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QUALITY OF LIFE AND MANAGEMENT OF LIVING RESOURCES PROGRAMME (1998-2002)

FIFTH PROGRESS REPORT OF PARTNER 06

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Contract number: QLK1-CT-2000-01017
Project acronym: Fishery by-products
QOL action line: 1.1.1 Novel and improved biological raw materials for high quality food

Reporting period: 01/6/2003 to 31/05/2004

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SECTION I: PROJECT IDENTIFICATION

Contract number: QLK1-CT-2000-01017
Title of the project: Utilisation of by-products from cod species
Acronym of the project: Fishery by-products
Type of contract: RTD project
QoL action line: 1.1; 1.1.1
Commencement date: 01-12-2000
Duration: 42 months
Total project costs: 2,590,169
EU contribution: 1,561,549
Project co-ordinator: ?? Name: Turid Rustad Assoc. Prof. ?? Organisation: Dept of Biotechnology, Norwegian University of Science and Technology (NTNU) ?? Postal address: N-7491 Trondheim, Norway ?? Telephone: +47 73 59 3320 ?? Telefax: + 47 73 59 12 83 ?? e-mail: Turid.Rustad@chembio.ntnu.nl
Keywords: by-products, cod species, protein, lipid, stability
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SECTION II: SECOND PROGRESS REPORT OF PARTNER 06

1. Overview of progress during the reporting period for partner 06

1.1 Introduction

The demand of collagen and gelatine from the industry throughout the world is considerable and still rising. By-products from fish processing are a potential source of collagen.
Collagen is the main component in the skin (Norland, 1989; Sikorski and Borderias, 1994), which can be collected separately from other by-products. The major collagen type in fish skin and bones is type I collagen (Sikorski and Borderias, 1994) and it belongs to the group of fibrous proteins.
Collagen in its purified form has found a number of pharmaceutical and cosmetical applications. Similarly, gelatine, the hydrolysed form of collagen, is an ingredient extensively used in the food industry. Gelatine is used as a food additive to increase the texture, the water-holding capacity and stability of several food products (Borderias et al., 1994). Both gelatine and collagen have been derived from fish skins and bones, but have been much less studied than mammalian gelatine and collagen (Norland, 1989; Gudmundsson and Hafsteinsson, 1997). The quality and specific application of the extracted collagen and or gelatine is highly related to their functional properties and its purity. Known problems with the extraction of collagen from fish skins are the abundance of pigments and the presence of fish odours, which would restrict its potential use. The uniqueness of fish collagen from cold water fish lies in the lower content of amino acids, proline, and hydroxyproline (Haard et al., 1994). Although fish gelatine does not form particularly strong gels, it is well suited for certain industrial applications, as for example micro-encapsulations, light-sensitive coatings and, low-set-time glues (Haard et al., 1994).

1.2 The main objectives of the project for this reporting period

Although fish skins are abundant, the annual catch of cod haddock and Pollack in Icelandic waters would produce around 8,000-10,000 metric tons of fish skins, there are only a few studies of the optimisation of processing of collagen and gelatine from fish skins. More research is needed to optimise the processing conditions in order to produce the best native collagen possible together with a high yield

The objectives of the workpackages are as follows:

WP 3.3
The development, optimisation and scaling up of a process to extract collagen from fish skins and bones originating from cod (*Gadus morhua*).

WP 2.6
Evaluation of functional properties of extracted collagen from cod and carp

WP 3.5
Industrial applications and marketing of extracted collagen

WP 4.4
Safety/HACCP of collagen extraction from cod skins and bones

WP 4.5
Increasing the heat stability of fish collagen and/or gelatine

1.3 Overview of the scientific progress

WP 3.3 Extraction of collagen from cod skins and bones

RIVO provided partner 8 with established protocols for extraction of.collagen from cod skins and bones. The protocols were used by partner 8 to extract collagen preparations from carp by-products. Details on the provided protocols are shown below and in Annex 2. For more details on the extraction of collagen from carp see the report of partner 8.

Skins
The skins were extracted with 0.1 N NaOH at 9°C to remove non-collagenous proteins, washed with distilled water and centrifuged. Then, the insoluble material was extracted with 10% butyl alcohol for one hour to remove fat, washed with distilled water and centrifuged. Subsequently, the insoluble material was mixed with water (1:7 w/v) and HCL was added continuously to a final pH of 4.0. The soluble fraction was stored at 4 °C for further analysis. The procedure is presented in figure 1.
It was decided to use HCl for extraction of collagen, as this can be removed completely from the final preparation. It is known that applying acetic acid or citric acid may results in an off-odour (Borderias, personal communication). The use of HCl requires that the pH is controlled to keep the value at 4. Therefore, the extraction with HCl was performed in a fermentor, equipped with a pH and temperature control. The temperature was kept at 9° C to avoid that collagen is converted into gelatine.
Bones
The bones were extracted with 0.1 N NaOH at 9°C to remove non-collagenous proteins, washed with distilled water and centrifuged. The bones were decalcified with 0.5 M ethylene-diaminetetraacetic acid (EDTA) (pH 7.4) for four hours and centrifuged. The pellet was washed with water and fat was extracted with 10 % butyl alcohol for one hour. The insoluble material was mixed with water and HCL was added according to the procedure described for skin. A schematic presentation of the procedure is shown in figure 2.

WP 2.6 Evaluation of functional properties of extracted collagen: cross-linked collagen from cod and hoki, and collagen extracted from carp

Rheological measurements
Rheological measurements were carried out using a dynamic stress rheometer SR200 (Rheometric Scientific Inc. Piscataway, USA). Before the measurements the measuring cell of the rheometer was cooled until 8°C before applying the samples. Rheology was measured in a chromium plate 40-mm parallel plate cell using a gap of 0.100 mm. A dynamic temperature ramp test was performed at a stress of 0.2 Pa and a frequency of 1 Hz. Starting at around 8°C the temperature was increased with a programmed rate of 2°C/min. The G' and G" were measured at intervals of 5 seconds. A vapour trap was used to minimise evaporation of water from the sample.
Cod and hoki collagen. The modulus of elasticity (G') and the modulus of viscosity (G") were determined for collagen from cod skins prior to treatment with transglutaminase (TGase) and after treatment for various periods (see table 1). The blank (not treated with TGase) was analysed prior to frozen storage and after to establish whether this could influence the physico-chemical properties. No influence was found. The control preparation, which was kept frozen and thawed prior to analysis, was converted into gelatine at 17°C, respectively (see figure 3). This result is the same as reported in the previous progress report.

Table 1: Treatment of cod and hoki skin collagen with TGase: experimental setup

	Blank	Blank: frozen and thawed	Frozen and thawed TGase (120 U/g used)	Frozen and thawed Tgase (120 U/g used)	Frozen and thawed Tgase (120 U/g used)
	Time = 0	*Time = 0*	*Time=0**	*Time = 4 h*	*Time = 22 h*
cod	x	x	x	x	x
hoki	x	x	x	x	x

x= preparation was made and analysed

It appeared that treatment with TGase resulted in inhomogenities in the aqueous cod collagen preparations. Therefore, treatment of cod collagen with TGase was repeated. It appeared that inhomogenities did not disappear. Visual inspection revealed that in the samples fibrous or sphere-shaped particles were present in the clear solution. These particles could not be dissolved, not even by heating at 80°C. These inhomogenities hindered the analysis.

In order to establish whether the inhomogenities could be caused by the extraction procedure used, collagen, which had been extracted by using a less complex procedure was also treated with TGas. For this purpose collagen from hoki skins was extracted with acetic acid dialysed and freeze dried by partner 4 and sent to partner 6 to perform the experiment (see table 1 for details on treatment with TGase). However, the inhomogenities were also present in the hoki collagen preparations after treatment with TGase. These inhomogenities hindered the analysis.

Carp collagen. Various collagen preparations were prepared by participant 8 and sent to participant 06 for analysis (see the report of participant 8 for details on extraction). It appeared that only for 1 sample, designated by participant 8 as fish collagen summer wild, the physico-chemical properties could be determined. This collagen preparation was converted into gelatine at 36°C (see figure 4). This temperature is significantly higher than for collagen from cod skins or bones. For the other samples transition temperatures could not be determined. Possibly, due to temperature abuse during transport. The samples were at room temperature upon arrival at participant 06.

Texture analysis

Texture measurements were performed with a Texture Analyser TA-XT2i (SMS, Goldaming, UK) equipped with a 25 N load cell and maintained at 1°C. Samples of were taken with a square punch (side length 2.2 cm). Samples height was that of the fillet, i.e. 3-4 mm. Special care was taken to obtain samples devoid of any bone, skin or lateral fat since these would influence measurements to a large extent. TPA measurement was performed with 40-80% compression (cylinder probe, 1.5 x 1.5 cm). The penetration test was also performed. For this the texture analyzer was equipped with a spherical probe (10 mm sphere). Samples were placed on the TA base. Hardness, i.e. the maximum shearing force (N) was recorded. For details on the samples see section WP 3.5.2

WP 3.5 Industrial applications and marketing of extracted collagen

WP 3.5.1 Marketability of extracted collagen for application in the food industry

Opportunities and threats for fish collagen or gelatine (seen Annex 1 for more details)

Fish gelatin has been available on the market for many years but the market for gelatin from fish is still a niche market. Recent volumes of fish gelatin are estimated at approximately 1,500 t on a global basis, which is small compared to the total gelatin market of 250,000 t worldwide. Despite BSE, the consumption of gelatin has grown over the past ten years with growth estimated at about 2% per year. Hoffmann-La Roche is believed to be the largest single

consumer of fish gelatin in the world. The rest of the market is very dispersed. Fish gelatin has not been able to penetrate the market due to its low availability, which prevents large volume consumers to come into the market, its high price, which prohibit inclusion in many products and its low melting point of cold-water fish grade gelatin, which excludes it from use as a gelling agent in solid, room temperature foods. Fish gelatin should be completely neutral in taste even when formulated. Not all producers have succeeded in purifying the gelatin to achieve this. To enter the market successfully and to gain a significant tonnage a supplier must address the above factors and limitations.

The greatest volume potential in the short-term is likely to be in the food industry. The pharmaceutical industry, especially in nutritional supplements, also holds potential. The photographic market is more specialized and will be more of a 'make or break' nature due to the small number of potential customers. There will always be some sectors of the food market, which will strongly resist fish gelatin as some marketing departments feel fish has a negative image for some products. But across the market there is potential providing that the price must be at least half of the price today and the availability can be guaranteed. When the melting point of cold-water fish gelatin can be modified to above room temperature the potential will than be greatly expanded. If the melting point of fish gelatin is limited to 10°C then the potential market will probably be small and primarily aimed at new applications such as health drinks and frozen or refrigerated goods. The potential for a given supplier is likely to stay around a few hundred tones, but if the melting point can be raised and the other issues with availability and price are addressed, the potential market is far greater. Grades of fish gelatin with higher melting points are already available on the market but are supplied by players, which have a stronger desire to prove the safety of beef gelatin and drive the market back towards bovine sources.

In the food and pharmaceutical markets, in applications where gelatin cannot be replaced, the vegetarian issue cannot be solved by fish gelatin, but fish as a source is acceptable to the portion of the vegetarian market that avoids red meat and poultry, but consumes fish.

Fish gelatin, provided it is kosher, is suitable for the Muslim and Jewish populations and so allows for the development of products, which can be sold in the Middle East. The question of labeling as an allergen is not answerable as the EU has not yet adopted this directive. Also it is not clear if the label will apply to gelatin from fish or to more directly derived products such as fish oils. Should fish gelatin fall into this Annex, it will be a negative factor. With or without a labeling requirement, heavy and active promotion will be necessary to raise the awareness of gelatin sources and the potential to avoid gelatin derived from pigs or cows. The bovine gelatin suppliers are expected to go directly to the press with their next round of marketing, expounding the safety of bovine gelatin, following the completion of the investigations last year. There is also expected to be a focus on the use of gelatin as a protein source for health benefits and hair care products. These offensives by current bovine gelatin suppliers are expected to create an atmosphere of awareness and a greater acceptance of gelatin in general. Once the acceptance of gelatin use is established the source becomes another issue. Fish gelatin will not become as widely used as bovine or porcine grades, but it could gain some 5% to 10% market share over time. In Europe alone, this would mean a market potential in the order of approximately 5,000 to 10,000 t.

International workshop held in Brussels on 3 May 2004

The coordinator organised a workshop for transfer of knowledge to companies. Participant 06 was present at the workshop.

Second Workshop held in the country of participant 06

RIVO subcontracted a SME, Moerdijk Van Oosten and Partners B.V., to organise a workshop in the Netherlands on utilisation of by-products from fish, especially collagen from cod skins and bones. The workshop was held 15 June 2004.

The following companies attended the workshop:

Matricel GmbH, HERZOGENRATH, Germany
Biomedical Company working on tissue engineering
L. Olde Damink (Head Collagen Production and Development)

A.van de Groep & Zn b.v., SPAKENBURG, The Netherlands
Trader in fish by-products, specialised in non fatty fish
(Absent with notice and in this case represented by Moerdijk van Oosten & Partners)

The aim of the meeting was to inform the participants concerning the results of the research project on collagen of cod fish and to explore the possibilities for a new CRAFT project on the exploitation of fishery by-products.

The following arrangements were made.

- Moerdijk van Oosten & Partners will prepare the minutes of the meeting.
- RIVO will inform the University College Cork (intends to be in charge of the preparation of a Craft project) about the results of this meeting.
- RIVO and the University College Cork will start a search for additional participants in order to strengthen the consortium.
- Both institutes will formulate more precise objectives for the proposal and subsequently, find end-users and more SMEs as partners for the consortium.

