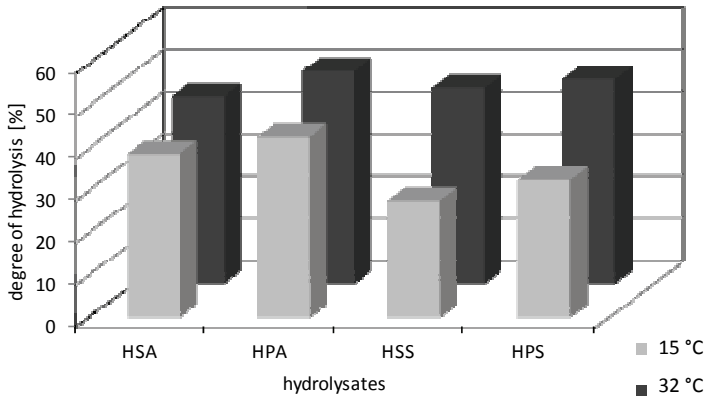

Fig. 3. Degrees of hydrolysis in silage after 30 days of storage at various temperatures (cf. Table 2)

In this study, the highest observed level of degradation of proteins fluctuated around 50% (Fig. 3). This value is lower than that expected based on literature data. According to Raa and Gildberg [21], in the production of hydrolyzates from fish and fish waste, 80% of the protein is converted into a soluble form after about a week. However, research conducted by other scientists has also not achieved such a high degree of protein degradation. It was reported that about 50% degradation of proteins was very often after 30 days, while Usydus [6] obtained at the same time, only 33% of the breakdown of protein.

As already mentioned, addition of post-coagulation sludge can affect changes in fat during storage. Lipid oxidation is an important factor that lowers the quality of fish silage. The temperature has also significant impact on the quality changes of lipids. Therefore, the level of hydrolysis and changes in fat oxidation were determined. Also

the differences, depending on the composition of the mixture of hydrolyzates were recognized.

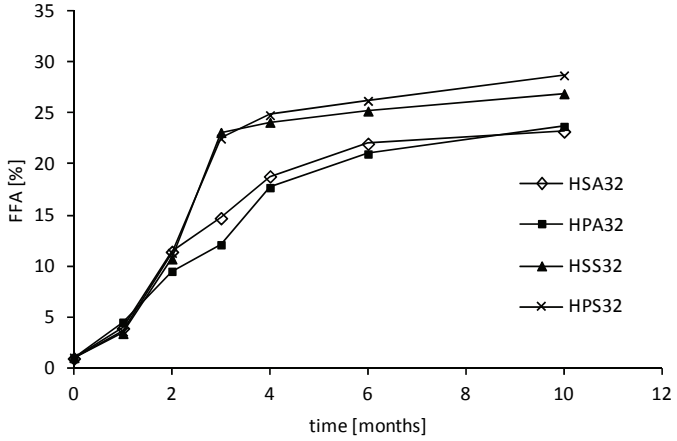

Fig. 4. Free fatty acids (FFA, % of oleic acid) of fish silages obtained with sulfuric or phosphoric acid during storage at 32 °C (cf. Table 2)

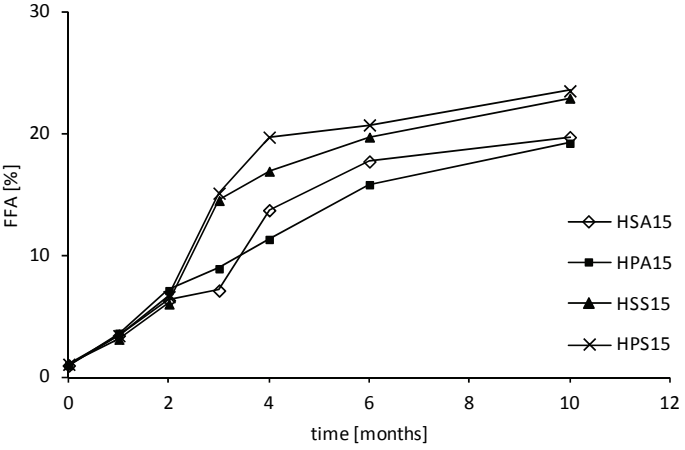

Fig. 5. Free fatty acids (FFA, % oleic acid) of fish silages obtained with sulfuric or phosphoric acid during storage at 15 °C (cf. Table 2)

In Figures 4 and 5, the changes in free fatty acid content (expressed as oleic acid; FFA, % of oleic acid) in the fat separated from hydrolyzates from raw sprat and with addition of post-coagulation sludge at various temperatures are presented. These changes are a measure of decomposition of glycerides under the influence of hydrolyses.

The study showed that fat decomposition was the slowest in hydrolyzates produced from sprat at 15 °C. After 10 months of storage, the content of free fatty acid

(FFA) was 19–20% regardless of the acids used (sulfuric or phosphoric acid (Fig. 5)). Higher content of free fatty acids of ca. 4–6% was observed after the same time in hydrolyzates produced at 32 °C (Fig. 4).

