sciendo

DOI: 10.2478/9788367405805-017

ICAMS
The International Conference on Advanced Materials and Systems

EFFECT OF LONG-TERM STORAGE ON THE PROPERTIES OF AN ENZYME-CONTAINING PREPARATION FROM FISH WASTE

IRYNA KOPYTINA, OLGA ANDREYEVA, DMYTRO DAVYDIUK
Kyiv National University of Technologies and Design, Mala Shyianovska (Nemyrovycha-Danchenka) Street, 2, 01011, Kyiv, Ukraine, emails: i.v.kopytina@gmail.com; wayfarer14@ukr.net; gysb356@gmail.com

The demand for fish products in Europe is gradually increasing, but at the same time fish stocks are declining due to stress factors for aquatic populations, as a result of illegal and unregulated fishing. At the same time, fish processing generates a significant amount of waste, the discharge of which is unprofitable both for the industry as a whole and for individual entrepreneurs, and, in addition, it provokes environmental pollution. Since fish waste contains microelements and various nutrients, it is a valuable raw material resource that is used in the production of fat, flour, animal feed, cosmetics and pharmaceuticals, etc. In this case, the main emphasis is on the mineral, protein- and fat-containing components of waste, but the presence of enzymes in them is practically not taken into account. Previously, it was reported on the production, study of the properties and the possibility of using an enzyme-containing preparation (ECP) obtained from fish waste for leather processing. The purpose of this study is to determine the effect of long-term (for 4 years, in a refrigerated chamber at a temperature of 7-8 °C) storage on the technological capabilities of this preparation. The experiment was conducted in laboratory conditions on the heifer skin, which was bated with ECP at a rate of 0.2, 0.4, 0.6 and 0.8% of the sample weight. The effect of ECP on the chrome leather indicators was studied using traditional and modern analytical methods. Based on the results of chemical analysis and physical and mechanical tests of leather, the suitability of ECP obtained from fish waste for processing leather at the bating stage was established even after such a long storage.

Keywords: enzyme-containing preparation from fish waste, storage, properties.

INTRODUCTION

Existing fish collection and processing methods produce a significant amount of fish waste worldwide each year. It is estimated that, on average, two-thirds of all fish catches are by-products that are discarded. Fish by-products and processing waste pose serious disposal problems for the fishing industry. Therefore, the waste generated from fish processing must be utilised and treated properly to sustain the industry and preserve the environment. In this regard, numerous instruments have been adopted in the European Union to minimise the environmental impact of fisheries within the framework of Integrated Coastal Zone Management (Sappasith *et al.*, 2024).

Processed fish waste has found many applications, among which the most important are animal feed, biodiesel/biogas, dietary products (chitosan), natural pigments (after extraction), food packaging (chitosan), cosmetics (collagen), chromium immobilisation, soil fertilisation and food moisture maintenance (hydrolysates) (Arvanitoyannis & Kassaveti, 2008). The main attention is paid to mineral, protein- and fat-containing components of wastes, the presence of enzymes in them is practically not taken into account, although fish processing wastes, especially digestive organs, have a huge biotechnological potential as sources of such enzymes as proteases, lipases, chitinase, alkaline phosphatase, transglutaminase, hyaluronidase, acetylglycos-aminidase and others. These enzymes can find a variety of applications in the seafood industry, including isolation and modification of proteins and marine oils, production of bioactive peptides, acceleration of conventional fermentation,

https://doi.org/10.2478/9788367405805-017

cleaning and separation of shellfish, scaling of fish, removal of membranes from fish roe, extraction of flavourings, shelf life extension, texture modifications, removal of extraneous odours, and quality control directly or as components of biosensors. Enzymes from fish and shellfish from cold habitats are particularly useful because they can function at comparatively lower temperatures, thereby saving energy and protecting food products (Venugopal, 2016; Shahidi & Janak Kamil, 2001; Imran, 2022).

