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(54) Benævnelse: **FREMGANGSMÅDE TIL FREMSTILLING AF DEHYDRERET FØDEVARE MED ET HØJT INDHOLD AF HYDROLYSEREDE PROTEINER FRA FISKELIMVAND**

(56) Fremdragne publikationer:
EP-A1- 0 990 393
ES-T3- 2 311 990
PE-A1- 07 492 005
US-A- 2 525 294
US-A- 2 857 278
US-A1- 2011 129 565
Tze-Kuel Chiou: "Desalting and Recovery of taste compounds from salted sardines and shrimp stickwaters by membrane electrodialysis", , 1 June 1996 (1996-06-01), pages 1-13, XP055375848, Retrieved from the Internet: URL:https://www.researchgate.net/profile/T ze-Kuei_Chiou/publication/264393532_Desalt ing_and_Recovery_of_Taste_Compounds_from_S alted_Sardines_and_Shrimp_Stickwaters_by_M embrane_Electrodialysis_in_Chinese_with_En glish_tables_and_figures/links/53db5b5c0cf 2a19eee8b6910/Desalting-and-Recovery-of-Ta ste-Compou [retrieved on 2017-05-24]
G. O'neill: "Concnetration of fish stickwater by mechanical vapour recompression", , 1 January 1989 (1989-01-01), pages 1-36, XP055375541, Retrieved from the Internet: URL:http://bookshop.europa.eu/hr/concentra tion-of-fish-stickwater-by-mechanical-vapo ur-recompression-pbCDNA12301/downloads/CD- NA-12-301-EN-C/CDNA12301ENC_001.pdf;pgid=y 8dlS7GUWMdSR0EAIMEUUsWb00000Bf8nXif;sid=Ta 9OtvjsDw1Ooau_oLTQEZrJ72wy5lovMf8=?FileNam e=CDNA12301ENC_001.pdf&SKU=CDNA12301ENC_PD F&Catalogu [retrieved on 2017-05-23]
PETER J. BECHTEL: "Properties of Stickwater from Fish Processing Byproducts", JOURNAL OF AQUATIC

Fortsættes ...

FOOD PRODUCT TECHNOLOGY, vol. 14, no. 2, 15 July 2005 (2005-07-15), pages 25-38, XP055375662, US ISSN: 1049-8850, DOI: 10.1300/J030v14n02_03
R A Macleod ET AL: "Studies on the conversion of fish stickwater to solubles. I. The bacterial decomposition of stickwater at high temperatures", Applied microbiology, 1 July 1955 (1955-07-01), pages 202-204, XP055375548, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/artic les/PMC1057098/pdf/applmicro00300-0010.pdf
JACOBSEN, F.: 'Effect of enzymatic treatment of stickwater on evaporator capacity and fouling' PROCESS BIOCHEMISTY vol. 20, 1989, ISSN 0032-9592
PEDERSEN, L.D., CRAPO, C. ET AL.: 'Membrane Filtration of Stickwater.' ADVANCES IN SEAFOOD BYPRODUCTS ALASKA SEA GRANT COLLEGE PROGRAM ? AK- SG -03-01 2003, pages 359 - 369, XP055316522
MEDINA, A.L. ET AL.: 'Desalado of los sueros lÃ¡cteos by electrodiÃ¡lisis.' INTERCIENCIA vol. 21, no. 4, 1996, pages 1 - 9, XP055316526 ISSN: 0378-1844 Retrieved from the Internet: <URL:http://www.interciencia.org/v21_04/com unicaciones01.html>
CROS, S. ET AL.: 'Desalination of mussel cooking juices by electrodialysis: effect on the aroma profile.' JOURNAL OF FOOD ENGINEERING vol. 69, July 2005, pages 425 - 436, XP027609653

DESCRIPTION

1. Technical Scope

[0001] This invention refers to a method for obtaining a dehydrated food with a high content of hydrolyzed proteins from stickwater, which can be used as food for human consumption directly or indirectly.

2. Background

[0002] During the manufacturing process of fish meal, the raw material (fresh anchovy) is cooked and then undergoes a pressing process, obtaining a press liquor from which the suspended solids and the fat fraction (oil fish) are separated by centrifuging, remaining at the end a liquid called stickwater or fish stickwater. Stickwater can also mean the liquid fraction obtained from fish or cephalopods cooking broth (hereinafter fish) in the production process of canned foods or precooked products, which can then be subjected to a freezing or cure process. Fish stickwater mainly contains a large amount of soluble proteins, insoluble solids, vitamins and minerals (including salt), and fat traces with values that vary depending on the species processed. The amount of stickwater produced basically depends on the composition of the raw material and a suitable cooking and pressing process, which can be 20 and 70% of the processed fish in the case of fish meal. The total solids content in this liquid usually varies between 4-9%, with a variable fat content of 0.3-1.0%, depending on the raw material, its freshness, and centrifugal equipment available. The salt content is also variable, although it is common to see concentrations above 1% (w / w), as in the case of the fishmeal process.

