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Inactivation of Anisakis simplex in hake viscera by acid autolysis.

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The removal and discard of hake viscera on board is a widely used method in commercial fleets to prevent the postmortem migration of the nematode larvae from the viscera to the muscle when the fish are stored onboard.

In 2018, the production of hake viscera by Basque vessels based in ports in the Basque Country was estimated at 545.6 t. The use and exploitation of fish viscera could be an interesting way to obtain new products and also it could also help reduce the discarding of viscera at sea.





Part of the project: 00001-IRB2018-33 - ANISAKIS:

"Knowledge of the ecology and minimization of the parasite load of anisakid nematodes in commercial species, from extraction to commercialization (*anisakis*)".



European Maritime and Fisheries Fund (FEMP)



Process: fish silage

Simple and low-cost process. No large means are required. Easily scalable. Possibility of doing it on board. Stable product for months. Improves digestibility: animal feed in dry or wet feed. If the quality is not enough it can be used as fertilizer.





For this study, the following works have been proposed:

- 1. Silage tests with hake viscera.
- 2. Verification of anisakis mortality during the process.
- 3. Allergenicity tests (ELISA) and characterization of potential allergens in silage products (Electrophoresis and Immunoblotting).
- 4. Analysis of the presence of anisakis DNA in silage products.





Characterization of the products of the silage of hake viscera.

3 silage experiments were carried out with freshly landed viscera.

The silages were made for 15, 7 and 11 days respectively.

At a temperature between 18 and 21°C

Different conditions were tested:

With and without added antioxidant to preserve the oil.

With intensive crushing and with simple chopping.

2 different pH conditions.

The composition of the silage and the oil obtained was analyzed.

The effect of silage and crushing conditions on *Anisakis* mortality was observed.







Most relevant conclusions of the characterization of the products of the silage of hake viscera.

In uncrushed silage the mortality of anisakis is 100% after 24 hours. In uncrushed silage at pH 3.6, the maximum degree of hydrolysis is achieved after 8-9 days.

	Mortality (%)	
Time	Non treated	Treated viscera
Initial	5%	50%
24 h	0%	100%
48 h	85%	100%
78 h	95%	100%
96 h	100%	100%







Allergenicity tests (ELISA) and characterization of potential allergens in silage products (Electrophoresis + Immunoblotting).

Objective:

1. Detect the presence of traces of anisakis allergens.

2. Identify the allergens present

In:

Aqueous fraction with soluble protein

Solid pellet

Floating solids.





Most relevant results of allergenicity tests and characterization of potential allergens in silage products.

The silage digests the Anisakis and releases allergens into the environment in the first days that they pass into the aqueous phase. Thus, in the solid pellet, and the aqueous fraction, an increase in allergenicity is observed as the silage treatment progresses.

Only a slight decrease in reactivity is seen beyond day 9.





Most relevant results of allergenicity tests and characterization of potential allergens in silage products.

Immunodotting confirms the results of allergenicity tests (A electrophoresis and B immunoblotting).



Days of silage

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Most relevant results of allergenicity tests and characterization of potential allergens in silage products.

As an additional test to observe the resistance of anisakis allergenic proteins to the action of digestive enzymes present in the silage process, experimental samples of three different silages (F2, F5 and V3) were analyzed **after 12 months of silage** (A electrophoresis and B immunoblotting).

Reactivity is still observed in some bands. It is not seen in small proteins (<15 kDa).





Presence of anisakis DNA in silage product

The analyses detected the presence of anisakis DNA only in the solid pellet samples at any time of silage, from 24 h to 264 h, while those of the soluble fraction did not present traces of parasitic DNA at any time.





Conclusions

Taking into account the estimated volume of annual production of viscera, it could be obtained with the maximum yields of silage theoretically: 72.8 t of proteins and 76.7 of fatty acids, with an EPA and DHA content of between 19 and 24%

Options

Animal feed option:

It should be checked whether resistant and larger fragments with allergenic potential can pass through the intestinal tract without being absorbed and incorporated into the edible fraction of animals fed with a feed incorporating silage.



Fertilizers option:

Silage or enzymatic hydrolysis of viscera can be a method of obtaining foliar fertilizers consisting of amino acid solutions.

They are being explored in the project







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