More details about the workshop are given in Annex 2.

WP 3.5.2 Improvement of texture of fish by-products by the application of collagen

The effect of collagen on textural properties on fish sausages of cod by-products was studied. For the preparation of the sausages we used four recipes in order to establish whether addition of collagen could influence textural properties significantly. In this experiment by-products generated by trimming of cod fillets was used.
The experiment was carried out as follows. The cod by-products were deboned, using a Baader 694 deboner, equipped with a drum with 3 mm pore size. After deboning NaCl (0.8 % w/w), cryprotectants (4% w/w sorbitol and 0.4% polyphosphate were added to the mince) and subsequently the mince was cuttered. In the next step the mince was divided into parts: one part with added aqueous cod collagen and one without. 0.5% w/w Collagen was added to minces not enriched with oil. For minces enriched with collagen the ratio added lipid and collagen was 120:1. In case of the enriched samples we decided to change the amount of collagen added, as we were interested to establish whether the emulsifying properties of cod collagen could influence the textural properties.
All samples were treated with TGase (Activa WM 0.4% w/w added to the mince) for 12 h at 2-4°C in order to obtain sausages of sufficient firmness. After the treatment all samples were pasteurised (core temperature 85°C). After pasteurisation the textural properties were analysed, as described under section WP 2.6
Prior to the treatment with TGase various samples were enriched by addition of oils. Details on the recipes are shown in table 2 below.

Table 2: Used recipes to establish effect of cod collagen on properties of fish

Recipes	without collagen	with collagen
1 minced cod trimmings: added water 1.7 % w/w for all samples	n= 5	n= 5
2 minced cod trimmings enriched with lipids (7.5% w/w olive oil 2.5% w/w hardened vegetable fat): added water 10% for all samples	n= 5	n= 5
3 minced cod trimmings enriched with 8% olive oil: added water 40% for all samples	n= 5	n= 5
4 minced cod trimmings: enriched with lipids 20% olive oil: added water 20% for all samples	n= 5	n= 5

The textural properties of the four recipes differed significantly, as measured by the TPA compression and penetration test (see figure 5 for effect of recipes on hardness). This is in accordance with our expectations. We decided use a two way model to establish interaction effects. Processing of the data revealed an interaction effect between the amount of oil and the use of collagen, which acts as an emulsifier. However, our experiment revealed no significant effect of using collagen on the texture of the fish sausages, as measured by the TPA compression and penetration test (an example is shown in figure 6).

WP 4.4 Safety/HACCP of collagen extraction from cod skins and bones

The aim was to investigate whether collagen from fish by-products could serve as an important raw material in high quality food. Since cod is a major commodity in western European countries, better use of by-products from filleting could result in reducing waste and producing a valuable ingredient for the food industry.

In the application of collagen as a food ingredient, it has to be ensured that there are no human health risks implicated in application of collagen as a food ingredient. EU legislation requires an analysis of hazards associated with the manufacturing of the ingredient, and that no toxic residues are present in the end product.

In order to establish the feasibility of this application, a hazard analysis of the production of collagen from cod skins and bones was carried out.

Evaluation of hazards revealed that the extraction step with butyl alcohol can not be controlled in practice. The chemical substance is not safe to apply in the production of foodstuffs, since the washing step cannot guarantee complete elimination of the toxic solvent. Therefore the butyl alcohol should be excluded from the process. During our work we used butyl alcohol to obtain a pure collagen preparation.

With the application of appropriate control instructions that can be developed the experimental process of extraction of collagen can be regarded as a basis for safe production of collagen from cod skins and bones (for more details see Annex 3).

WP 4.5 Increasing the heat stability of fish collagen and/or gelatine

A protocol was established (see figure 5). Results from the analysis are presented in section WP 2.6 “Evaluation of functional properties of extracted collagen: cross-linked collagen from cod and hoki, and collagen extracted from carp”.

1.4 Compare the progress achieved against the activities planned for the period

Deliverables

The subcontracted SME Moerdijk Van Oosten en Partners B.V. organised a second workshop, which was held on 15 June 2004 in the Netherlands (see Annex 2). The workshop and the information presented in Annex 1 comprise deliverable 12. The planned month of delivery of deliverable 12 was 25.

Work for WP 4.5 (cross-linking of the collagen preparations) and WPs 4.4 (Safety/HACCP) has been carried out as planned. The Deliverables of WP 4.4 and 4.5 are 15 (planned date of delivery month 32) and 25 (planned date of delivery month 40), respectively.

Deliverable 16 of WP 3.5.2 (date of delivery month 40) is shown section WP 3.5.2 “Improvement of texture of fish by-products by the application of collagen” in this report.

With regard to Deliverable 15 (Methods to increase the heat stability of collagen) a protocol was tested to treat cod and hoki skin collagen with transglutaminase. It appeared that the samples were inhomogeneous and this hindered analysis.

Deliverable 25 is shown in Annex 3 of this report.

1.5 Update of tables 1, 2, and 3 from the technical annex of the contract, as reported in the previous progress reports

Updates of the tables are presented as tables 3, 4 and 5 in this report, respectively. At the first plenary meeting in Trondheim it was decided to extend the duration of workpackage 2.6 from 13 to 17 months (see table 3). The number of person months for partner 06 was not changed. Due to this extension the start month of workpackage 2.6 and 4.5 was changed into month 15 and 22, respectively. The former months are shown between brackets. The end workpackage 2.6 and 4.5 will, therefore, be month 25 and 33, respectively.

The changes also affect the availability of milestones 1 and 2. The former months, which indicate the time of availability, are given in brackets in table 4.

Due to these changes deliverables 10, 13 and 15 will become available in month 23, 28 and 32, respectively (table 5). The former months for these deliverables are given between brackets in table 5.

2. Contribution of partner 06

2.1 Work accomplished

WP 3.3 Extraction of collagen from cod skins and bones

Extraction of collagen from skins and bones
Participant 06 provided participant 8 with detailed information on extraction methods for collagen from skins and bones.

WP 2.6 Evaluation of functional properties of extracted collagen

Physico-chemical properties of cod and hoki skin collagen are present prior and after treatment with transglutaminase. Carp collagen samples provided by participant 8 were analysed.

WP 3.5 Industrial applications and marketing of extracted collagen

3.5.1 Marketability of extracted collagen for application in the food industry

Participant 6 attended an national and international workshop on utilisation of by-products and contributed to organising the national one. An overview on the opportunities and threats of the use of fish collagen and gelatine is presented in Annex 1. Participant 6 contributed to forming of a consortium for a Craft project proposals on fish by-products. Participant 4 and 6 intend to send in the proposal in the course of 2005.

3.5.2 Improvement of texture of fish by-products by the application of fish collagen
The effect of collagen on the textural properties of fish sausages from cod by-products was studied.

WP 4.4 Safety/HACPP of collagen extraction from cod skins and bones
A report on HACCP of collagen extraction is presented in Annex 3.

WP 4.5 Increasing the heat stability of fish collagen and/or gelatine

Participant 6 studied the application of transglutaminase with cod and hoki collagen.

2.2 Deviations

None

2.3 Actions taken to remedy significant problems encountered

Not applicable

2.4 Changes in the scientific team

None.

2.5 Activity of subcontractors

The SME Moerdijk Van Oosten en Partners B.V (subcontractor 04). organised a workshop on collagen. The workshop was held on 10 April 2003. A second workshop was held on 15 June. The subcontracted Agotechnology and Food Innovations (formely known as ATO) performed the rheological measurements.

2.6 Indication of resources used

The number of person months devoted to each WP during the reporting period are: WP 2.6: 2 person month; WP 3.3: 0.1 person months; WP 3.5: 2 person months; WP 4.4: 2 person months and WP 4.5: 8 person month.

3. Exploitation and dissemination activities

A second workshop on collagen was held for industry (food and non-food). Participant 6 also was present at an international workshop, which was organised by the coordinator and held in Brussels on 3 May. Results obtained by partner 06 were presented at the First Joined Trans Atlantic Fisheries Technology Conference 10-14 June 2003, Reykjavik, Iceland (see Annex 4).

Oral presentation

Van Pelt-Heerschap, H., Kotterman, M.J.J., Shaw, N., Kals, J. and Van de Vis, J.W. (2003): Extraction and characterization of collagen extracted from skin and bones of cod (Gadus morhua). In *Proceedings of the First Joined Trans Atlantic Fisheries Technology Conference 10-14 June 2003, Reykjavik, Iceland*, Icelandic Fisheries Laboratories, Reykjavik, Iceland, pp 355-356, ISBN 9979-74-005-1.

Poster presentation

Rustad, T., Aursand., M., Arason, S., Shaw, N., Pommer., K., Van de Vis, H. and Berge, J.P. (2003): Utilisation and stabilisation of by-products from cod species. In *Proceedings of the First Joined Trans Atlantic Fisheries Technology Conference 10-14 June 2003, Reykjavik, Iceland*, Icelandic Fisheries Laboratories, Reykjavik, Iceland, pp. 367, ISBN 9979-74-005-1.

4. Ethical aspects and safety provisions

Not applicable

5. Mid-term review

Not applicable.

6. Plans for the next reporting period

None

7. Requests to the Commission

None

8. References

Borderias, J., Angeles M., Montero, P. (1994). *Z. Lebensm Unters Forsch* 199: 255-261.

Gudmundsson, M. and Hafsteinsson, H. (1997). *Journal of Food Science* 62 (1), 37-39.

Haard, N.F., Simpson, B.K. and Sikorski, Z.E. (1994). Biotechnological Applications of Seafood Proteins and Other Nitrogenous Compounds. Chapman and Hall, New York, pp 195-216.

Norland, P.E. (1989): Fish gelatine. In: Advances in Fisheries Technology and Biotechnology for Increased Profitability. (M.N. Voigt and J.R. Botta, Eds), Technomic PublishingCo. Inc., Lancaster, Pennsylvania, US.A. pp 325-333.

Sikorski. Z.E. and Borderias, J.A. (1994): Collagen in the muscle and skin of marine animals. In Seafood Proteins. In Seafood Proteins. Chapman and Hall, New York, pp 58-70.

Table 3: Work package list for partner 06

Work package	Work package title	Responsible partner	Person months	Start month	End month	Deliverables
WP 2.6	Evaluation of functional properties	6	3	15 (11)	25 (21)	10
WP 3.3	Collagen extraction on small and large scale	6	41	1	17 (13)	13
WP 3.5	Industrial applications and marketing of extracted collagen	6	3	12	25	12, 13, 16
WP 4.4	Safety/HACCP	6	7	22 (22)	36	25
WP 4.5	Increasing the heat stability of fish collagen and/or gelatine	6	2	22 (19)	33 (30)	15

Table 4: List of milestones for partner 06

Milestone no.	Title and description	Deliverable date	Partner
1	Characterisation of chemical composition of extracted collagen (including seasonal, habitat and species variations)	21 (17)	06
2	Documentation on efficient method for extraction of collagen	21 (17)	06
8	HACCP protocol for processing of collagen and/or gelatine	36	06

Table 5: List of deliverables for partner 06

Deliverable no	Title	Delivery date (month)	Nature	Partner 06	WP	Dissemination level	Dissemination target
10	Data on seasonal variation in functional properties of extracted collagen	23 (21)	R	x	3	PU/CO[1]	Industry, scientific publishing
12	Description of the market situation regarding gelatine and collagen	25	R	x	3	PU	Industry
13	Documentation on extraction of collagen	28 (26)	O	x	3	PU	Industry, scientific publishing
15	Methods to increase the heat stability of collagen	32 (30)	O	x	4	PU	Industry, scientific publishing
16	Evaluation of the use of by-products in food systems	30	O	x	3	PU	Industry, scientific publishing
25	HACCP protocol	36	R	x	4	PU	Industry