Enzymatic methods have become an important and indispensable part of the processes used by modern industry to produce a large range of goods, including leather goods (Kopytina *et al.*, 2022; Kopytina *et al.*, 2023). The production, properties and potential use of an enzyme-containing preparation (ECP) derived from fish waste for leather treatment have been previously reported. The preparation was obtained according to the following scheme: grinding - degreasing - extraction - coarse fractionation of extract proteins with separation from other proteins and impurities by filtration or centrifugation - fractionation - purification. The preparation obtained was a powder of brown coloured fine fibrous substance, which dissolved reasonably well in warm water and had an activity of 450 units/g according to the precipitation method. No complications were detected when using the preparation for bating sheepskin, the obtained leather semi-finished product (pelt) was soft, plastic, with a clean surface grain by organoleptic evaluation (Atamanova *et al.*, 2020). Using chromatographic and electrophoretic analysis methods, the presence of several fractions of active proteolytic enzymes in the preparation was established, and using the IR spectroscopy method, its multifunctional nature (the presence of certain functional groups and bonds), the ability to interact with collagen and the reagents used for its processing (Andreyeva *et al.*, 2001), Since the effectiveness of enzymes and enzyme preparations largely depends on many factors, including the duration of storage, the purpose of this study was to determine the effect of long-term (four years, in a refrigerated chamber at a temperature of 7-8 °C) storage of enzyme-containing preparation obtained from fish waste on its properties and technological capabilities.

MATERIALS AND METHODS

Materials

The work uses the above-mentioned enzyme-containing preparation from fish waste, as well as leather and chemical materials common in leather production:

- *pelt* obtained from cattle hide (skin) after soaking and liming processes according to the method of producing chrome-tanned leather for footwear uppers; mass part of moisture – 28.4%, shrinking temperature – 57 °C, thickness –1.84 mm;
- *chrome tanned leather* for shoe uppers without finishing (Crust), made from this pelt;
- *enzyme-containing preparation* from fish waste (ECP), which was stored under polyethylene film in a refrigerator at a temperature of 7-8 °C for four years;
- *other chemical materials used in leather production:* ammonium sulphate (DSTU 7370:2013), sodium chloride (DSTU 8056:2015), sodium bicarbonate (DSTU 3893:2016) and sodium carbonate (DSTU 7274:2012), sulphuric (DSTU 2184:2018) and acetic (DSTU 13189: 2019) acids, dry chromium tannin (DSTU 2726-94), Sulphirol C – anionic fatliquor based on sulphated fish oil (Smit & Zoon, the Netherlands), condensed tannins of quebracho extract (China).

Methods

To achieve this goal, traditional physical and chemical methods of analysis and a modern spectroscopic (spectrophotometric) method were used in the study. For physical and mechanical tests and chemical analysis, samples of raw materials and Crust were selected and analysed in accordance with the requirements of regulatory documents: preparation and sampling – ISO 2588:2022; determination of the mass part of moisture – ISO 4684:2005; determination of the mass part of minerals (ash) – ISO 4047:2006; determination of nitrogen content (mass fraction of carbonaceous matter) – ISO 5397:2006; determination of chromium oxide content – ISO 5398-1:2007; determination of the content of substances extractable by organic solvents – ISO 4048:2006; determination of tensile strength and relative elongation – ISO 3376:2008; determination of porosity – ISO 2589:2019; measurement of thickness – ISO 2589:2019; determination of shrinkage temperature – ISO 3380:2008.

To evaluate the effect of the enzyme preparation on the structure of the biogenic material (leather semi-finished product) during enzymatic treatment, a modified gelatin melting method was used to control the liming and bating processes, which are aimed at removing soluble protein substances from biogenic raw materials. The essence of the method is that a 0.5 g weight of the crushed semi-finished product (in terms of absolute dry matter) is placed in a 100 mL flask with a lapped lid and 50 mL of distilled water is poured in. The flask is closed and kept in a thermostat at 70 °C for 2 h with periodic stirring, and then cooled. The contents of the flask are filtered through a cotton filter into a 100 mL volumetric flask, thoroughly rinsed with distilled water, which is poured onto a filter to wash the melted gelatin. Then add 1 mL of 10% sodium hydroxide solution and 5% copper sulphate solution to the volumetric flask. The volume is made up to the mark with distilled water. The flask was transferred to a dark place, where it was kept for 20 min, after which the optical density of the solution was measured using a spectrophotometer at a wave length $\lambda = 520$ nm in a 10-mm-wide cuvette (ULAB 102 UV spectrophotometer, China).

The gelatin concentration, g/L, is determined by extrapolating from the calibration curve based on the results of determining the optical density of gelatin solutions of the set concentration: 0.10, 0.15, 0.20, 0.25, 0.30, 0.42, 0.52 and 0.75 g/L.