[0003] What are the uses of stickwater?

[0004] Usually during the preparation of fish meal, stickwater undergoes a concentration process from 6 - 9% of solids to a final varying concentration of 35 - 45%, depending on the operational variables and additives used in this process. This concentrate is then incorporated into the production flow of fish meal, which is reflected in a higher volume of product made, with an increase of around 25%. As a result, stickwater recovery, in addition to solving an environmental problem, is highly profitable from the economic point of view. Alternatively, concentrated stickwater can be directly spray dried to a 5% level of humidity, in order to be used separately as an important additive of high biological value on CHI specialized food formulation. In general, concentrated stickwater provides among other components a series of nucleotides and genetic material involved in biochemical processes of attractability, immune stimulation and vital energy, as well as free amino acids, vitamins and minerals, where - according to recent research - the unknown growth factor generally attributed to fish meal can be found.

[0005] On the other hand, stickwater obtained from the cooking process in the canning and freezing industry is usually derived to effluent treatment systems, where oil with a high degree of oxidation and sludge are usually obtained and then subjected to a dehydration process to recover fish meal of very low quality.

[0006] What problems result from the use of stickwater?

[0007] Beyond the environmental and economic benefits that entail the use of this subproduct, the concentration process of stickwater, besides condensing the present soluble protein, can concentrate other solid compounds, including salt. By adding this concentrate to fish meal, salt is added at levels that often exceed the standard set by users for this compound (3.5%), lowering the quality of the final product. Depending on the quality of the processed raw material, stickwater concentrate incorporated to fish meal may also carry decomposition products like biogenic amines including histamine, and volatile nitrogenous bases that greatly lower the quality of fishmeal. The problem is further emphasized when the stickwater concentrate is dried directly and the final product achieves salt levels up to 15% (dry basis), making the percentage protein content relatively low (67%), which reduces the competitiveness of the product when compared with fish protein concentrates from other sources that are offered with a protein content greater than 80%.

[0008] Generally, the current use of stickwater favors the underutilization of a major source of protein that, when used as a subproduct in the fish meal process, can lower the quality of this product and, if used as a separate consumable, it has low protein contents due to the salt content accumulated during its process.

[0009] Is there a technique used for the removal of salt (demineralized) from fish stickwater?

[0010] Now in the fishing industry, stickwater plants are evaporators and therefore tend to concentrate the compounds contained in this subproduct, increasing the salt content in the dehydrated concentrates obtained; i.e., no method or technique is applied to remove salt from fish stickwater.

[0011] Is there a proprietary technology for the production of hydrolyzed stickwater protein and what are the differences when compared to our proposal?

[0012] There is research on some processes, such as electrodialysis, hydrolysis and evaporation, for the use of various raw materials. For example, Ramirez Reid (2011), in the patent application US 2011/129565 A1, refers to a process for the production of wet solutions of peptides concentrated to 50% or dry peptides, for animal or human use, starting from residues or secondary products from the protein food industry for human consumption.

[0013] Although a hydrolysis process for concentrating peptides is carried out, it used residues from the food industry as raw material -unlike the present invention-, such as residues of land animals or fishes that were used for producing fresh, frozen, preserved, and cured products.

etc. (pieces of meat, meat with bone, skin and/or connective tissue in general), so it requires conditioning prior to the hydrolysis process, for example, the residues must be ground or crushed, given its solid nature. This translates into the dynamics of the process that should be used, as well as the type of hydrolysis carried out, which is a combination of acid and enzymatic hydrolysis.

[0014] On the other hand, G. O'neill in "Concentration of fish stickwater by mechanical vapour recompression" dated 1989, describes a project for the installation of an equipment for concentrating stickwater, through a system of mechanical vapor recompression. Although it is mentioned some stickwater concentration, concentration systems, such as descending film evaporation, are not used, which is closely linked to the purified stickwater (with no solids or fat) and to the final product obtained by the inventor of the present application after concentration.

[0015] Peter J. Bechtel in "Properties of Stickwater from fish processing byproducts", published in 2005, describes a chemical and nutritional characterization of single-stickwater samples and then concentrated by a method of lyophilization. There are no references, as in the present invention, about the application of electrodialysis or the use of enzymes for promoting the enzymatic hydrolysis of the present protein.