[1]Provided that there are no patents pending, the results will be published

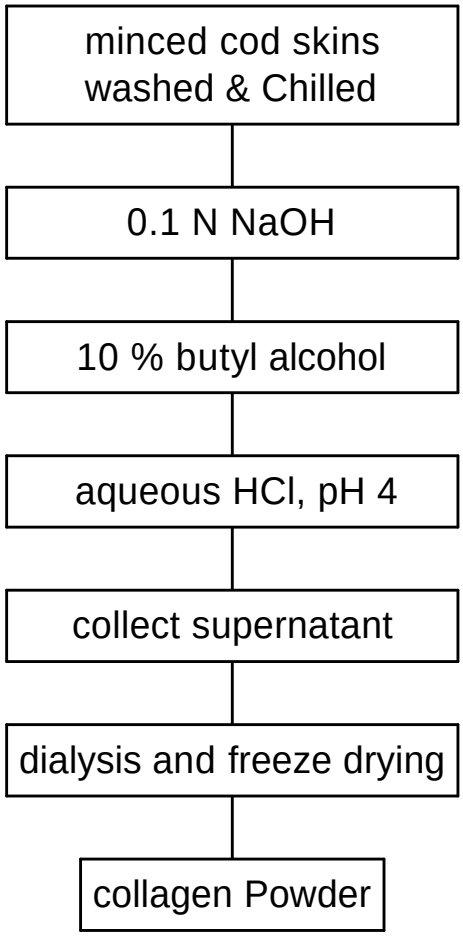

Figure 1: Flow sheet for extraction of collagen from cod skins

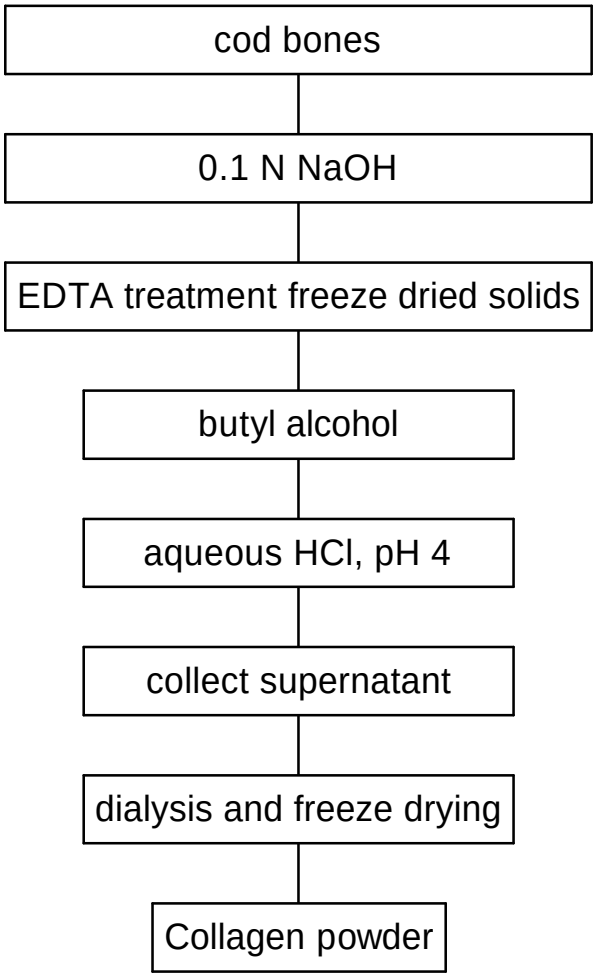

Figure 2: ***Flow sheet for extraction of collagen from cod bones***

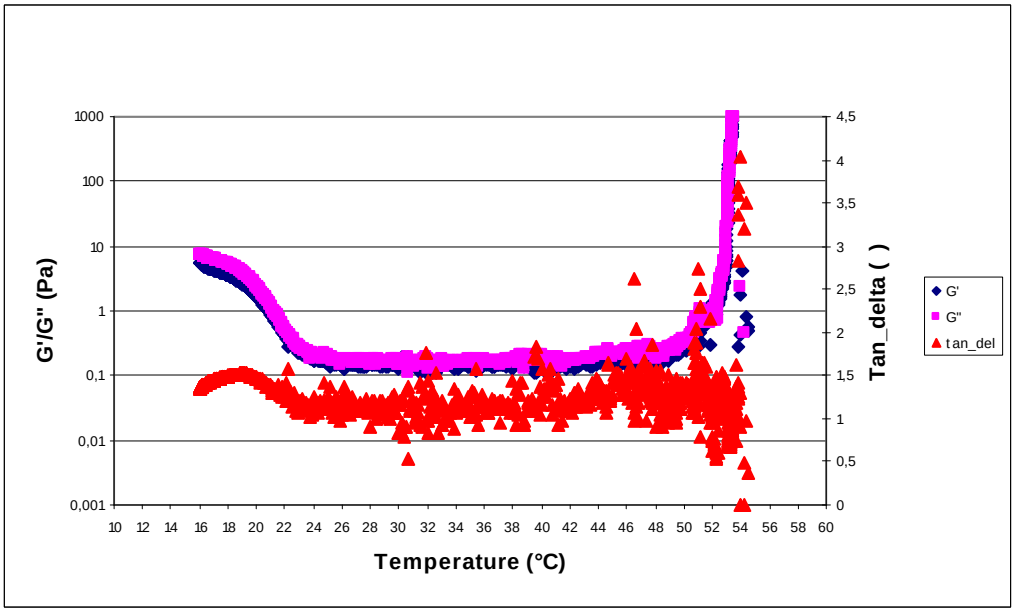

Figure 3: Rheological properties of collagen from cod skin

G_1= elastic modus
G_2= viscosity modus

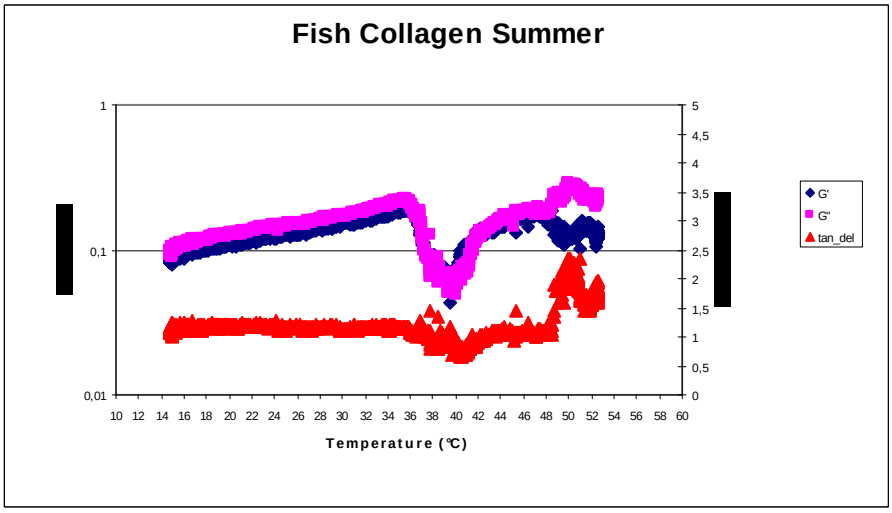

Figure 4: Rheological properties of collagen from carp

G_1= elastic modus
G_2= viscosity modus

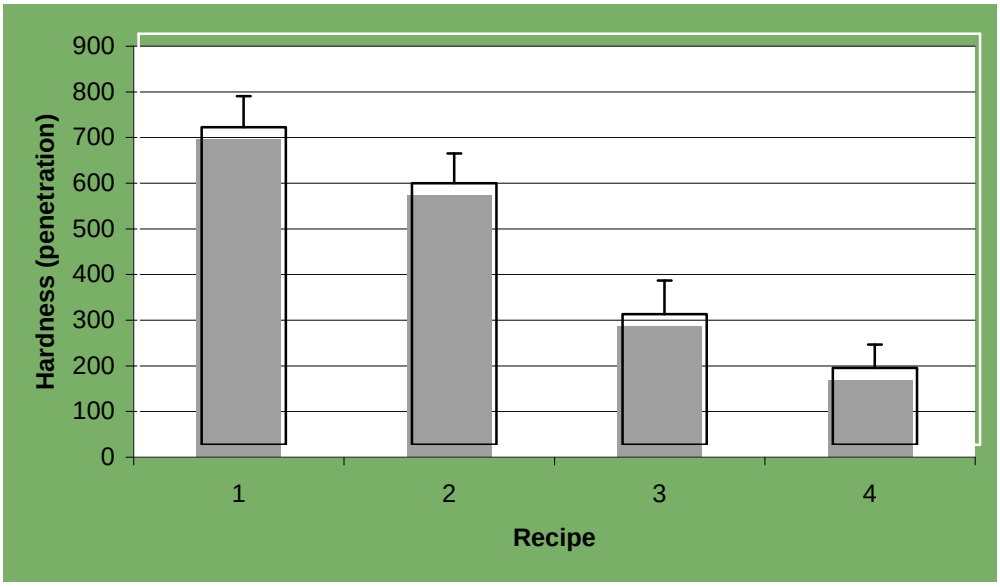

Figure 5: Effect of the four recipes on hardness: penetration test.

The x-axis depicts the different recipes and the y-axis the hardness. Its obvious that there is a significant difference between the different recipes (F=120 Fcrit 2.92, p=0).

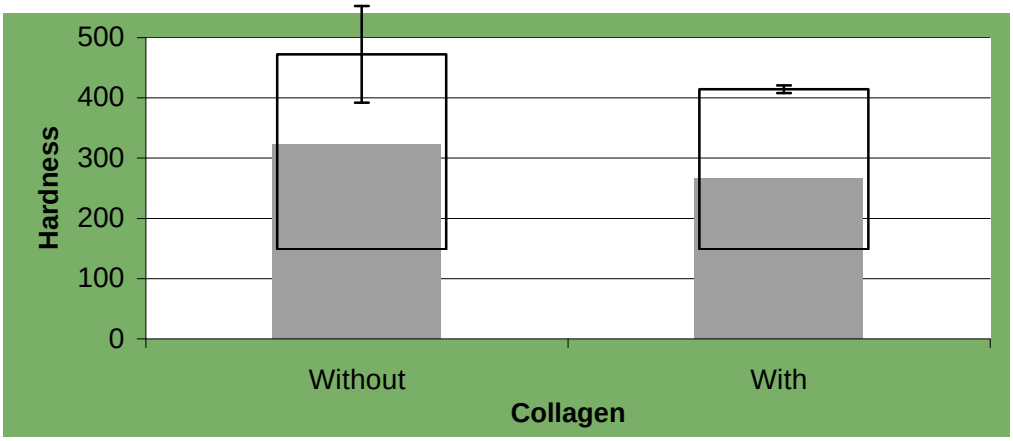

Figure 6: Effect of collagen on the hardness of sausages (recipe 3; 40% water and 8% olive oil): penetration test.

The x-axis depicts the recipe with or without collagen and the y-axis the hardness in g (F(1,7) = 2,03 p< 0,20)

Protcol for collageen modification

20 g collagen solution

?

Estimate collagen concentration measuring the absorbance (280 nm). The pH of the collagen solution must be adapted with y ml NaOH until pH 5.0 ± 0.2

?

Dissolve MTGase at ratios (0 and 120) U/g collagen (Activity ActivaWM 100 U/g) in 2 ml demi-water, and add to collagen solution while stirring.

?

Keep collagen solution with enzyme at 10 ?C ± 2?C. Collagen is not allowed to denature.

?

Reaction times: (0, 0* and 4 hr), with slow stirring.

?

After the specific reaction time the samples are immediately frozen by –25?C.

?

Samples must be thawed in water (10?C) just before the visco-elastic measurements will be performed to prevent the enzyme to start working again.

?

Visco-elastic measurements will be performed using a rheometer with a cone-plate geometry (cone angle 4?, gap 0.15 mm). Temperature ramp implemented are from 14 to 55?C with oscillating applied stress of 3.0 Pa. The elastic modulus (G'), viscosity modulus (G'') and the relation between the two, i.e. phase angle will be represented as function of the temperature.

Experimental setup:

	Niet vriezen	**Zonder enzym**	**Met (120 U/g)**	**(120 U/g)**	**(120 U/g)**
	Time = 0	*Time = 0*	*Time=0**	*Time = 4 uur*	*Time = 22 uur*
RIVO extr	A	1	3	5 a, b	5 c, d
UCC extr	B	2	4	6 a, b	6 c, d

Figure 7: Protocol for cross-linking of extracted collagen from cod and hoki skins

Variables:

- pH: 5.
- Reaction temperature: optimum reaction temperature of the enzyme is 50 ?C, which cannot be used in this situation. In this experiment the highest possible reaction temperature of 10?C ± 2?C is taken, to be on the safe site to prevent that cod collagen is changing into gelatin.
- Reaction time: (0* and 4 hours).
- Enzyme concentration: (0 and 120) u/g collagen. Standard activities of Activa transglutaminase, measured by hydroxamate method (EB 50 U/g and WM 100 U/g).
- Slow stirring due to modification.

Note: the substrate specificity of MTGase is different between collagen and gelatin, which is reactable respectively very reactable. This could give problems, when the extract is not purely collagen, but contains a fair amount of gelatin as well.
* 0 is the minimum time possible to add and get rid of the enzyme.

Needed amount of solution: 6 treatments in duplo = 12*20ml=240 ml

Figure 7, continued

Annex 1 Opportunities and threats for fish collagen and gelatine

The first commercial uses of gelatin were recorded in Holland in the 17th century. Today the global consumption of gelatin rose to approximately 250,000 t, worth 1.2 billion US dollars according the Gelatin Manufactures Association [1]. The biggest amount, approximately 40-45% of the total production in produced as well as consumed in Western Europe (table 1 and figure 1) with an annually growth at about 3% [2].

Table 1: Estimated global consumption of gelatin by region [3]

Region	**Consumption (t)**
Western Europe	110,000
United States	80,000
Other	60,000
Total	**250,000**

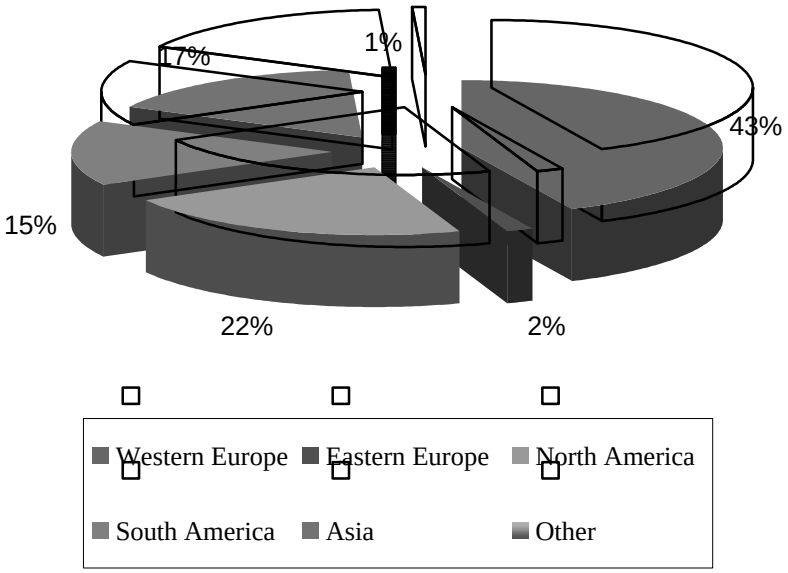

Figure 1: Production of gelatin per region [1]

Gelatin is currently extracted from bovine, porcine and fish sources. In Europe, porcine gelatin now accounts for 58% of the total volume (table 2). The high volume of bovine gelatin still used, despite BSE, is due to the preference for bovine gelatin for encapsulation in pharmaceutical applications. The producers only use raw materials from bovine sources, which are from countries certified as free of BSE. Porcine gelatin offers an option on avoiding BSE completely, but the availability of raw material can be problematic[3].

