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USE OF PROTEIN ISOLATE FROM FISH WASTE "*SARDINA PILCHARDUS*" IN THE SYNTHESIS OF A CULTURE MEDIUM

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Abstract:

Fish processing is a crucial sector to meet the nutrient needs for humans, but it generates a significant amount of the estimated 50% of total waste. It follows the same time a problem of dumping of waste in different environments which increases environmental pollution and in particular the water environment. Our work aims to study the physicochemical quality of sardine waste (edges, heads and guts). Then, we seek to exploit these coproducts as medium culture for microorganisms such as *Lactobacillus* and *E. coli.* In this case, two universal mediums were prepared with the isolate obtained from fish in the place of peptones of the conventional mediums. The physicochemical analyzes showed that the isolate obtained has a high biological value (rich in essential amino acids). The results indicate that the use of this isolate must be accompanied with other essential elements of culture (vitamin compounds, essential amino acids, minerals) as growth factors to optimize the growth of bacteria involved. This work allowed us to have a recovery method that reduces the risk of pollution caused by discharges of different fish processing industries and we contribute to the development of biotechnology.

Keywords: Evaluation; Fish waste; isolate; *Lactobacillus*; *Sardina pilchardus*.

1. Introduction:

People consume more than three-quarters of world fish production. The remainder is mainly fed to animals, primarily in the form of fishmeal. Half the fish is consumed by humans in their fresh form, while the other half is subjected to any processing. Processed fish is often frozen or canned, dried, salted, smoked (Comby, 1994). However, many developing countries have increasingly often use fish processing to meet demand for domestic or export markets requirements. Fish also plays an important role in the production of animal fodder and compounds for the pharmaceutical industry (Andrieux, 2004). Fish processing is a crucial sector to meet the nutrient needs for humans. However, it generates a large amount of waste

estimated at 50% of the total volume. It follows the same time a problem of dumping of waste (co) in different environments which increases environmental pollution and in particular the water environment. Co-products are defined as "unused parts and recoverable in the traditional production operations" (AFSSA, 2003). During the processing of fish for human consumption, co-products including heads, viscera, falling trimming (thread), skin, scales, fins and tails are generated (Adieux, 2004; Gerard *et al.*, 2004). These products are described as derivatives and not of finished products because they are generally marketed as ingredients, i.e. in the form of intermediate products for human nutrition, animal feed, dietetic, cosmetic **(**Guérard *et al.*, 2004).

The research is therefore directed towards the development of methods for obtaining more sophisticated products preserving in possible functional properties of native proteins and can thus find new opportunities in more sophisticated preparations (Kim & Mendis, 2006).

Our work therefore aims to recover the waste (edges, heads and guts) of a species of pelagic fish is the common sardine "*Sardina pilchardus*". So, we seek to study the quality of waste sardine and on this basis to explore the different possible ways of valuation and know the efficiency of these products as culture medium for microorganisms. Then, we determine the possibility of using waste as a source of bacteria of industrial interest among other lactic bacteria.

2. Materials and methods:

2.1. Biological material:

The biological material includes the waste of one species of fish, widely consumed, which is the common sardine (*Sardina pilchardus*). The species *Sardina pilchardus* belongs to the family *Clupeidae*. This is a small fish whose length does not exceed 20 cm, has a fusiform body, slightly compressed on the flanks and covered with large scales. She wears a short dorsal fin lacks spines.

2.2. Microorganisms tested:

They represent lactic bacteria strains (MC9) and one strain of E. coli reference (ATCC25922) from the laboratory collection LRSE, University of Oran-ALGERIA-.

2.3. Preparation of protein isolate

The protein isolate refers to a product which is characterized by its high protein content with low ash content (Bourgeois & LE Roux, 1982). Preparing a protein isolate is made by a succession of steps:

Eviscerate and grinding: 3 kg of sardines washed. The flesh is removed and only takes the heads, fins and entrails. The waste is crushed to facilitate hydrolysis.

Hydrolysis: is a chemical hydrolysis with a solution of Sodium hydroxide (NaOH).

Bleaching: it is produced by hydrogen peroxide H_2O_2 (pH 3.5-4.5)

Precipitation and centrifugation: After adding a concentrated acid such as hydrochloric acid, centrifugation is performed to separate the precipitate from the liquid.

Extraction of fats: 2-3 extraction tests of the fat have been made using an isopropanol solution.

Washing and drying: the product is dried in an oven of 50 ° C. At the end of these steps, we have a product as a powder which can be called "protein isolate."