[0016] The patent application PE20030108320, filed by Pesquera Diamante, describes a method for obtaining soluble fish concentrate from stickwater. Although the raw material is similar, the final product, unlike the present invention, is obtained through filtering with press filters, which does not release the protein from other components that affect its quality. In this document, other metal ions, including heavy metals, remain in the concentrate, which affects the quality of the final product.

[0017] The invention patent request (ES 2 311 990 T3) for "Product of hydrolyzate marine protein, process for its production and application" discloses the obtaining of marine protein from any source and fish and shellfish species that generally includes subproducts such as stickwater. The method describes how the protein is obtained by separating it through filtering operations (Ultra and Nano), for which ultrafiltration systems with ceramic membranes are used and then nanofiltration systems that allow the removal of a smaller proportion of salt and biogenic amines.

[0018] In this regard, there are a number of differences between the above-mentioned patent and our proposal:
The procedure described in said patent registration (item 6 of claims) does not apply, unlike our proposal, an enzymatic hydrolysis process to stickwater and is only limited to filtering operations (Ultra and Nano).

[0019] Another substantial difference is that authors of said patent use ultrafiltration systems with ceramic membranes to remove salt from stickwater and then use nanofiltration to remove the salt and biogenic amines. In our case, our technology only involves electrodialysis to

remove with greater efficiency the ions comprising sodium chloride and we do not use conventional filtration methods.

[0020] Finally, we also found another difference: the filtration systems applied allow part of the protein components of low molecular weight to pass through the filtration membranes and they are lost along with the water and undesirable compounds such as salt, smell and taste components. This results in the loss of protein components with important biological and technological functionality and reduces the process performance.

[0021] Instead, our procedure deals first with the demineralization of stickwater by electrical loads (electrodialysis) in order to make sure not to remove any protein component, because they all are larger than the electrodialysis membranes pores, thus ensuring a greatest number and better quality. Subsequently, hydrolysis allows us to reduce the molecular weight of the protein fractions to give them the desired functionality with the best possible performance.

3. Brief description of the invention

[0022] To resolve these drawbacks, by means of this invention, a new method for obtaining food with a high content of hydrolyzed protein obtained from fish stickwater is proposed. First, solids and fat remaining in stickwater are separated, and then begins the demineralization through a selective salt removal method, applying the electrodialysis technique, so that a greater quantity and quality of protein components are concentrated, avoiding loss of protein fractions due to not being subjected to filtration processes; then, the demineralized stickwater is pumped to an equalizer tank, where the temperature and pH is adjusted by adding NaOH: an enzymatic hydrolysis process reduces the molecular weight of the protein fractions to the desired functionality with the best possible performance; subsequently, the product obtained is subjected to an evaporation process in an evaporator plant; concluded the evaporation step, the hydrolyzate concentrate is stored in a buffer tank to be then subjected to a spray drying process; finally, the dried product is packaged.

4. Brief description of the figure

[0023] Figure No. 1 shows the flowchart to carry out the method for obtaining food with a high content of hydrolyzed proteins from fish stickwater.

5. Detailed description of the Invention

[0024] The following describes in detail the method for obtaining food with a high content of hydrolyzed proteins from fish stickwater.

[0025] The process according to the invention consists of the following steps:

1. 1- Stickwater from a fish with a high degree of freshness is subjected to a separation process of insoluble solids and fat traces by means of a separation equipment that may be a Sedicanter or a centrifugal disc operating at a speed from 3000 to 6000 xg for 5 minutes, in order to remove impurities contained in the stickwater, thus preventing these compounds from interfering with the subsequent operations of physical separation or concentration.
2. 2- The resulting liquor - consisting of refined stickwater - is placed on a conventional electrodialysis equipment consisting of a stack of anion and cation exchange membranes arranged alternately and separated by spacers that form two circuits, which allow on one hand a constant stickwater to be demineralized (circuit 1) and at the same time in a separate compartment there is a constant flow of weak brine (circuit 2), that additionally receives salt ions (sodium and chloride) that have been permeabilized by the above-mentioned membranes. The driving force of the process is a direct current between anodes and cathodes housed in the two ends of the stack, inside electrode plates.
   When stickwater with the original salt content reaches the active area of the membranes, the DC voltage causes the positively charged cation (Na^+) to migrate toward the cathode, while the anion (Cl^-) goes to the anode system. When any of these ions reaches the ion exchange membranes, the properties of the membranes will determine whether the ion is rejected or accepted through them. The ions that can pass through the membranes are retained in the next compartment, since the next membrane has the opposite charge. Accordingly, while the stickwater flow runs through a demineralizing circuit, there is at the time a water flow parallel to another circuit that accumulates salt, forming concentrated brine.
3. 3- Demineralized stickwater is pumped to an equalizer tank, where the temperature is adjusted to 60 °C and pH of 7.5-9 with the addition of NaOH. Under these conditions, stickwater is subjected to an evaporation process in an evaporator plant - which may be a falling film, vacuum evaporators or other means of evaporation -, adding at the beginning of this process and continuously an alkaline protease (endopeptidase) of bacterial origin (*Bacillus licheniformis*) in a proportion of 1% with respect to the protein content of stickwater, in order to hydrolyze the peptide bonds inside the protein molecules. The evaporation process - which takes 30 to 60 minutes depending on the type of evaporator plant used - ends when the concentrate reaches 40 to 45% of solids, and then subjected to a final heating process of 85 °C for 15 minutes to inactivate the enzyme.
4. 4- After the evaporation step, the hydrolyzed concentrate - with a hydrolysis from 18 to 20% - is temporarily stored in a buffer tank to be subsequently pumped into a "spray dryer", allowing the dehydration of the product until it achieves a solids content from 90 to 95% and a moisture content between 3 and 5%.
5. 5- The dry product - with 92% of protein, with molecular weights below 1000 daltons and a digestibility degree greater than 98% - is finally packaged with the use of flexible bags made of a moisture-resistant barrier, in order to avoid the rehydration of the product.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US2011129565A1 **[0012]**
- PE20030108320 **[0016]**
- ES2311990T3 **[0017]**

Patentkrav

1. Fremgangsmåde til opnåelse af dehydreret fødevare med højt proteinindhold og høj biotilgængelighed, fremstillet ud af fiskelimvand; idet fremgangsmådens karakteristika indbefatter følgende trin:

a. tilvejebringelse af en limvandskvantitet,

b. separering af limvand fra uopløselige faststoffer og fedtspor ved hjælp af et separationsudstyr, der kan være en Sedicanter eller skivecentrifuge, der kører ved en hastighed på 3000 til 6000 xg i 5 minutter,

c. fjernelse af salt fra det tidligere raffinerede limvand med et elektrodialyseudstyr; hvor limvandet med salt når det aktive område for elektrodialyseudstyrets membraner; hvor jævnstrømsspændingen påføres indtil det positivt ladede kation, Na+, migrerer mod katoden, mens anionen, CI-, vandrer til anodesystemet; når en hvilken som helst af disse ioner når ionbyttermembranerne, vil membranernes egenskaber bestemme, om ionen skal afvises eller kan passere gennem dem; de ioner, der kan passere gennem membranerne, tilbageholdes i det næste rum, idet den næste membran har den modsatte ladning; hvor limvandsstrømmen, der løber gennem et kredsløb, demineraliseres, indtil reduktion af ledeevnen, og samtidigt bliver en anden vandstrøm eller svag saltopløsning ved med at akkumuleres, indtil der er dannet en opkoncentreret saltopløsning med høj elektrisk ledeevne,

d. hydrolysering af det demineraliserede limvand. Til dette formål pumpes limvandet ind i en udligningstank,

hvor temperaturen justeres til 60 °C og pH på 7,5-9 med tilsætningen af NaOH. Under disse forhold udsættes limvandet for en fordampningsproces i et fordampningsanlæg - der kan være en faldende film, vakuumfordampere eller andre fordampningsmidler -, tilsætning i starten af denne proces og kontinuerligt af en alkalisk protease, endopeptidase, af bakteriel oprindelse, *Bacillus licheniformis,* i et forhold på 1 % i forhold til limvandets proteinindhold, for at hydrolysere peptidbindingerne inde i proteinmolekylerne. Fordampningsprocessen - der varer 30 til 60 minutter afhængigt af typen af anvendt fordampningsanlæg - slutter når koncentratet når 40 til 45 % faststoffer med et hydrolyseniveau på 18 - 20 %, og derefter udsættes for en endelig opvarmningsproces ved 85 °C i 15 minutter for at inaktivere enzymet,

e. midlertidig oplagring af det hydrolyserede koncentrat i en buffertank for efterfølgende pumpning ind i en "spraytørrer", der muliggøre dehydrering af produktet, indtil det opnår et faststofindhold fra 90 til 95 % og et fugtindhold mellem 3 og 5 %, har 92 % protein med molekylvægte under 1000 dalton og et fordøjelighedsgrad på mere end 98 %,

f. pakning af det hydrolyserede koncentrat i fleksible poser fremstillet af en fugtbestandig barriere for at undgå rehydrering af produktet.

DRAWINGS

FIGURE N° 1