Table 2: West European consumption of gelatin by source [3].

Source	**% of total**
Porcine	58
Bovine	42
Fish	<1
Total	**100**

European Consumption of gelatin by source

Volumes of fish gelatin are relatively minor. Total volume of fish gelatin consumed in Western Europe is estimated at about 500 t. Global consumption of fish gelatin is believed to be around 1,000 to 1,500 t. The market for fish gelatin is a niche market, which mainly serves the market for specific food and pharmaceutical applications. Limiting factors for further growth in applications are the perceived lack of availability and security of raw material. Also pricing levels of fish gelatin remain relatively high and thereby limiting consumption even more.

Properties

Gelatin is multifunctional. The most important characteristics, which define the behavior of gelatin in a final product are gelling power, viscosity, melting point, color and odor. In addition to the influence of the source of the raw material the production process will also affect the properties. Bovine bone gelatin is typically produced using an alkaline process and these grades are referred to as B type gelatins. Porcine and bovine gelatin from skins are produced using an acidic process and are called A types. Fish gelatin is predominantly an A type. The melting point does vary depending on the grade and greatly affects the use of the specific gelatin. Lower melting point gelatins dissolve faster in the mouth, thereby releasing the flavours more quickly for an instant taste sensation. For most applications, gelatin is required to be solid at room temperature so that the final product holds form even when removed from the fridge. Also colour and odour are very important. The gelatin should be as clear as possible and in addition no odor should be generated by its incorporation into the final product.

Fish gelatin

Fish gelatin is different to other gelatins. Fish gelatin can be extracted from cold or warm water fish species. The type of gelatin will vary depending on the fish type, environment and production process. Gelatin and or collagen extracted from cold-water fish species are particularly different as the melting point is below 10°C. A typical cold-water fish for the production of gelatin is Cod. Gelatin from cod has the same range of amino acids as bovine and porcine gelatins, but the levels of proline and hydroxyproline are lower [3,4]. These amino acids are believed to be responsible for the forming of H-bonding and the gelling. Cold-water fish gelatin can be used in applications, which do not require gelling at room temperature, in which the function of gelatin lies in its other abilities such as prevention of syneresis and texturization. Further these kinds of gelatins can be used in frozen or refrigerated products, which are consumed quickly following removal from the fridge or defrosting[3].

Presuming a favorable pricing structure they could be used in large volume consumer-price driven products such as low fat spreads and yogurts. In these products, often cold-water soluble grades of porcine gelatins are used which are available at a price premium of approximately 25%. Alternatively, normal grades are used as in yogurts and considerable agitation is required to prevent clumping. Fish gelatins with low melting point would be easier to incorporate.

Gelatin and or collagen extracted from warm water fish species more closely resemble bovine or porcine gelatin, which melts at 32-35°C. Tilapia gelatins have a melting point of 25-27°C, therefore are suitable for products at low room temperature. Warm water fish gelatin grades can therefore more readily compete in the traditional gelatin markets.

A limiting factor in the production of fish gelatin is the availability of raw materials. Producers have found it difficult to source adequate quantities of a particular fish type on a guaranteed basis. This primarily applies to warm water fish. It is also difficult to obtain certification on the raw material, which is required for the traceability, which becomes a necessity for food additives especially from animal sources. Other limiting factors, although the producers started to get these resolved, are residual odor and differences in product characteristics compared to gelatins available from bovine or porcine sources.

Prices for fish gelatin vary considerably in the market from €12 to €18/kg [3]. Under current conditions, producers of fish gelatin may find it difficult to lower the prices due to the low yield from fish skins and the lack of economy of scale in the production process. An advantage of fish gelatin is that it is *kosher* as long as the fish has scales and fins.

Applications for gelatin

Gelatin is used in a wide variety of applications including ingestible applications and technical applications. Gelatin is available in several grades and the source and grade will determine the specific behavior. Local conditions, such as temperature and pH will also affect the properties of the gelatin in the final product. Gelatin competes with several other food additives but it has some specific advantages, which gives gelatin its unique properties, such as formation of elastic thermo reversible gels, control of crystal formation, film formation, fat substitution, water-binding, improved mouth feel and thickening[3].

Table 3: The following table lists major applications and functions for gelatin by category ([1,3]):

Application	Function
Food	Gelling, stabilization, emulsification, water-binding and prevention of syneresis
Pharmaceutical	Capsule production and coatings
Photographic	Support of silver halide systems
Micro encapsulation	Vitamins/additives encapsulation
Cosmetics	Delivery systems
Technical	Paintball, coatings, viscosity control micro-encapsulation of vulnerable compounds

Food

Fish gelatin is used to a limited extent in food applications. It is regarded as a niche product as the industry cannot support the high price for general products. Low melting point grades are used in high-energy drinks and some refrigerated or frozen products such as yogurt or sour cream. Fish gelatin is also used in icing.

Pharmaceutical

Minor volumes of fish gelatin are used to make soft gel capsules. Most encapsulators have developed the expertise to handle fish gelatin in this process, but it is more difficult to handle than other gelatins. Fish gelatin soft capsules are most popular for nutrition supplements for the French market.

Micro-encapsulation

Fish gelatin is used by the micro-encapsulation of vitamins and pharmaceutical additives such as azoxanthine or colorants.

Photographic industry

Fuji Photo is currently researching grades of fish gelatin. The aim of the research is to identify a gelatin grade with improved functionality rather than a desire to move away from bovine gelatin. In fact, most of the consumption is expected to continue to be dominated by bovine bone gelatin. An advantage of fish gelatin is that it is de-ionized which leads to lower conductivity.

Grades of fish gelatin under research are free of reducing impurities, as these would interact with the silver ions. A concern of Fuji is the batch production cycle for fish gelatin as batch-to-batch consistency is considered greater for continuous production programs. The company indicated that, should there be an economic advantage in using fish gelatin it would consider using it more widely, should it prove successful.

Competitive products

Gelatin has strong competition in the food industry from various agents, many of which are vegetable-sourced. In pharmaceutical and photographic applications, alternatives are hard to find. Pectins, carrageenan, alginates and guar gums in the food industry and povidones, acrylates and cellulosics for the pharmaceutical industry could be competitive in respect to gelatin. But gelatin is almost unique in its ability to fulfil the requirements of many industries. Advantages are excellent gelling properties, reversible gel formation, foam stabilization, properties highly manipulative-available in a wide variety in viscosity and melt temperature, transparent, taste and odour less, availability in sufficient volumes, relatively inexpensive, biocompatible and biodegradable.

Market volumes

The food industry accounts for the greatest amount of gelatin, with a consumption estimated around 70.000 t or 64% of the total in Europe. The pharmaceutical industry is the second most important application consuming around 18% of the total. Volumes consumed are shown by application in table 4.

Table 4: Volumes consumed by application [3.]

Application	Volume in t	% of total
Food	**70,000**	**64**
Pharmaceutical	**20,000**	**18**
Photographic	**10,000**	**9**
Other	**10,000**	**9**

Food

The food industry uses mainly porcine gelatin, having changed in the past ten years from a predominance of bovine gelatin. There are some applications where the properties of bovine gelatin cannot be matched by porcine grades and therefore bovine gelatin has not been completely dropped. At this point in time, only minor volumes of fish gelatin are used, as the cost is prohibitive for many foods. Current applications are believed to include health beverages and dietetic foods.

Gelatin, a traditional food ingredient, is used in a wide variety of foods. Its neutral-taste, coupled with its excellent properties in gelling, stabilization, binding, and emulsification make it an ideal food additive for many foods. In addition gelatin is almost unique in its ability to stabilize foam, an important requirement in many confectionery items and chilled and non-chilled desserts.

In more recent years another important function of gelatin has been identified and developed. Its performance in giving fat-like properties allows gelatin to replace some of the fat content in many applications. One of the main product groups in which this property is used is in low-fat table-margarine and spreads.

Gelatin prices

Prices for gelatin vary by source and by application, but the determining factor for price tend to be viscosity. Prices in the photographic industry, which predominantly use bovine gelatin, vary widely depending on the grade. Average prices are higher than other applications due to the level of purity. Special grades can be between €12-13 as illustrated in table 5:

Table 5: Prices of gelatin divided by source and application[3]

Bovine/porcine gelatin	**Price range €/kg**
Cosmetics	4-5
Food	3-4.5
Pharmaceutical	4.5-8
Photographic	7-13
Fish gelatin	
All applications	12-18

The major producers of gelatin

There are approximately 10 major gelatin producers. The larger producers use many different sources of raw material and employ both acidic and alkaline extraction processes. The most important producer of fish gelatin is Norland in the United States/Canada [5], but many of the traditional producers have invested in the production of fish gelatin. Some major producers are described below.

Croda [3,6]

Croda was formed in 1925 and produces gelatin from a variety of sources including bovine, porcine and fish [6]. Initially, Croda offered fish gelatin as a niche product for the kosher market. Due to the new level of interest found in the market, the company now foresees an opportunity to build it up to a reasonable level. Croda produces its gelatin mainly from warm water fish. The company indicated its product had very different properties to types offered by Norland. Problems with taste are solved, but odour is not completely absent [3,6].

Rousselot [7]

Rousselot is currently the leading supplier of gelatins worldwide, accounting for approximately 20% of the market. The company is the number one player in pharma-ceutical and photographic applications and ranks second in the food industry. All Rousselot facilities are ISO certified, and have implemented HACCP programs. Rousselot™ gelatins comply with the international food standards (FAO / WHO, AFNOR), and/or pharmacopoeia (USP, European Pharmacopoeia, Japanese Pharmacopoeia). All sources are used, for the production of around 50,000 t of gelatin. The company is using a variety of fish species. Rousselot regards its fish gelatin as a niche product and production volumes are small (<100t). The company does not expect a strong growth for fish gelatin due to the lack of availability of raw materials and high production costs [3,7].

Miquel Junca [8]

Miquel Junca is located in Spain and produces approximately 4,000 t of gelatin per year. Pigskin is the main source of raw material. The company produces less than 100 t of fish gelatin. Miquel Junca has found greatest interest in fish gelatin from France. The main application areas are dietetic food with low sugar content and pharmaceutical capsules. The company does not expect fish gelatin to account for a great share of its production in the future as it has found a strong limiting factor to be the availability of raw material [3,8].

Figli di Guido Lapi [9]

The Italian company Figli di Guido Lapi produces approximately 2,400 t of gelatin from bovine and porcine skin. The company also claims to have commenced production of an odourless, tasteless fish gelatin very recently. Due to the high price, the expectation for volume growth is limited but the company anticipates some interest in niche markets. [3,9]

Reinert Gruppe [10]

Reinert produces approximately 6,000 t gelatin, most of which is produced form porcine sources. The company attempted to market fish gelatin and invested in some new equipment for the production of fish gelatin under high pressure. However, the product, although tasteless when dry, gave off-flavours once incorporated into foodstuffs. Reinert could not find any interest in its product, even in the fish products industry [3,10].

Fibrogen[11]

Fibrogen is an American-Finnish company and is offering 'synthetic' gelatin to the market as a development product. The company has developed a method to express the genes for collagen with prolyl hydroxylase so it can produce recombinant collagen and gelatin. Fibrogen is working on the development of the use of recombinant or synthetic gelatin for vaccines as a stabilizer, the use of gelatin in plasma expanders, hard and soft gel capsules, gel tabs and wound care. This product has not yet been commercialized, but the success of current projects has led to great expectations. As recombinant gelatin will be very expensive the company is directed to the higher end of the pharmaceutical market. Recombinant gelatin, as it is synthetic, will have a defined amino acid characterization, molecular weight, structure and other parameters. All other gelatins are natural and so can vary by species or due to diet by age etc. Batch to batch consistency and reproducibility can be very difficult to achieve. This offers scope for recombinant gelatin to enter this market [3,11].

Weishardt[12]

Weishardt founded in 1839 operates two factories for gelatin production producing up to 10,000 t from pigskin and up to a max of 2,500tonnes from bovine skin. Weishardt decided two years ago to start the production of gelatin from fish. A batch was produced two years ago, which is now being sold off in small volumes. Weishardt's product is priced at about €18/kg. There are no plans for another production batch as the high price is limiting sales [3,12].

Norland [5]

Norland founded in 1960 is specialized in gelatin used in the electronics industry and is believed to be producing approximately 500 t of fish gelatin. Norland has 5 to 6 different grades; Dry Fish Gelatin, high molecular weight fish gelatin, high pure liquid gelatin, photo engraved glue and high tack fish glue [5]. Currently, Norland markets its products for technical, food and pharmaceutical applications. Norland can vary the molecular weight of fish gelatin and is known as a supplier of high quality, high viscosity fish gelatins. The company has been producing fish gelatin for forty years and has been marketing fish gelatin for ingestible applications for twenty years. Norland has done considerable work in the characterization of its

gelatins. Typically, its grades have lower proline and hydroxyproline levels than other grades available on the market. Norland sources its fish gelatin from cold, deep-water fish such as cod, haddock and pollack. Norland sells its products directly to customers in Europe. Norland indicates that it can only grow its business for cold-water gelatin by finding its own markets, as its grades cannot compete directly with bovine or porcine gelatins. Photosensitive applications seem to be a key focus of Norland's activities. The company has developed considerable expertise in the use of fish gelatins in photoresists (light sensitive coatings) for the electronic industry-in televisions and colour video cameras. Photoengraving applications were the first applications researched by Norland [3,5].