2.4. Physicochemical analysis:

2.4.1. Water content: The method used is the evaporative drying method (Audigie *et al.,* 1978).

2.4.2. Ash content: The method used by the calcination mineralization method (Pinta *et al.,* 1980).

2.4.3. Electrophoretic analysis of the protein isolate:

To prepare the tapes, paper Whatman No. 3 is cut into strips 2 cm wide. A median line is drawn on the strip. Then, the amino acids (lysine, glycine, methionine, isoleucine, and tryptophan) and a drop of solution of the isolate are deposited on this line. Fill each compartment of the container of 400 ml of acetate buffer 0.1 mol / L, pH 5. The generator is plugged in and set the voltage of 150 volts. Then, the solution is allowed to migrate for 1 hour. Electropherogram is dried with hot air and then it is dipped in the ninhydrin reagent.

2.5. Microbiological control of the isolate:

It is important to check if the preparations comply with microbiological requirements specified in his monograph in the Pharmacopoeia (Rouquette, 2002).

Kinds microbiological analysis were applied; a count of the total aerobic mesophilic flora (FMAT) and yeasts and molds (TYMC) and looking for some pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*). As of fecal contamination germ, a search for *Escherichia coli* is useful.

2.6. Preparation of culture media:

To this end, the work is divided into two areas: A culture medium was prepared for fastidious bacteria such as lactic acid bacteria. In this case, a universal medium (MRS) was prepared and another modified culture medium with which we have introduced a protein isolate obtained from fishing waste). Then, a culture medium was prepared for not demanding bacteria such as *E. coli*. In this case, a universal medium (TGEA) was prepared and another modified culture medium with which we have introduced a protein isolate obtained from fishing waste).

Once the various media are prepared and autoclaved, the reference strain (lactic acid bacteria and *E. coli*) are seeded on the surface of prepared media.

After incubation at 30 ° C / 24h, reading of the results is done through the following points:

- The colony growth on various agar
- Macroscopic and microscopic identification (Gram staining)
- The catalase test.

2.7. Fish waste as a source of lactic acid bacteria:

To this point we have adopted the same standard protocol for research of this type of microorganisms which comprises: an enrichment, isolation, identification, purification **(**Larpent-Gourgaud, 1997) and conservation**.**

3. Results and discussion:

3.1. Physicochemical analysis:

3.1.1. Water content: The water content is measured for different samples (sardines, waste and protein isolate) (figure1).

From the results, the water content in the whole sardine is 72.26%. It appeared consistent with the work of Soudan (1995) which indicated that the average value of the water content of the sardine is between 66% and 78%.

For cons, the water content of the waste sardine is low compared to the whole sardine. This decrease is probably due to the lack of fish flesh part (rich in water), knowing that the majority of fish waste is composed of the head and bones (Volatier, 2007).

The water content of the protein isolate (34.5%) is high due to insufficient drying step carried out during the preparation of the protein isolate protocol.

3.1.2. Ash content:

The ash content of the whole sardine (1.3%) seems consistent with the results reported by other work. The ash content for sardine can take a value between 1.2% and 1.5% (Soudan, 1995). For the waste sardine ash content is 2.2%. It appear higher compared to the ash content of the entire sardine (figure 2). This difference is related to the ratio of the bone mass (heads and bones) and viscera.

For the protein isolate, there is a value of 0.75%. This level indicates that our protein isolate is poor in minerals.

3.2. Electrophoretic analysis of the protein isolate:

Each amino acid is an isoelectric pH (pHi). Outside of this "isoelectric point" amino acids are generally charged and migrate under the influence of an electric field (Bossuyt & Marien, 2005).

The results of electrophoretic analyzes indicate the presence of the following amino acids: lysine, methionine, isoleucine, tryptophan, and glycine (figure 3).

Our results are confirmed by the work of Howell (1985) concerns the composition of different animal tissues in essential amino acids and indicate that the sardine is an important source of this type of acid.

3.3. Microbiological control of the isolate:

In this study, it is useful to evaluate the microbial load in our sample to estimate the risk that may incur during a discharge of waste such as fishing waste and consequently explore the different recycling methods. The enumeration MTAF and TYMC on preparations gave loads of 76 and 5 CFU / ml, respectively. A total lack of desired pathogen was well noticed. These results are consistent with the standards of the European Pharmacopoeia.