Process Recipe for Leather Semi-Finished Product

The research was carried out in laboratory conditions in glass containers on a special installation, the design of which allows maintaining the required temperature and constant rotation. Production parameters of chrome tanned leather for shoe uppers:

1. Washing: water – 200 %, temperature 28-38 °C, duration 40 min;
2. Deliming: water – 200 %, ammonium sulphate 2.2%, temperature 38 °C, duration 60 min;
3. Bating: in deliming bath; product ECP 0.2% (group 1), 0.4% (group 2), 0.6% (group 3), 0.8% (group 4); temperature 38 °C, duration 60 min;
4. Washing: water – 200%, temperature 30-25 °C, duration 30 min;
5. Pickling: water – 80%, temperature 20-23 °C, sodium chloride 6.0%, sulphuric acid 0.8% 10 minutes after the salt dosage, duration 90 min;
6. Tanning: in a spent pickling solution, dry chrome tanning agent (basicity 36-42%) 2.2%, sodium bicarbonate 0.4% 3 h after the start of tanning, temperature 20-23 °C, duration 9 h;
7. Laying: duration12 h;
8. Washing: water – 100-150%, temperature 35 °C, duration 20 min;
9. Neutralization: water – 250%, temperature 35 °C, sodium bicarbonate 1.5 %, duration 60

min;
10. Washing: water – 200%, temperature 35 °C, duration 40 min;
11. Fatliquoring: water – 200%, temperature 55 °C, anionic fatliquor 6.0% (100 % fat), acetic acid 0.3% by weight of fatliquor (at the end of fatliquoring), duration 60 min;
12. Retanning: in the spent fatliquoring bath, temperature 35 °C, quebracho (in terms of tannins) 2.0%, duration 40 min;
13. Washing: water – 200%, temperature 30 °C, duration10 min.
14. Laying, sammy-out, drying, tempering, staking.

Material consumption was calculated from the weight of semi-finished product samples.

RESULTS AND DISCUSSION

After long-term storage in polyethylene film at a low temperature, the preparation did not lose its appearance: there was no change in consistency and color, nor the appearance of an unpleasant smell. pH of the 10% solution was 4.5.

When assessing the activity of the preparation by the precipitation method, a slight decrease in its activity was found relative to the initial value at a temperature of 40 °C (420 units/g vs. 450 units/g), which indicates a certain stability of the structure and properties of the studied object. The effect of temperature on the activity of the preparation was evaluated in the range of 20-60 °C, which is quite acceptable for liquid physico-chemical processes of leather production. It was found that the activity of the preparation increased with an increase in temperature from 20 to 40 °C. With a further increase in temperature, there was a tendency to decrease this indicator (Fig. 1).