Consumers of fish gelatin

Hoffmann-La Roche is believed to be the only big consumer of fish collagen. The company is estimated to account for 60% of the total European fish gelatin consumption, purchasing approximately 300 t annually.

Attitudes towards gelatin

Attitudes to the consumption of gelatin have been strongly influenced by BSE. In food applications where gelatin's properties are not unique, it has been largely replaced by other hydrocolloids. But, often a combination of other agents is required in order to fulfil the multi-functional behaviour of gelatin and in some products, properties such as drip control are difficult to achieve without gelatin. The food industry appreciates the advantages and versatility of gelatin. Appreciation of the properties of gelatin, is evident in the switch by some parts of the food industry from bovine gelatin to porcine grades rather than dropping gelatin completely or dropping products, which could only be made with gelatin during the BSE crisis. In applications where the properties could not be met by other additives, the industry now uses porcine grades. Some food manufacturers changed to porcine gelatin as a temporary measure and eventually plan to avoid it completely, but as some applications for gelatin will disappear new ones will appear especially in the field of low fat products. Gelatin is low in calories and melts in the mouth to give excellent sensory properties resembling fat, making it ideal for low fat products. The capsule producers regard gelatin as ideal raw material for capsule formation. However, the industry is looking for alternatives with film forming properties, which offer the same flexibility and dissolution profiles. The only major disadvantage of gelatin is the animal nature of the source. Vegetarians will choose tablets over capsules to avoid gelatin. Muslims and Jewish populations have specific constraints too. Therefore, as capsules are based on gelatin from bovine and porcine sources they cannot be offered on a global basis to encompass all races and minorities [3].

In photographic applications gelatin is expected to continue to be a major component. Its behaviour and gelling properties are unique and there is little pressure due to BSE or the animal origin of gelatin to change to other agents. The shift to digital photography will probably lead to some decrease in gelatin consumption. Gelatin is also used in digital photography, but it is expected that the net effect may lead to only a slight decrease in volume. The **cosmetics** industry is not very concerned by the use of gelatin in bath pearls with the exception of BodyShop, which highlights the use of animal derived gelatin in its bath pearls as the only animal-sourced ingredient used in the BodyShop product range. Protein use in cosmetics changed dramatically some years ago towards vegetable-based proteins, but should the efficacy of gelatin be proven in skincare or hair care, it may regain market share. However, volumes are unlikely to be large due to the wide variety of proteins available to cosmetic formulators.

Trends and driving forces

BSE has been a negative force in consumption of gelatin for ingestible applications. But it is not the only one. Other forces such as desire for vegetarian, halal and kosher standards are also important. The debate over BSE tends to be cyclic in Europe with issues over beef arising with

each new case identified. Recent cases in Japan have raised concerns over the use of gelatin outside Europe and there is a rumour in the industry that BSE will be identified in America as well. At the moment food manufacturers plan to avoid gelatin where possible. Currently, in the pharmaceutical industry, even technicians believe in the scientific data to show BSE is contractible from gelatin.

There is a desire to be save, and so the industry is adopting a policy of zero risk. If there is even the slightest chance of a consumer contacting the human form of mad cow disease from gelatin then it must be eliminated. As the majority of gelatin used in the pharmaceutical industry is derived from beef, these concerns have led to intensive research in Europe to identify and develop alternatives. Only a few alternatives are available and therefore it has not been possible to eliminate gelatin. Vegetarians are more and more accommodated in the marketplace and an increasing amount of products are made suitable for them. Similar kosher or halal products are provided for avoiding the use of porcine or bovine gelatin respectively. However, this is seen as a stop-gap solution as it leads to the development of different products for different markets. Companies would rather use a raw material with global acceptance, thereby allowing the production of global products and brands, which are suitable for all sectors of the population. There is a tremendous level of research to identify or discover potential materials or blends of hydrocolloids to replace gelatin. These could be of plant origin or synthetic. Synthetic options are of interest in particular to ethical pharmaceutical companies-the non-natural basis would not be considered a problem, as many drugs are synthetic. In foods the preference is still for natural products. There is a belief that more cases of BSE will arise in the coming years and therefore the concerns over BSE will not dissipate for some time. Therefore the industry has invested in the generation of scientific data to prove that the human form of mad cow disease is not contractible from gelatin. Recently a new study was published and the results are accepted by scientists as evidence of the lack of risk. The manufacturers now hope that the EU will accept these findings and raise some of the limitations on allowable sources of gelatin for food uses [3].

Gelatin manufacturers hope that this publication will finally stop the mad cow debate as they can then market all grades of gelatin as completely safe. Gelatin producers plan to launch a new marketing offensive on the food industry, backed up by this new scientific data in which there is absolute trust. There is belief that gelatin holds much potential as a functional food and will benefit from the current focus on identification and development of new products in this fast-growing market. The industry is always busy to find new sources of gelatin. There is much interest across the market in gelatin from fish and poultry. Fish gelatin is already available on the market. Poultry skin and bones are also expected to yield gelatin in the near future. Currently, these are niche products.

A potential problem with fish gelatin is in new labeling requirements[3]. In September 2001, the European Commission adopted a proposal to amend the existing EU framework on food labeling (Directive 2000/13/EC) in order to ensure mandatory labeling of certain allergenic substances and to provide more information to the consumer on all ingredients contained in food by abolishing the so called “25% rule” for compound ingredients.

Current EU food labeling rules require all food ingredients to be listed with the exception of compound ingredients (ingredients composed of several ingredients), which constitute less than 25% of the product. In its proposal, the Commission seeks to abolish this exemption. In addition, current EU food labeling rules do not require the labeling of allergenic substances. Under this proposal for an amendment to the directive, labeling of certain allergens listed under Annex IIIa would be mandatory. This list includes fish and fish products. Currently, gelatin is indicated on the label of food products, but the source of gelatin is not included. Should this amendment go through, fish as the source of gelatin would have to be indicated. The amendment guidelines indicate gelatin would be included in the list of ingredients, rather than specifically indicated as a potential allergen [3].

Attitude to fish by products

In the food industry, the attitude to fish or fish products varies depending on the sector being investigated. For example in fish products fish gelatin would be very acceptable, but in other sectors acceptability is related to the labeling requirement. If the gelatin source must be indicated, there is considerably more importance attached to the source of gelatin and the consumer acceptance of the source. In low fat spreads, fish was regarded as acceptable, but it would not allow for the production of vegetarian products. In desserts there is an expectation of a negative attitude to fish products due to the taste and odour from fish, which does not fit creamed desserts. In ready meals the attitude varied –overall fish is regarded, as 'healthy' but the vegetarian label requirement would not be met for non-meat based products. The source of the fish would be important. Recently, there have been reports in the press on the unhealthy state of fish, particularly salmon, bred in fish farms. Fish farming methods have become intense, leading to the development of various fish diseases and unnatural states of health. This has led to fears and concerns over the value of looking to the fish industry as a favourable health option to red meat. Food processors appreciate the distance between gelatin from fish and the actual fish in terms of purity. Providing the fish gelatin is taste-free and odour-free, fish gelatin would be regarded as a very acceptable alternative to current grades, especially if it offered advantages in functionality. However, if the fish source must appear on the label then the consumer perception will play an important part in their decision on use of fish gelatin. This attitude of consumers will vary by food sector.

In the pharmaceutical industry, the opinion on fish gelatin varies. Some see gelatin from fish as an attractive alternative as fish is perceived to be 'healthy', but for the production of capsules, pharmaceutical and nutritional supplement houses are concerned that fish would not offer the marketing advantage of vegetarian status. On the positive side though, other than vegetarianism, fish gelatin would allow for the production of capsules, which could be sold to all markets globally, including the Muslim region. Considerable volumes of gelatin are used in capsules production, which if the industry will change to gelatin produced from fish, may lead to concerns over sufficient supply of high quality on a continuous basis.

In photographic applications, there are concerns over the source of fish gelatin due to the importance of purity. Photographic consumers must know exactly what is present in the gelatin grade. Bovine animals tend to have a very constant diet, which is controlled. There are fears that as the fish diet is dependent on surrounding waters, which are subject pollution, the fish could pick-up undesirable components, which may be difficult to remove and could be detrimental. However, if the purity of the grades can be guaranteed these fears can be overruled. In fact, as some grades of fish gelatin are de-ionized, they have even lower conductivity than current grades used.

Value

Fish gelatin has been available on the market for many years but the market for gelatin from fish is still a niche market. Recent volumes of fish gelatin are estimated at approximately 1,500 t on a global basis, which is small compared to the total gelatin market of 250,000 t worldwide. Despite BSE, the consumption of gelatin has grown over the past ten years with growth estimated at about 2% per year. Hoffmann-La Roche is believed to be the largest single consumer of fish gelatin in the world. The rest of the market is very dispersed. Fish gelatin has not been able to penetrate the market due to its low availability, which prevents large volume consumers to come into the market, its high price, which prohibit inclusion in many products and its low melting point of cold-water fish grade gelatin, which excludes it from use as a gelling agent in solid, room temperature foods. Fish gelatin should be completely neutral in taste even when formulated. Not all producers have succeeded in purifying the gelatin to achieve this. To enter the market successfully and to gain a significant tonnage a supplier must address the above factors and limitations.

The greatest volume potential in the short-term is likely to be in the food industry. The pharmaceutical industry, especially in nutritional supplements, also holds potential. The photographic market is more specialized and will be more of a 'make or break' nature due to the small number of potential customers. There will always be some sectors of the food market, which will strongly resist fish gelatin as some marketing departments feel fish has a negative image for some products. But across the market there is potential providing that the price must be at least half of the price today and the availability can be guaranteed [3]. When the melting point of cold-water fish gelatin can be modified to above room temperature the potential will than be greatly expanded[3]. If the melting point of fish gelatin is limited to 10 °C then the potential market will probably be small and primarily aimed at new applications such as health drinks and frozen or refrigerated goods. The potential for a given supplier is likely to stay around a few hundred tones, but if the melting point can be raised and the other issues with availability and price are addressed, the potential market is far greater. Grades of fish gelatin with higher melting points are already available on the market but are supplied by players, which have a stronger desire to prove the safety of beef gelatin and drive the market back towards bovine sources [3].

In the food and pharmaceutical markets, in applications where gelatin cannot be replaced, the vegetarian issue cannot be solved by fish gelatin, but fish as a source is acceptable to the portion of the vegetarian market that avoids red meat and poultry, but consumes fish. Fish gelatin, provided it is kosher, is suitable for the Muslim and Jewish populations and so allows for the development of products, which can be sold in the Middle East. The question of labeling as an allergen is not answerable as the EU has not yet adopted this directive. Also it is not clear if the label will apply to gelatin from fish or to more directly derived products such as fish oils. Should fish gelatin fall into this Annex, it will be a negative factor. With or without a labeling requirement, heavy and active promotion will be necessary to raise the awareness of gelatin sources and the potential to avoid gelatin derived from pigs or cows. The bovine gelatin suppliers are expected to go directly to the press with their next round of marketing, expounding the safety of bovine gelatin, following the completion of the investigations last year. There is also expected to be a focus on the use of gelatin as a protein source for health benefits and hair care products. These offensives by current bovine gelatin suppliers are expected to create an atmosphere of awareness and a greater acceptance of gelatin in general. Once the acceptance of gelatin use is established the source becomes another issue. Fish gelatin will not become as widely used as bovine or porcine grades, but it could gain some 5% to 10% market share over time. In Europe alone, this would mean a market potential in the order of approximately 5,000 to 10,000 t [3].

Acknowledgements

This part is largely compiled from a market report written by Rubin (http://www.rubin.no) in 2002 and the website of the most important gelatin producers.

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Annex 2 Report second workshop on collagen

Summary of the 2nd Workshop on the Industrial Application of fish collagen

Date: June 15 2004
Location: Kasteel Maurick Vught
Participants: W. van de Groep, A. vd Groep & Zn BV, Spakenburg, The Netherlands
L. Olde Damink, Matricel GmbH, Aken, Germany
Dr. J.W. van de Vis, RIVO, IJmuiden, Netherlands
Dr. J.T. van Konijnenburg, Moerdijk van Oosten & Partners BV, Mijdrecht, Netherlands
Mr. E. van Heerewaarden, Smits Vuren BV, The Netherlands was unable to participate due to production problems at the plant

1. Aim of the meeting

The aim of the meeting was to inform the participants concerning the results of the research project on collagen of cod fish and to explore the possibilities for a new CRAFT project on the exploitation of fishery by-products.

2. CRAFT

The background of the CRAFT projects was discussed. RIVO gave some examples of projects underway. A summary of the CRAFT rules is given below.

Scope
The CRAFT program is part of the 6th Framework program. The aim of CRAFT is to support the research and development activities of Small and Medium Sized (SME [Midden- en KleinBedrijf]) companies in the EU and the new members.

The R&D activities can be found in all scientific areas.

The actual research and development activities have to be carried out by research institutes or by the SMEs themselves. The SME's will out source the R&D activities to one or more institutes, in case they do not qualify for research partner.

Project-budget
The project-budget should be between € 0.5 – 2 Million. Please, note that a research partner (research institute or SME that qualifies for this) will get paid 100% of their total costs. The SMEs will get paid 50% at the most of their costs.

Project-period
One to two years.

Intellectual Property
The Research institutions have no right regarding the intellectual property developed during the project.

Composition of the consortium (Minimum)
- Three independent SME's from two different EU countries of associated countries, of which at least one from a member state or an associated member states.
- Two independent research organisations from two different EU countries of associated countries, of which at least one from a member state or an associated member states.
- Other companies or end users may participate.
- In the overall consortium participants have to be from three different members states or associated member states.