3.4. Preparation of culture media:

The results of seeding the reference strain of lactic bacteria (MC9) give us white colonies, convex, smooth, with even edge and small size with a diameter of about 2 mm (figure 4). All tested strains are Gram positive, stick shape and have a negative catalase test.

The figure illustrates perfectly the reduction in the number of settlements in the media. This result can be explained by the need for an adjustment period for new

substrates which are difficult to degrade. So, it needs some time to prepare enzymatic equipment.

The results of seeding the reference strain E. coli (ATCC25922) give us white colonies at regular board and the cells are Gram - in the form of bacillus (figure 5). It was noted that growth on agar TGEAM2 is less abundant, which means that our isolate cannot cover alone the absence of tryptone and meat extract.

The protein isolate added to different agars is not able to completely replace the basic elements which are the peptone (source of amino acids), meat extract (source of vitamin) and yeast extract (soluble vitamins and amino acids). Therefore, the action of the isolate requires elements or growth factors such as vitamins and certain essential amino acids to promote the lactic acid bacteria to survive normally.

3.5. Fish waste as a source of lactic acid bacteria:

The seeding of a 0.1ml of sample on the acidified MRS medium resulted, after incubation at 30 °C / 24-48 hours, typical colonies of lactic acid bacteria. The identification results have presented 02 different groups of lactic bacteria:

- A group of bacteria (LR4), heterofermentative grow at 15 ° C and 45 ° C, ADH positive, ferment lactose, raffinose, sucrose, ribose but mannitol, arabinose and xylose. These bacteria are pre- identified *Lactobacillus fermentum.*
- A group (LR6), has atypical characters of *Betabacterium* group. They do not grow at 15 °C, hydrolyze esculin ferment mannitol, rhamnose, xylose and sorbitol. They are pre-identified to the genus *Lactobacillus spp.*

4. Conclusion:

Based on the results obtained through the physicochemical analyzes of our substrate (protein isolate) extracted from co-products, we find it is rich in essential amino acids which gives a high biological value for our sample. Thus, the results prepared culture media allow us to conclude that the use of this isolate must be accompanied with other essential elements to culture (vitamin compounds, essential amino acids, minerals, ...) as factors growth to achieve optimal desired culture bacteria.

Acknowledgements:

We express our sincere gratitude to all staff of the Directorate of Education of Mascara.

References:

1. Comby, B. 1994. Mangez mieux, vivez mieux. Ed : les éditions de l'homme, une division du groupe Sogides. 326 pages, pp : 88-91.
2. Andrieux, G. 2004. La filière française des co-produits de la pêche et de l'aquaculture: état des lieux et analyse. *Etudes de l'Ofimer,* 63 pp.
3. AFSSA. 2003. Fatty acids of the omega 3 family and the cardiovascular system: nutritional benefits and claims. Press AFSSA, 70 pp.
4. Guérard, F., Batista, I., Pires, C., Thorkelson, G., et Le Gal, Y. 2004. Repport on sources and selection criteria for raw material. Rapport établi pour le programme SEAFOOD plus, 57 pp.
5. Kim, S.K. et Mendis, E. 2006. Bioactive compounds from marine processing by-products - A review. *Food Res. Int., 39:* pp 383-93.
6. Bourgeois, C.M. et LE Roux, P. 1982. Protéines animales. extraits, concentrés et isolats en alimentation humaine. Paris: Lavoisier. Original hardcover. XVIII, 366 pp.
7. Audigie, C., Figarella, J., Zonszaain, F. 1978. Manipulation d'analyse biochimique. Doin (Ed).Paris, 274p.
8. Pinta, M., Bourdou, B., Rousselet, F. 1980. Spectrophotométrie d'absorption atomique .Masson et Arston (Eds).Paris, 478p.
9. Rouquette, C. 2002. Médecine, chirurgie et soins infirmiers. Paris: Lammare: 357.
10. Larpent-Gourgaud, M. 1997. Microorganismes eucaryotes et procaryotes. Structure. Métabolisme. Systématique. Applications industrielles. Milieux de culture et réactifs.
11. Soudan, F. 1995. Encyclopédie du froid: la conservation par le froid des poisons, crustacés et mollusques. Ed: Paris, 514 pages.
12. Volatier, JL. 2007. Enquête INCA Individuelle et Nationale sur les Consommations Alimentaires. Lavoisier (Editions TEC&DOC), Paris.
13. Bossuyt, X., Marien, G. 2005. False-negative serum protein electrophoresis in a sample with an IgM monoclonal protein by semi-automated gel electrophoresis. Clin Chem.