Addition of post-coagulation sludge had visible effect on free fatty acid content during storage. In hydrolyzates produced with 20% residual after 10 months of storage at 15 °C the content of FFA was ca. 22.9% (for HSA15) and about 23.5% (for HPA15). At 32 °C FFA content in hydrolyzates prepared from the raw fish material was higher and amounted to 26.9% and 28.7%, respectively

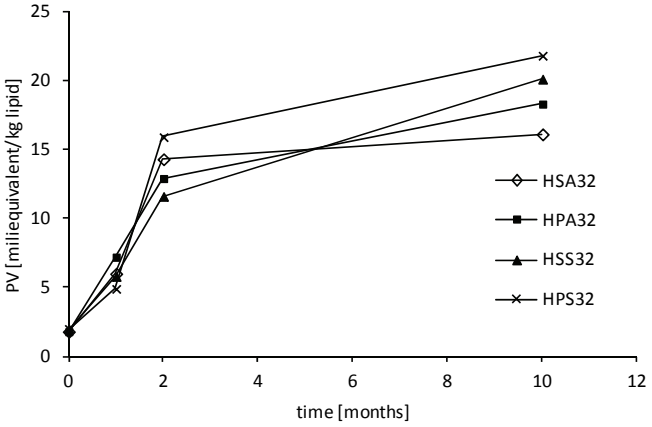

Fig. 6. Peroxide values (PV, miliequivalent/kg of lipid) in oil extracted from silage by various methods at 32 °C (cf. Table 2)

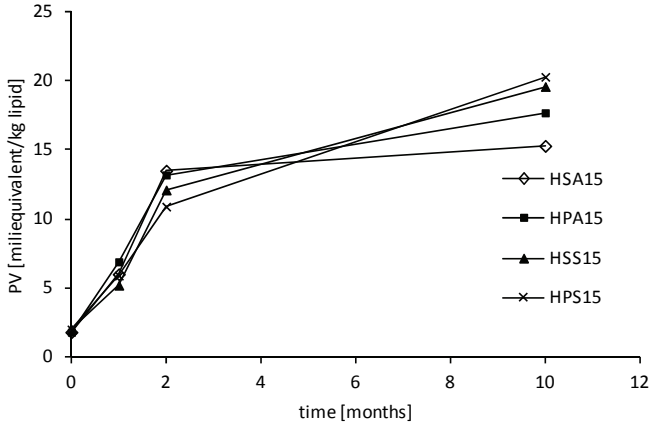

Fig. 7. Peroxide values (PV, miliequivalent/kg of lipid) in oil extracted from silage by various methods at 15 °C (cf. Table 2)

Hydrolysis of fats in hydrolyzates occurred most intensely during the first four months of storage, then the rates of changes slowed down. FFA content in hydro-

lyzates with addition of post-coagulation sludge differed from that without addition (10–15%). In Figures 6 and 7 changes in peroxide value (PV, miliequivalent/kg lipid) are presented.

As can be seen, during storage there are marked lipid oxidation in all hydrolyzates. The rate of oxidative changes was the highest in the first two months. Peroxide value increased in this period from 1.8 to ca. 10–16 (depending on the raw material from which the hydrolyzate was prepared). Further storage (up to 10 months) did not result in significant changes in peroxide value. The lowest level (16.3 miliequivalent/kg of lipid) of the peroxide value was observed in the silage with sulphuric acid without sludge supplementation. The peroxide value increased more intensively in hydrolyzates with the addition of post-coagulation sludge. The value increased by 20–30%. The highest level of the peroxide value (21.8 miliequivalent/kg of lipid) was observed in the silage with phosphoric acid with the sludge.

4. CONCLUSIONS

Utilization of post-coagulation sludge produced in fish processing wastewater treatment plants is one of the most challenging problems due to the high content of protein ant fats. The treatment and disposal of the post-coagulation sludge are very costly in comparison to the total wastewater treatment cost. On the other hand, the disposal of sludge is banned because of high content of organic matter. Therefore, the main attention is focused on developing new technologies for minimizing the post-coagulation sludge production.

Post-coagulation sludge can be used as a supplementary material for fish hydrolyzates production. As expected, a high degree of hydrolysis was obtained for the hydrolyzates when the hydrolysis was carried out at 32 °C. Comparing the process of hydrolysis of the raw fish with the process with addition of post-coagulation sludge it can be stated that in the presence of sludge there is no inhibition of the enzymatic activity (at 32 °C). At 15 °C, negative effect of addition post-coagulation sludge was observed (especially silage with sulphuric acid).

Regardless of the temperature of the process, a high degree of hydrolysis finally was obtained in hydrolyzates prepared with phosphoric acid. The quality of produced silage changed during storage. The increasing peroxide value and amount of free fatty acids pointed at the hydrolysis and oxidation of fats. The higher increasing (ca. 10–20%) was observed for the hydrolysates with post-coagulation sludge addition.

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