SME definition (Commission Recommendation 96/280/EC)

An SME (Small or Medium-sized Enterprise) is an enterprise which:
has fewer than 250 employees,
has *either*,
an annual turnover not exceeding 40 million euro,

or

an annual balance-sheet total not exceeding 27 million euro,
and conforms to the criteria of independence.

An independent organisation is an organisation, which is not owned for 25% or more of the capital or the voting rights by one enterprise or jointly by several enterprises falling outside the definition of an SME. This threshold may be exceeded in the following two cases:

if the organisation is held by public investment corporations, venture capital companies or institutional investors, provided no control is exercised either individually or jointly,

or

if the capital is spread in such a way that it is not possible to determine by whom it is held and if the organisation declares that it can legitimately presume that it is not owned as to 25% or more by one enterprise, or jointly by several enterprises, falling outside the definition of an SME.

3. Possible subjects for a CRAFT project

With the participants ideas for a CRAFT project were discussed. Firstly both companies gave a short introduction on their activities.

A vd Groep & Zn. BV
Van der Groep is family owned company, which started in 1873. Nowadays the company supplies 75,000 tonnes of fish remains for Mink farming.
VD Groep owns production facilities in The Netherlands, Belgium and Germany.
VD Groep is interested in new products and processes for better exploitation of fishery by-products. VD Groep is willing to invest in new technology to produce high added value products out of fishery by-products for food, non-food and animal food applications.

Matricel
Matricel is a small company established in 2001 and is active in the field of tissue engineering for medical applications. Pig collagen is used as a raw material. Marticel is interested in alternative materials for the pig collagen.
The amounts needed for the production of Matricel products is limited to a few hundred kg's per annum.
For Matricel traceability and high quality standards of the product are key issues.
General ideas for a project on fishery by-products

The general scope for a project can be formulated as follows:
To investigate the possibilities to use fractions of fishery by-products as high quality ingredients for food, medical and animal food applications, such as food supplements, collagen films and others.
In principle there are three areas of application: food (e.g. collagen films), non-food (medical applications of collagen) and feed.

The University College Cork, that has the intention of heading the preparation of a Craft project proposal, gave already a first indication for a project regarding food applications of collagen from fisheries by-products.

Food applications
Objectives of Collagen research project:
From the research arising from an EU consortium on applications for fishery by-products, it emerged that fish collagen was an excellent new ingredient for use as a biodegradable/ edible packaging substance.
Edible film research has increased in activity in the past few years since the use of biodegradable matter for packaging would alleviate the rising concerns of waste disposal throughout Europe. Much work has focused on forming packaging from existing food ingredients such as whey protein, gelatin, soy, and other protein systems. Little work has focused on using existing waste products to form such edible films. There is a lot of scope for researching the use of fish skin in the production of edible film/ biodegradable packaging.
The use of food proteins as packaging/ edible films has shown that this material may have many functions:

- To provide a barrier for foodstuffs against moisture migration between layers of food in a food product.
- To provide a barrier for foodstuffs against gas transfer across a foodstuff
- To increase the shelf life of a foodstuff by coating food products with such edible films and coatings
- To add food preservatives, anti-microbial agents or colourings to foods

The objectives of establishing a research programme to assess the feasibility of fish collagen as an edible film-forming agent will require expertise from a variety of industries and we are interested in seeking support from such companies.

Non-food applications
Martricel is interested to assess whether fish skins can serve as source of collagen for medical applications The following remarks were made in this respect:

1. In order to end up with a usable collagen it is recommended to limit this “task” in a future project to “tropical” fish only (from waters of about 20°C, e.g. sea bass, which farmed in the Mediterranean). Using skins from these type of fish will probably result in a more stable product, compared to cod collagen.
2. In the project attention has to be paid to the use of the remains after the collagen extraction, which will be about 60 % of the skin input weight. In order to use this material as an animal food contamination with acids is not allowed.
3. The amounts of material needed, will be too limited for an economical extraction process. Therefore, more applications in for instance the food industrial have to be found.

Feed applications
To be worked out in more detail.

4. Feasibility of a CRAFT project

The criteria for a feasible CRAFT project can be summarized as follows:

- *Innovative*; The participants believe a project with the given scope can be innovative.
- *Beneficial for SME's*; It is of importance to build a project consortium with participant, who will benefit of the project, i.e. the SMEs will be able to expand their business by using the results generated in a Craft project. More participants will be needed in order to form a strong consortium. The following table indicates a possible structure of a Craft project proposal.

Type of participant		**Fishery by-products**	**VD Groep**
	Food	**Non-food**	**Animal feed**
RTD	University college Cork, RIVO	Matricel	
SME	To be found	Matricel	VD Groep
End users	To be found	To be found	To be found

- *If possible*, we should include end-users as this demonstrates that the proposed research if of interest for stakeholders in the whole chain. An end-user could be for instance a supermarket, which sells products with fish collagen as essential ingredient. An end-user is larger than an SME and cannot have a dominant role in the project
- *Balance between costs and profits;* It is of great importance to find a good balance between the projects and the potential profits of the results. Special attention to financial aspects has to be given, as a research partner, who is using a full cost model gets paid 50% by the EU and the remaining 50% is paid from the budget of the SMEs, which is paid by the EU. Please, note that the SMEs do not have to pay the research partners. The participants feel that this will be possible.
- *A minimum of 2 EU countries;* two countries are present.

RIVO indicates that the preparation of project with a high potential will take about six months. Therefore, RIVO proposes to send the proposal to the commission in the tender period early 2005.
In that way more participants can be found and It can be ensured that a good Consortium with a good underlying contract between the participants can be prepared.

5. Agreements

Moerdijk van Oosten & Partners will prepare the minutes of the meeting.
RIVO will inform the University College Cork (intends to be in charge of the preparation of a Craft project) about the results of this meeting.
RIVO and the University College Cork will start a search for additional participants in order to strengthen the consortium.
Both institutes will formulate more precise objectives for the proposal and subsequently, find end-users and more SMEs as partners for the consortium.

Annex 3 HACCP analysis of extraction of collagen

Contents

1. Introduction

The aim of the European research project "UTILISATION AND STABILISATION OF BY-PRODUCTS FROM COD SPECIES" (QLK1-CT-2000-01017 QLRT-2001-02829) is to investigate whether collagen from fish by-products could serve as an important raw material in high quality food. Since Atlantic cod is a major commodity in western European countries, better use of by-products from filleting could result in reducing waste and producing a valuable ingredient for the food industry.

In the application of collagen as a food ingredient, it has to be ensured that there are no human health risks implicated. EU legislation requires an analysis of hazards associated with the manufacturing of the ingredient, and that no toxic residues are present in the end product.

In order to establish the feasibility of this application, a hazard analysis of the production of collagen from cod skins and bones is carried out.

This paper presents the results of this analysis following the principles of Hazard Analysis and Critical Control Point (1), as explained in more detail in Annex 2. Based on experimental research and literature research on similar extraction processes, hazards are evaluated and critical steps in the experimental process are identified.

The process under study is still at an experimental level. It is important to note that when the process is expanded to a plant-scale level of operation, critical steps, and operational procedures have to be reviewed to effectuate measures of control and verification.

2. Hazard Analysis

2.1 Introduction

To identify hazards that will occur in the extraction of collagen from cod skins and bones, the end product will be formulated in terms of application, product characteristics, safety aspects, and shelf life. Subsequently raw material and processing steps are defined to meet these specifications. A process description and a flow chart will be used as an aid in the hazard analysis. Potential hazards will be ranked based on risk and severity of occurrence and preventative measures will be suggested to control the risks.

2.2 End product specification

The end product is aimed to be suitable as a processing aid for food manufacturing for the improvement of texture and structure of fishery products.

Table 1. End product specifications

Application	Food constituent (food grade): for the improvement of texture and structure of fishery products
Consistence	freeze-dried or spray-dried powder
Appearance	white/opaqe colour
Odour	free of odour or as much as possible
Shelf life	6 months at 20°C
Application concentration	maximum: 1-3% g/100g
Water content	maximum water content in end product: <1%

2.3 Raw material

Collagen will be extracted from raw, frozen or defrosted skins and bones, hygienically collected as by-product from filleting fresh cod. The raw material should be hygienically stored at a temperature between min. –30°C and max. 1°C.

2.4 Initial process description

At this stage, the extraction process is still on laboratory scale (3). In scaling-up, process conditions may change the occurrence or severity of a hazard. Therefore, a redesigned process should be reviewed according to the analysis described in this article.

The process can be divided in 3 different stages resp. Preparation, Extraction and Preservation (see Annex 1). Activities are presumed to be carried out in 3 different separated production rooms to ensure control of temperature and prevention of recontaminating the product.

The process is designed to treat both cod skins and bones. Whenever the treatment for bones is different to that of skins, specific treatment for bones is described separately. See also Annex 1: Process description.

The process description starts from point of reception of the raw material entering the site for extraction. The site is considered to be a land-based factory, since application of the process on a boat is highly inappropriate, due to safety and practical limitations.

PREPARATION

Reception of raw material

At time of reception a batch of raw material will be determined for origin of capture, sensory and microbiological quality.

Thawing (if frozen) and washing

If the raw material is frozen, it will be thawed in air overnight, at 10°C, fresh material will be stored at 4°C. Defrosted and fresh material will be washed with water at 4°C.

Mincing

The fish skins will be minced with a Finis Meat Mincer at $\leq$4°C. Temperature will be controlled by by addition of flake ice.

Temporary storage

Temporary storage before further processing may take place at 0°C for a maximum of 3 days.

EXTRACTION

All extractions are carried out with a 2 liter Erlenmeyer.

Extraction with NaOH

Minced skins (or bones) will be extracted by a NaOH solution in a fermentor order to remove non-collageneous protein. The mince will be continuously mixed with 0.1 N NaOH for 1 hour at 4°C; at a weight/volume ratio of 1:7.

The solution will be centrifugated with a Sorvall Superspeed RC2-B at 10.000 rpm in 10 minutes to separate the treated skins or bones from the dissolved non-collageneous material.

Washing

Stirring for 1 hour at 4°C with 2l Erlenmeyer and 1l distilled water.

Extraction with EDTA (only for bones)
In order to remove calcium the solution will undergo an extraction with 0.5M EDTA for at 1 hour at 4°C; at a weight/solute ratio: 1:7 followed by a centrifugation with Sorvall Superspeed RC2-B at 10.000 rpm in 10 minutes (100g isolate).

Washing (only for bones)
Stirring for 1hour at 4°C with distilled water.

Extraction with butyl alcohol
In order to remove fat and aroma components the solution will be mixed with 10% butyl alcohol for 10 minutes at 4°C. Centrifugation of about 100g of isolate with Sorvall Superspeed RC2-B at 10.000 rpm in 10 minutes.
Important: this extraction can only be used when its absence in the final product can be guaranteed.

Washing
Stirring for 1 hour at 4°C with 2l Erlenmeyer and 1l distilled water.

Extraction with HCl
In order to remove the insoluble fraction:
Prior to extraction: 125g isolate + 875ml distilled water adjustment with 0.4M HCl to pH4
Max 500ml 0.4M HCl to maintain pH 4, 24h at 6-9°C in 2L fermenter, continuous chilled, with stirrer at 200 rpm. Centrifugation of about 100g of isolate with Sorvall Superspeed RC2-B at 10.000 rpm in 10 minutes.

PRESERVATION

Freeze drying
Purpose: Drying of soluble fraction.
In a Virtis Freeze mobile 25SL 100g of collagen extract is frozen to by chilled air to a temperature of -40°C. Then the temperature is gradually increased to 0°C after 5 days and a water content of <1% is achieved. The dried product is packed in airtight plastic bags.

2.5 Flow diagram

The flow diagram (see Annex 1) shows all processing steps as described in 2.4. It shows the flow of raw materials, ingredients, and equipment as input into the process as well as the separate rooms where processes take place.

2.6 Identification of potential hazards

There are several hazards that may threat the safety of a product of marine origin. These can be categorized by chemical, physical, and biological origin. Hazards may be present due to the presence of agents naturally present in the environment where the fish has been caught, due to increase in concentration or formation of hazardous components during processing, handling, transport and storage.

Chemical residues are pesticides, toxic heavy metals, and PCB's, antibiotics and growth hormones, either from the natural environment or ingested by feed as well as processing chemicals like hydrogen chloride or sodium hydroxide. Butyl alcohol may remain present due to improper processing. A side effect of hydrogen chloride is the oxidation of stainless steel production equipment. This can be overcome by application of coated stainless steel (i.e. 'inox').

Physical hazards are hazards occurring due to bad separation or insufficient mincing of the raw material (bones) or elements introducing the process like glass, wood, metal, insects, plastics, jewellery, paper/cardboard, cigarette ends, flaked paint, string, and hair.

Biological hazards are the presence or activities of pathogenic micro-organisms (indigenous as well as non-indigenous), biotoxins, pathogenic viruses, parasites, and formation of biogenic amines like histamine.

Biological Hazards

The raw material before processing is highly perishable, causing decomposition of proteins, formation of biogenic amines, production of off-odours. The extraction with NaOH will provide an accurate reduction of viable micro-organisms (see Table 1). However, toxins produced before this treatment may not be inactivated by this treatment. Therefore strict hygiene and time-temperature control should be applied to keep the concentration of micro-organisms within limits.

Table 1: Microbiological analysis of the experimental process

Processing step	**Analysis**	
	Total Count (cfu/g)[1)]	**Spore forming bacteria (cfu/g)[2)]**
Raw material	1.2*10e5	<10
After NaOH extraction	<10	<10
After Butanol extraction	<10	<10
After HCl extraction	<10	<10

1) Total mesophilic aerobic count (30°C)
2) Sulphite reducing Clostridia

Enzymatic hydrolysis

Hydrolysis of collagen by constitutional enzymes or produced by micro-organisms can reduce the yield of collagen. This is considered to be only a quality aspect and therefore will not be discussed in this hazard analysis.