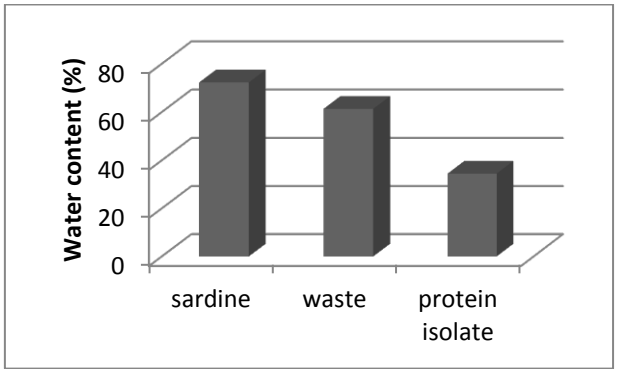


Figure 1: The water content (%)

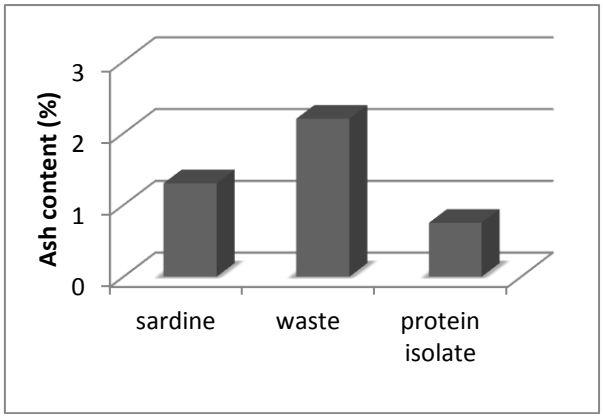


Figure 2: The ash content (%)

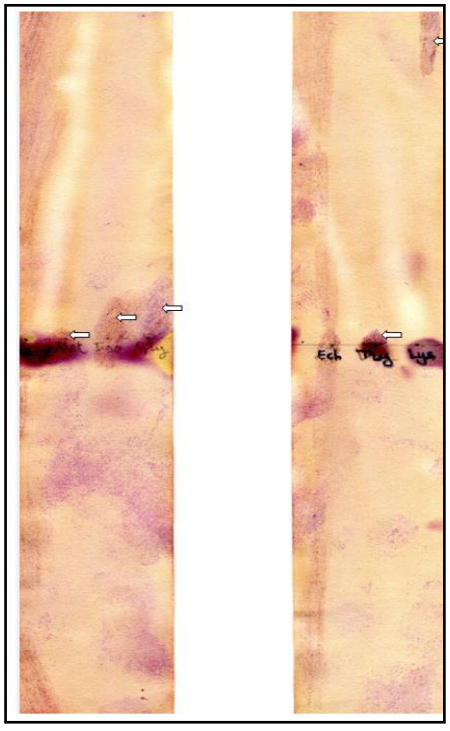
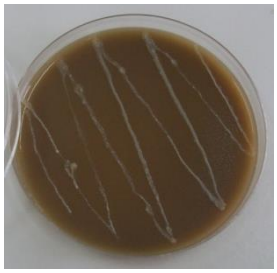
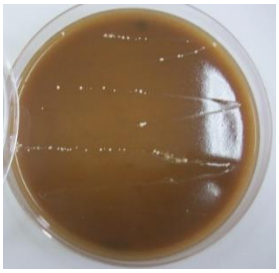


Figure 3: electropherogram of the protein isolate

MRS sample



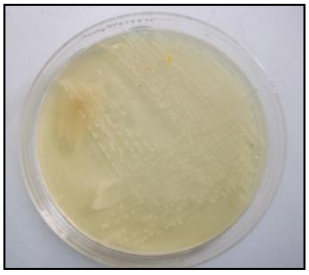
MRS modified 1



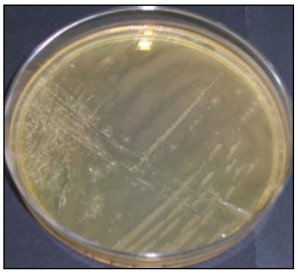
MRS modified 2

Figure 4: Appearance of colonies of the strain MC9 on the three prepared medium.

TGEA sample



TGEA modified 1



TGEA modified 2

Figure 5: Appearance of colonies of *E. coli* strain on the three prepared medium.

Figure 6: Macroscopic appearance of lactic bacteria isolated on MRS medium