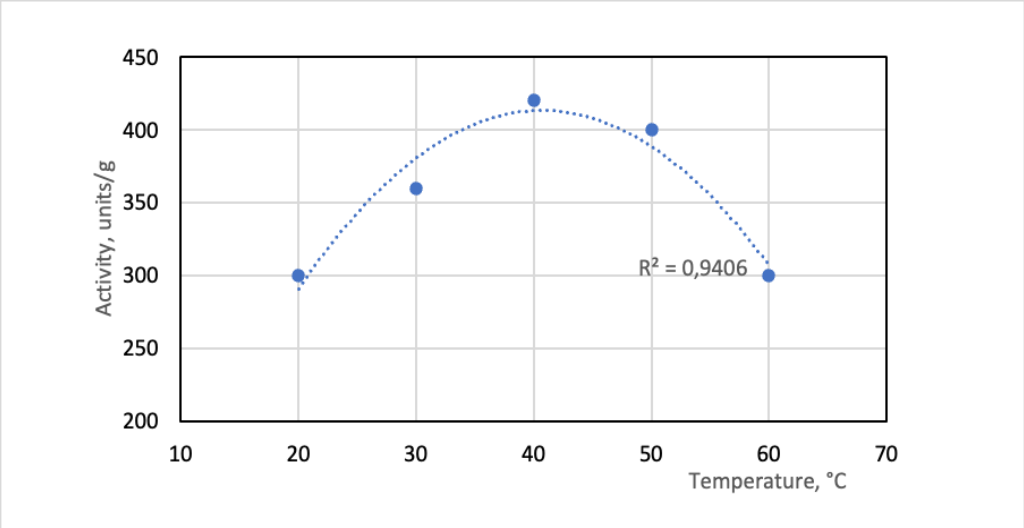

Figure 1. Effect of temperature on the ECP activity after storage for 4 years

The next series of experiments was devoted to determining the technological capabilities of the investigated enzyme-containing preparation after long-term storage. It should be noted that when determining the melting of gelatin, even before working with the photospectrometer, the color of the solutions under study signaled different amounts of nitrogen in different groups (Fig. 2). This indicated the influence of the conditions of enzymatic treatment on the degree of removal of interfibrous proteins from the structure of the dermis.Indeed, with an increase in the consumption of the enzyme preparation from 0.2 to 0.8 %, gelatin melting (i.e., loosening of the structure) doubled, regardless of the duration of

bating. At the same time, the more active effect of the ECP is detected after 0.5 h of bating, and after bating for 1.0 h, the gelatin melting rate decreases by 31.8-37.7 % (Fig. 3).

Figure 2. Flasks with analytical solutions prepared for use on the spectrophotometer (first four flasks on the left – after 0.5 h of bating, last four flasks on the right – after 1.0 h of bating)

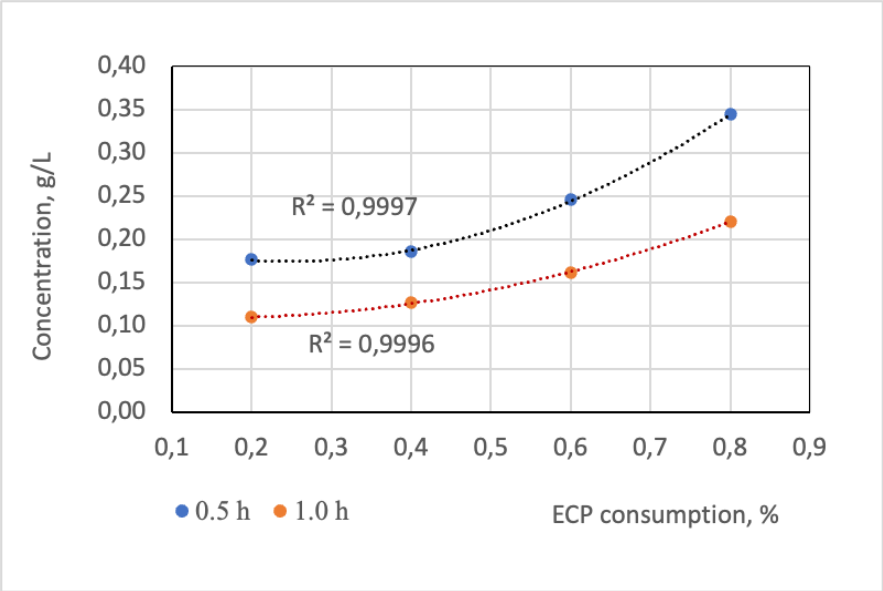

Figure 3. Effect of bating conditions on gelatin melting

The results of chemical analysis and physico-mechanical tests of leather materials in the form of pelt and Crust are shown in Table 1, which shows that the use of the ECP for bating, even after prolonged storage, allows to obtain high-quality leather material that fully meets the requirements of regulatory document in terms of consumer properties (strength, elongation, hydrothermal resistance, chemical analysis).

The absence of a difference between the strength of the leather as a whole and the strength of its front layer indicates a more even distribution of components in the dermis and is an indirect confirmation of the improvement of the cutting properties of the leather, i.e. more rational use of scarce raw materials.

Table 1. Indicators of pelt and finished leather (Crust)

Indicator	Value				Regulatory document **
	group 1	group 2	group 3	group 4	
PELT:					
Mass part of the pelt substance, %*	78.6	77.4	76.9	76.0	-
CRUST:					
Mass part, %:					
- moisture	15.2	15.2	15.6	15.7	10-16
- chrome oxide*	5.3	5.3	5.0	5.5	≥3.5
- substances extracted with an organic solvents*	7.2	7.0	7.5	7.8	3.7-10
Tensile strength, MPa	15.6	15.5	15.3	15.1	≥15.0
Tension at the appearance of cracks in the grain, MPa	15.6	15.5	15.3	15.1	13.0
Elongation under tension 10 MPa, %.	27.0	29.0	30.8	31.0	20-40
Porosity, %.	58.0	66.0	67.0	68.5	-
Thickness, mm	1.78	1.80	1.81	1.75	-
Shrinking temperature, °C	117	118	117	118	-

Note: *In terms of absolutely dry substance;
**DSTU 2726-94. Leather for shoe uppers. Technical specifications (for cow, bovine, pigskin).