Environmental contaminants ingested by feed

Dioxins, 'old' style pesticides, and PCB's are fat-soluble. They persist in tissues, which are rich in fat. Fish skin may contain fat tissue. Cod contains low levels of fat, and apart from specific organs as the liver, low levels of these compounds (5). The process is targeted to concentrate the protein fraction and not the fat fraction. The proposed butylalcohol will lead to a reduction in levels, because of the release of fatty substances. Therefore, risk of concentration of these contaminants is not expected.

Antibiotics and growth hormones will not reside in the fat but in the other matrix, causing a potential problem, because the level of acid and alkali treatment is too mild to inactivate these substances. Wild catch appears to have neglectable levels of these contaminants, so the hazard is limited to farmed fish, which is excluded from this study.

Heavy metals will be mainly present in bones. In the decalcification step they will be attached to EDTA en therefore sufficiently removed from the isolate.

Note: If it is decided to include cod liver as raw material, there will be a serious problem, because of high concentrations of dioxins and PCB's.

Residues of processing chemicals

In the process of collagen extraction, low concentrations of NaOH and HCl are applied to the extraction steps. They will be washed away in the washing steps following the extraction steps. However, butyl alcohol may not be eliminated completely, while traces are not allowed to be present in the final product. It is suggested that the extraction step with butyl alcohol should be removed from the process.

2.6.1 Formation of immuno-active products during modification of the collagen

Modification of the isolated collagen is not within the scope of this process.

2.6.2 BSE pathogens/Transmissable spongiform encephalopathies (TSE's)

There is no evidence available that the occurrence of BSE is associated with the consumption of fish. EU funded research has recently started to evaluate the possible transmission of prions (scrapie and BSE) to differerent fish species (4). New insights from these studies may result in a reassessment of this hazard.

3. Identification of Critical Control Points

From the hazard analysis and the description of the process the following Critical Control Points can be identified:

- Microbiological evaluation of raw material
- Sanitary monitoring programme
- Hygiene control
- Time-temperature control
- Process control: strict control of process parameters to prevent residues of extraction aids

Table 2: Hazard analysis worksheet for the extraction of collagen from cod skins and bones

Process step	**Potential Hazard**	**Risk / severity**				**Preventative measures**	**CCP**
		contamination					
			growth				
				severity			
					risk		
PREPARATION							
1. Reception of raw material	microbiological contamination - spoilage bacteria - pathogenic bacteria Environmental contaminants	 +++ +++ +++	 low low low	 low high high	 high high high	 Sensory evaluation and Microbiological evaluation Sanitary monitoring programme	 x
2. Thawing and washing	microbiological contamination - spoilage bacteria - pathogenic bacteria	 +++ +++	 high high	 low high	 high high	Hygiene control Water quality Time-temperature control	X x
3. Temporary storage	Microbial growth	++	low	low	high	Hygiene control Time-temperature control	x x
4. Mincing	Microbiological contamination	++	low	high	high	Hygiene control Time-temperature control	x x
EXTRACTION							
5. NaOH extraction	Residues of NaOH	+++	low	low	high	Process control	x
6. Butyl alcohol extraction	Residues of Butyl Alcohol	+++	low	low	high	Process control	x
7. HCl Extraction	Residues of HCl	+++	low	low	high	Process control	x
PRESERVATION							
8. Freeze drying	Microbial contamination and growth	++	low	low	low	Hygiene control	
9. Storage	Microbial growth	+	low	low	low		

The extraction step with butyl alcohol cannot be controlled: The component is not safe to apply in the production of foodstuffs, since the washing step cannot guarantee complete elimination of the toxic solvent. Therefore the butyl alcohol should be excluded from the process.

With the application of appropriate control instructions that can be developed based on Annex 2. This experimental process of extraction of collagen can be regarded as a basis for safe production of collagen from cod skins and bones.

Acknowledgements

The following scientists are acknowledged for their contributions:
Hans van de Vis, Stefan van Leeuwen, Pim Leonards, and Michiel Kotterman.

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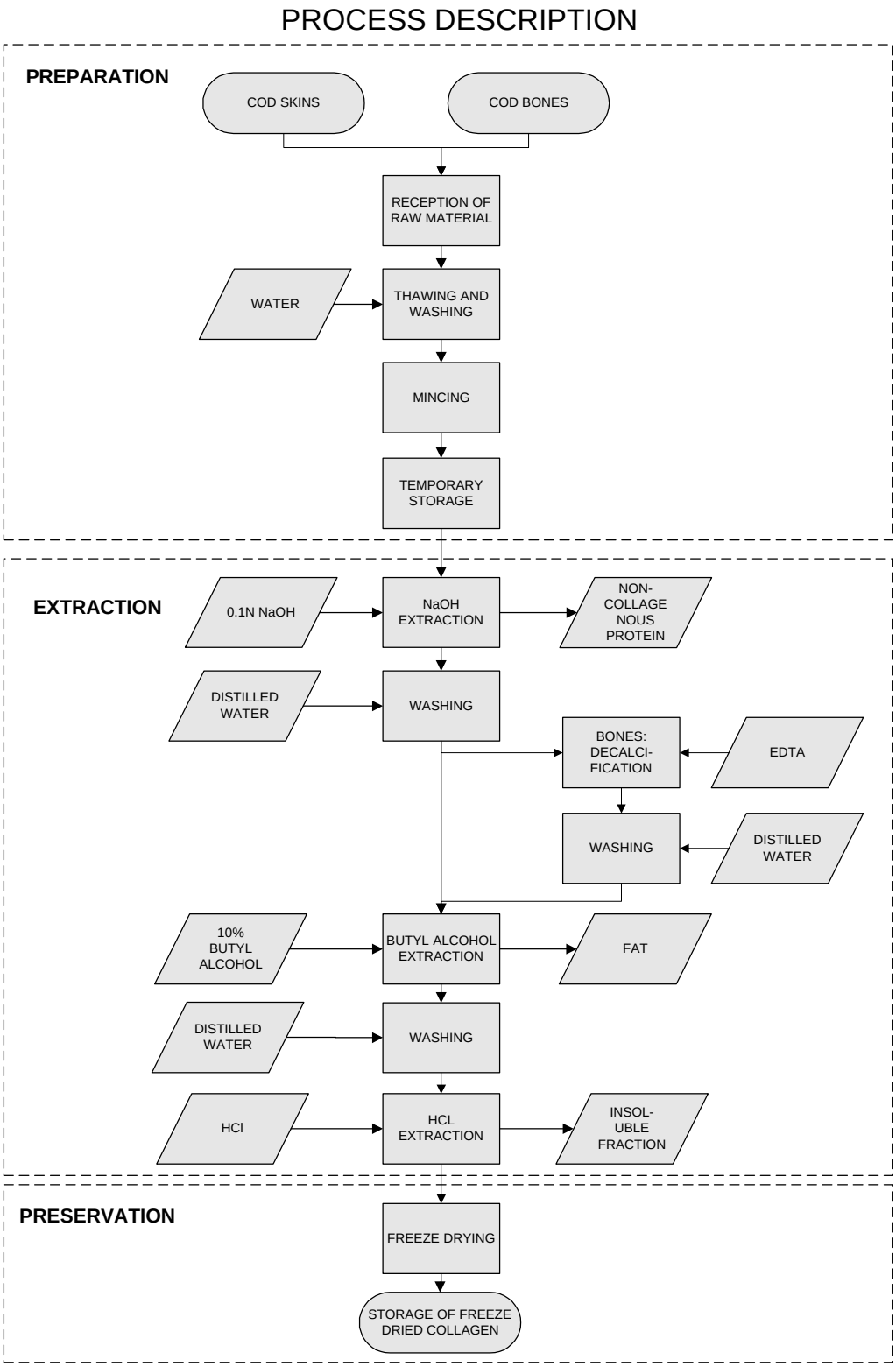
Annex 1: Process flow chart



Annex 2: Introduction to Hazard Analysis and Critical Control Points

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Introduction and regulatory needs/laws for Hazard Analysis of Critical Control Points

In the past regulatory authorities for food products had a duty to ensure that foods offered to the consumer are at least safe to eat. The authorities required a positive approach of using Good Manufacturing Practices (GMP), producing food in a hygienic manner, and by inspection of finished product. It is now realised that inspection of finished product gives a poor control over the safety of foods. Therefore, since 1 January 1993, regulatory authorities in Europe required that companies take a preventative approach to safety based on the principles of Hazard Analysis and Critical Control Points (HACCP). European Countries have food legislations which are placing full responsibility for food quality on the producer (EEC Council Directive 91/493/EEC (EEC 1991b)). These requirements might be incorporated in primary legislation on food control, or be applied by executive action of the regulatory authority. The management of the company must then be able to produce for the regulatory authority a documented HACCP plan, and be able to demonstrate that the plan is being effectively implemented.

HACCP is therefore a major change for companies as it is a food safety management system, which concentrates prevention strategies on known hazards, occurring at specific points in the food chain, rather than end product testing with the chance of rejecting complete production lots. The major difference of this quality system, compared with final product check systems, is that by using HACCP a company is able to prevent problems before they occur. It controls all production steps and prevents food safety problems, which can occur. There will, however, always be a need for some end product testing, particularly for verification purposes.

Anyone exporting fish products to Europe or North America will have to implement a programme based on HACCP. If a company cannot demonstrate to the satisfaction of regulating agencies in importing countries that it has an effective programme operating in their processing plant, importers will not be permitted to accept the products.
The United Nations food standard group Codex Alimentarius Commission has recommended HACCP's adoption as a system for ensuring the safety of foods (including finfish and shellfish) and the prevention of foodborne diseases (ref: http://www.fao.org/DOCREP/005/Y1579E/y1579e00.htm#Contents)

Scope

HACCP is a powerful system, which can be applied to a wide range of simple and complex operations. For manufacturers to implement HACCP they must investigate not only their own production methods, but must also apply HACCP to their raw material supplies and to final product storage, and must consider distribution and retail operations up to and including the point of consumption.
It can be concluded that HACCP is not a 'stand alone' process control system but may be a part of a larger system, of viz. integral quality assurance.

HACCP step by step

Commitment

First of all the management of the companies must be committed to provide all the necessary resources for the study for implementation of HACCP. This includes appointing team members, provide time for HACCP analysis, writing the HACCP plan, implementation of the system, training and instruction of personnel, reviews and updates. Without such commitment there is no point in beginning the study. Everybody in the organisation must be aware of the needs of the company to comply with HACCP regulation.

HACCP team

It is important that a multi-disciplinary team, with knowledge and expertise required for the specific product line being considered carries out the study. The use of such team is known to improve greatly the quality of data considered and, therefore, the quality of decisions reached. The team can for example consist of:

- A chairman who has knowledge of HACCP and should be responsible for managing the study.
- A quality assurance/quality control specialist: an individual who understands the microbiological and/or chemical hazards and associated with a particular product group (fish).
- A production specialist: an individual who has responsibility for, or is closely involved with the process under study. It is essential that this individual is able to contribute details of what actually happens on the production line throughout all shift patterns.
- An engineer: an individual who has a working knowledge of the hygienic design and engineering operation/performance of the process equipment under study.
- Others with special knowledge e.g. microbiology, hygiene, food technology, plant construction/maintenance, operations, market requirements etc.
- Sales representative: to consider quality expectations of the end product

Terms of reference

The HACCP study should be carried out on a specific product- or process-line, in this case the production of collagen from by-products of fresh filleting of cod. In order for the study to proceed quickly it is essential that the terms of reference be outlined clearly at the start. It is necessary to decide upon the process line, product and whether physical, chemical and microbiological hazards (or any combination of these) and whether product safety and/or microbiological quality aspects (i.e. spoilage) are to be considered with respect to food legislation. It may also be necessary to take into account demands of buyers of manufactured products. It is also to be considered when the product is judged as safe: the point of consumption or the point of manufacture with clear storage and use instructions.
It is to advise to keep it simple when you start to make it a successful operation; when a system is working, it can be further developed. At this stage the terms of reference is based upon the experimental production of collagen from skins and bones. As experience increases, and upscaling will take place, the terms of reference will be more applicable to an industrial process.

Product information

A full description of the product under study, or intermediate product if only part of the process is to be looked at, should be prepared.
Product information should contain:

- Composition
- Structure and physical characteristics

- Description of the processing (whether the product has been heated and to what extent)
- Packaging
- Storage and distribution conditions
- Required shelf-life
- Instructions for use

Identify the intended use

The intended use of the product by the consumer and the consumer target groups should be defined. This can be done in combination with the other product information you just made. Some groups of the population, elderly, very young, sick or immune compromised are much more susceptible to some hazards. For instance, it might be necessary to label the products with the text: 'not recommended to be eaten during pregnancy' when there is a risk of *Listeria monocytogenes* being present. The intended consumer group may affect your 'level of concern'. Are there specific requirements imposed by the importer or the importing country?

Process overview

Show all specific steps in the manufacturing process, from the time raw materials are received until the end product is on the market; receiving, preparation, processing, packaging, storage, distribution.

The more specific the flow-chart, the easier to understand the possible source of hazards. Take into account the delays that may occur during the process. Include sufficient technical data for the study to proceed.

Examples of information that might include:
- All raw materials and ingredients and packaging used (microbiological, chemical, physical data)
- Floor plans and equipment layout
- Sequence of all process steps (including raw material addition)
- Time/temperature history of all raw materials, intermediate and final products. Including potential for delay
- Product recycle/rework loops
- Equipment design features (including presence of void spaces)
- Efficiency of cleaning and disinfecting procedures
- Environmental hygiene
- Personnel routes
- Routes of potential cross-contamination
- High (dirty)/low (clean) risk area segregation
- Personal hygiene practices
- Storage and distribution conditions
- Consumer use instructions.

Confirm the flow chart and all recorded details during operating hours to verify that it is accurate and that all recorded details show what actually happens rather than what is wished to happen by the HACCP-team.

Hazards-analysis of each processing step

The flow chart, which was prepared, can now be used for assessment of hazard at each processing step.
Hazards have been defined as the unacceptable contamination, growth or survival of bacteria in food that may affect food safety or quality (spoilage) or the unacceptable production or

persistence in foods of substances such as toxins, enzymes or products of microbial metabolism.
The team may decide in its terms of reference to include only particular groups of hazards, e.g. infectious pathogens or toxin forming pathogens. Equally the team may decide to study all potential microbiological, chemical, physical and economical hazards.
Hazard analysis requires two essential ingredients. The first is an appreciation of the pathogenic organisms or any disease agent that could harm the consumer or cause spoilage of the product, and the second is a detailed understanding of how these hazards could arise. Thus the hazard analysis requires thorough microbiological knowledge in combination with epidemiological and technological information.
In order to be meaningful, hazard analysis must be quantitative to assess both severity and risk. Severity means the seriousness of the consequences when a hazard occurs, while risk is an estimate of the probability or likelihood of a hazard occurring. It is only the risk, which can be controlled. It is however difficult to estimate risk, as it cannot be predicted what the chances are when an employer makes a mistake during processing. Therefore, we will not estimate chances for a hazard to occur.

Hazard Analyses:
a) Identify hazards
b) Identify contamination point
c) Determine the probability
d) Asses severity
e) Determine preventative measures.

A Identify hazards:
Identification and classification of hazards should be carried out. Different classifications (e.g. Food Safety, Other legislation, Other Quality aspects, Commercial aspects) are set, and in the terms of reference decide whether these hazards are considered in this study or not.

B Identify contamination points:
Identify contamination points by a so called 'cause -> effect' analysis.
The principal causes are:
Man power (skills, training, attitudes, and knowledge)
Method (procedures, inspections),
Machines (processing, engineering)
Materials (attributes of the product and its components).

C Determine probability:
It is to advise to determine the probability by using historical data from quality controls or failures occurred in the past.
The potential for cross-contamination in food preparation is built by: food raw materials, cleaning methods, raw material preparation, equipment, environment, post cooking handling, people and personal hygiene.

D Asses severity:
Within the context of HACCP, risk can be defined as the likelihood that a hazard will occur. Within food safety it is helpful to consider food-risk-categories being high, medium or low.
Products of high risk: product not heated prior to consumption, containing fish, egg, vegetable, cereal and/or dairy ingredients which need to be refrigerated. Raw meat, fish and dairy products. Infant feed.
Products of medium risk: dried or frozen products containing fish, meat, egg, vegetable or cereal and/or dairy ingredients or any substitutes for these and other products excluded in the food hygiene regulations and heated prior to consumption.
Products of low risk: not relevant for fish products.
The rationale behind the allocation of foods to these groups is a consideration of: Is the fish likely to contain and/or support the growth of potential pathogens? Will the product undergo any

additional heat processing? Will future storage conditions provide opportunities for the growth of pathogens or further contamination? Is the population consuming the fish especially susceptible?

E Preventative measures:

Control measures are actions and activities that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level. More than one control measure may be required to control a specific hazard and more than one hazard may be controlled by a specific control measure.

Identify critical control points

A CCP is identified as a point, step or procedure at which control can be applied and a food safety hazard can be prevented, eliminated or reduced to an acceptable level. Thus for every step, location or procedure identified as a CCP, a detailed description of the preventative measures to be taken at that point must be provided. For the manufacturer those CCP's highlight where particular care has to be concentrated in the implementation of preventative measures. There are two levels of control, and therefore two kinds of CCP's: CCP1 is a Critical Control point where a food safety hazard is eliminated (for example sterilisation), where as CCP2 is a Critical Control Point where a food safety hazard is reduced to an acceptable level (for example pasteurisation).

In any operation many control points (CP) could be necessary but not critical due to low risk or low severity of the hazard involved. Some of these control points are a result of company rules for good manufacturing practice, product reputation, company policy or aesthetics. Such distinction between CP's and CCP's is one of the unique aspects of the HACCP-concept, which set priorities on risks and emphasises operations that offer the greatest potential for control. Thus the HACCP points out what is necessary while further control may be nice.

It is not always easy to determine if a certain processing step is a CCP.
Examples of CCP's are: a specified heat process, chilling, specific sanitation procedures, prevention of cross contamination, adjustment to food to a given pH or NaCl content. When considering a possible increase in levels of the hazard the team should be aware that it is possible that a single process step will not allow development of the hazard to unacceptable levels. Over a number of process steps however, the amount of increase may reach unacceptable levels due to the cumulative time and temperature of holding the product during processing. The team must therefore take account of not only the specific process step under discussion, but also the accumulated effect of subsequent process steps when answering the question.

Target levels and tolerance

Proceed the HACCP system by identifying target levels (and specified tolerance) for the control measures at each CCP. The specific target levels and tolerance set for each CCP/control measure must represent some measurable parameter related to the CCP.

Monitoring procedures

Monitoring is the series of observations or measurements to ensure that the preventative measures being implemented correctly. The CCP's are 'in control'. Monitoring should provide this information in time for corrective action to be taken to regain control of the process before there is a need to segregate or reject the product. Therefore those that can be measured relatively easy and quickly are preferred. Examples of these measurements suitable for monitoring are: temperature, time, moisture level, metal detection, pH, aw, in some cases chemical analysis, visual assessments of product and management/operational practices. Unfortunately this is not always possible. Microbiological monitoring systems have the

disadvantage of having to interpret the results in the light of the known distribution of organisms in the product and are therefore only suitable for verification of CCP's.

Corrective actions

The HACCP plan should contain written details of:

- Immediate action to be taken when there is (a trend to) loss of control
- Who is to be informed and the type of report to be produced.
- What to do with the product that has been produced.
- Investigations of how loss of control has occurred (prevention of recurrence should be an essential element of any HACCP plan).
- Who is responsible for decision making.

Verification

How to verify that the HACCP-system is working effectively:
- Methods that might be used to verify random sampling and analysing (microbiological analysis and chemical analysis (for example TVB-N) and trend analysis. Reinforced analysis or tests at selected critical control points. Intensified analysis of intermediate or final products. Take surveys on actual conditions during storage, distribution, sale and use of products.
- Verification procedures: Inspection of operations, validation of critical limits, with specialists, experts and standards setting organisations. Review of deviations from the set critical limits and of corrective actions. Audits by consulting agencies or government inspection authorities.

Documentation

In a HACCP system all activities from production to safety and quality control are described in procedures and instructions, so it will be clear what action is needed at every step of processing and when problems occur. Operating Instructions (OI) cover working activities, whereas Control Instructions (CI) explain which controls have to be carried out, how they are to be carried out and by whom, what to do when control limits are exceeded, what to record. As production data is important for control of production, so is quality and safety data important for control of safe processing. These data are recorded on Registration Forms (RF). Production and quality aspects of raw material, intermediary products, end products, and any material needed for processing (packaging, ingredients) need to be specified in Product Specifications (PS). Documents are identified by an abbreviation of the type of document (OI, CI, RF, PS) and a number, referring to a specific topic. Table 2 shows at which point, which documents are in use.

Operating instructions and Control Instructions have to be available to the persons responsible for the tasks in those instructions. They should be present and accessible at the point where the tasks take place, so they serve as quick reference. Registration forms have to be collected and managed by the Quality Manager.

Review and update the HACCP plan

When HACCP is completed, it is necessary to review the plan.
It is essential that change to any of the following should automatically act as a trigger for a HACCP review and update:
Change in raw material/product formulation
Change in processing system
Change in factory layout and environment

Modification to process equipment
Change in cleaning and disinfecting programme
Change in packaging, storage and distribution system
Change in staff levels and /or responsibilities
Anticipated change in consumer use
Receipt of information from the market place indicating a health or spoilage risk associated with the product, etc.

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EXTRACTION AND CHARACTERIZATION OF COLLAGEN FROM SKIN AND BONES OF COD (*GADUS MORHUA*)

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Abstract

Collagen is the main component in fish skin. In fish bones collagen is the major protein. The skins and bones can be collected separately from other by-products. Collagen from mammals in its purified form has found a number of pharmaceutical and cosmetical applications. The quality and specific application of the extracted collagen is highly related to the functional properties and its purity. Known problems with the extraction of collagen from fish skins are the presence of pigments and fish odours, which restrict its potential use.
Here, we will present isolation methods for collagen obtained from skin and bones of cod and the properties of the collagen preparations.
A fermentor was set up for the extraction of collagen below 10°C. For skins, extraction methods using HCl and acetic acid were compared. The extraction with HCl was more efficient and resulted in a higher yield of collagen. To remove the non-collageneous proteins, pigments and odours the skins were extracted with NaOH and butyl alcohol. The freeze-dried preparation was colourless and pigments were absent.
Cod bones were treated with NaOH to remove non-collageneous proteins. Subsequently, the bones were freeze-dried and ground to facilitate the extraction of collagen. EDTA was added to bind calcium. The concentration of EDTA and extraction time was optimized. NaOH, butyl alcohol and HCl were used similarly as for the skins.
The physico-chemical characteristics of the isolated collagen are described.

Introduction

The demand of collagen and gelatine from the industry throughout the world is considerable and still rising. By-products from fish processing are a potential source of collagen.
Collagen is the main component in the skin (Sikorski and Borderias, 1994), which can be collected separately from other by-products. The major collagen type in fish skin and bones is type I collagen (Sikorski and Borderias, 1994).
Mammalian collagen in its purified form has found a number of pharmaceutical and cosmetical applications. Similarly, gelatine, the hydrolysed form of collagen, is an ingredient extensively used in the food industry. Gelatine is used as a food additive to improve the texture, the water-holding capacity and stability of several food products. Both gelatine and collagen have been derived from fish skins and bones, but have been much less studied than mammalian gelatine and collagen. The quality and specific application of the extracted collagen and or gelatine is highly related to their functional properties and its purity. Known problems with the extraction of

collagen from fish skins are the abundance of pigments and the presence of fish odours, which would restrict its potential use. The uniqueness of fish collagen from cold water fish lies in the lower content of amino acids, proline, and hydroxyproline. Although fish gelatine does not form particularly strong gels, it is well suited for certain industrial applications, as for example micro-encapsulations and light-sensitive coatings.
Therefore, the objectives of this study were to develop efficient extraction methods to obtain collagen from cod skins and bones and to characterise the preparations.

Materials and Methods

By-products from processing of cod were purchased from a Dutch fish processor. Preparation of collagen from skin and bones. The preparative procedures were performed below 9°C.

Skins
The skins were extracted with 0.1 N NaOH at 9 °C to remove non-collagenous proteins, washed with distilled water and centrifuged.
Then, the insoluble material was extracted with 10% butyl alcohol for one hour to remove fat, washed with distilled water and centrifuged. Subsequently, the insoluble material was mixed with water (1:7 w/v) and HCL was added continously to a final pH of 4.0. The soluble fraction was stored at 4 °C for further analysis.

Bones
The bones were extracted with with 0.1 N NaOH at 9°C to remove non-collagenous proteins, washed with distilled water and centrifuged.
The bones were decalcified with 0.5 M ethylene-diaminetetraacetic acid (EDTA) (pH 7.4) for four hours and centrifuged. The pellet was washed with water and fat was extracted with 10 % butylalcohol for one hour. The insoluble material was mixed with water and HCL was added according to the procedure described for skin.

Rheological measurements
Rheological measurements were carried out using a dynamic stress rheometer SR200 (Rheometric Scientific Inc. Piscataway, USA). Before the measurements the measuring cell of the rheometer was cooled until 8°C before applying the samples. Rheology was measured in a chromium plate 40-mm parallel plate cell using a gap of 0.100 mm. A dynamic temperature ramp test was performed at a stress of 0.2 Pa and a frequency of 1 Hz. Starting at around 8°C the temperature was increased with a programmed rate of 2°C/min. The G' and G" were measured at intervals of 5 seconds. A vapour trap was used to minimise evaporation of water from the sample.

SDS-polyacrylamide gelelectrophoresis (SDS-PAGE)
The collagen samples from bones and skin were dissolved in SDS-sample buffer and electrophoresis was preformed according to protocols of the manufacturer (Biorad).

Results and Discussion

The effect of acid-extraction of collagen has been described for several fish species. Mostly, weak organic acid acids were used. If the pH of the organic acid solution is the most important factor favouring the solubilization of collagen, also other acids, like HCL, could be used. For the practical use in an applied method HCL is cheaper and easier to remove from the collagen extract. Experiments showed that the pH is an important factor in solubilization of the collagen from cod skins and bones. Here, we developed an extraction method for collagen, using a fermentor, with an automatic pH controller.
The HCL soluble fractions were analyzed on SDS-polyacrylamide gels to identify the collagen types. The patterns were similar to type I collagen from Bovine Achillus Tendus. Two bands corresponding to a 1 and a 2 components were observed. These results are consistent with the collagen type found in the bones and skin of other fish species.

The viscoelastic properties of the collagen in the acid soluble fractions were studied using a rheometer viscometer. The modulus of elasticity (G') and the modulus of viscosity (G") were determined during heating. Changes were observed at 17 and 18-19 °C for collagen from skins and bones, respectively.

References

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