CONCLUSION

Enzymatic methods have become an important and indispensable part of the processes used by modern industry for the production of a wide range of goods. The aquatic environment contains a huge pool of diverse genetic material and, therefore, represents a significant potential for various sources of enzymes. In recent years, research has been carried out on the study of enzymes of fish and aquatic invertebrates, interesting applications related to marine enzymes have appeared. Enzymes obtained from fish processing waste may have distinctive features that make them more suitable for industrial applications, as fish live in a wide range of temperature conditions and have characteristics that distinguish them from warm-blooded animals. Thus, to maximise the efficient use of aquatic resources, these enzymes can be extracted from fish processing waste and used as value-added products or processing aids in a variety of economic applications.

The effectiveness of enzymes and enzyme preparations largely depends on many factors, including the duration of storage. We have experimentally confirmed the stability in time of the structure and properties of the enzyme-containing preparation obtained from fish waste, its compatibility with dermal collagen, i.e. the possibility of using it for skin treatment. Further research in this area is planned. The expected results include expanding the range of materials for the production of natural leather, more rational use of natural resources, and reducing the harmful impact on the environment as a result of the production activities of fish processing and tanneries.

REFERENCES

Andreyeva, O., Atamanova, A., Maievska, T., Raksha, N. & Savchuk. O. (2001). Utilization of Enzyme-Containing Products Obtained from Fish Waste in Leather Production Processes. *Fibres and Textiles*, *28*(4), 3–10. Retrieved from: http://vat.ft.tul.cz/Archive/VaT_2021_4.pdf

Arvanitoyannis, I.S. & Kassaveti, A. (2008). Fish Industry Waste: Treatments, Environmental Impacts, Current and Potential Uses. *International Journal of Food Science & Technology*, *43*(4), 736–745. https://doi.org/10.1111/j.1365-2621.2006.01513.x

Atamanova, A., Andreyeva, O. & Savchuk, O. (2020). Study of the Properties of Enzymes Derived from Industrial Waste. Achievements and Prospects of Modern Scientific Research. Abstracts of the 1st International Scientific and Practical Conference, Editorial EDULCP. Buenos Aires, Argentina, pp. 144–149. Retrieved from: https://sci-conf.com.ua/i-mezhdunarodnaya-nauchno-prakticheskaya-konferentsiya-achievements-and-prospects-of-modern-scientific-research-6-8-dekabrya-2020-goda-buenos-ajres-argentina-arhiv/

Imran, S. (2022). Enzymes from Waste Products after Fish Processing and Their Uses, *Pakistan Research Journal of Biological Sciences*, *2*, 16–24

Klomklao, S., Kuepethkaew, S., Benjakul, S., Zhang, Y. & Simpson, B.K. (2024). Enzymes from Fish Processing Waste Materials and Their Commercial Applications. In: S. Maqsood, M.N. Naseer, Benjakul, S., Zaidi, A.A. (Eds.) *Fish Waste to Valuable Products*, Springer, Singapore, 147–194. https://doi.org/10.1007/978-981-99-8593-7_8

Kopytina, I., Andreyeva, O. & Mokrousova, O. (2023). Innovative Approaches to Enzimization of Leather Production. *Herald of Khmelnytsky National University. Technical Sciences*, *5*(325), 141–149. https://doi.org/10.31891/2307-5732-2023-325-5-141-149

Kopytina, I., Andreyeva, O., Mokrousova, O. & Okhmat, O. (2022). Enzymes and Approaches to Their Application in the Leather Production. *Herald of Khmelnytsky National University. Technical Sciences*, *5*(313), 227–232. https://doi.org/10.31891/2307-5732-2022-313-5-227-232

Shahidi, F. & Janak Kamil, Y.V.A. (2001). Enzymes from Fish and Aquatic Invertebrates and Their Application in the Food Industry. *Trends in Food Science & Technology*, *12*(12), 435–464. https://doi.org/10.1016/S0924-2244(02)00021-3

Venugopal, V. (2016). Enzymes from Seafood Processing Waste and Their Applications in Seafood Processing, *Advances in Food and Nutrition Research*, *78*, 47–69. https://doi.org/10.1016/bs.afnr.2016.06.004