Rapport nr. **204**

Utvikling av konkurranse-dyktig prosessering av marint biråstoff til smaks- og luktfrie fiskeprotein-ingredienser.

Forprosjekt

RUBIN

RAPPORTTITTEL

Marked

Utvikling av konkurransedyktig prosessering av marint biråstoff til smaks- og luktfrie fiskeprotein ingredienser. Forprosjekt.

RAPPORTNUMMER	204	**PROSJEKTNUMMER**	4654
UTGIVER	RUBIN	**DATO**	September 2011

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SAMMENDRAG OG KONKLUSJONER

På oppdrag fra RUBIN har Altavida tidligere gjennomført en kartlegging av potensialet for fiskeproteiner i det amerikanske helse- og ernæringsmarkedet. Prosjektet identifiserte et stort potensial for fiskeprotein som et alternativ til melke- og soyabaserte proteiner med betydelig bedre priser enn det som oppnås for dagens fiskeprotein ingredienser (mel og hydrolysater). Imidlertid vil det være et krav hos potensielle brukere om ingen lukt/smak av fisk eller lukt/smak som følge av oksidasjon av fiskefettsyrer i ferdige applikasjoner (i ferdigproduktets holdbarhetstid på 18-24 mnd). Ingen norske produsenter som kan levere fiskeprotein ingredienser som vil i dag tilfredsstille disse kravene.

På denne bakgrunn ble det igangsatt et innledende utviklingsprosjekt for å teste ut aktuelle prosesser for fjerning av lukt/smak fra proteinprodukter basert på marint biråstoff. Målet har vært å evaluere og komme med anbefaling til de best egnede råvarer og prosesser for industriell produksjon av smak og luktfrie fiskeprotein ingredienser for bruk i humane helse og ernæringsprodukter. Prosjektet ble ledet av Altavida, og SINTEF og Chalmers har uavhengig av hverandre foretatt en praktisk uttesting av alternative metoder/prosesser. Videre er det estimert produksjonskostnader og foretatt sammenligninger av priser for de mest sannsynlige substitutter i markedet for å avsjekke potensiell konkurransedyktighet.

SINTEF har benyttet kjøtt mekanisk separert fra lakserygger, og foretatt en enkel prosess med denaturering, vasking og etanolekstraksjon, samt en sensorisk test av produkter. Resultatene viste at utbyttet lå på ca. 10% tørt produkt av inngående våte lakserygger, og med en svak karakteristisk smak, sammenlignbart med soya –og myse isolat. Chalmers benyttet en pH-shift prosess (behandling ved høy pH) kombinert med ”pervaporation” (avdamping pluss membranfiltering) på torskerygger, der hele råstoffmengden går inn i prosessen. Utbyttet lå på samme nivå som SINTEF-prosessen, mens produktet ble bedømt å ha for sterk smak etter 2 måneders lagring til å være kommersielt interessant. Et betydelig optimaliseringsarbeid vil være påkrevet for å få akseptabel kvalitet.

En grov kalkyle av SINTEF-prosessen viser god lønnsomhet. Med en kilopris på 60 kr/kg er fortjeneste-marginen beregnet til 74%. Selv med pris ned i 30 kr/kg er det god fortjeneste.

Rapporten foreslår en videreføring av prosjektet med utgangspunkt i SINTEF-prosessen. Det pekes på 3 viktige utfordringer. 1) Optimal separasjon av kjøtt fra bein. Kan evt. gjøres etter denaturering 2) Uttesting og valg av tørkemetode 3) Reduksjon av fettinnhold i produktet.

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Rapport:

Utvikling av konkurransedyktig prosessering av norsk marint biråstoff til smaks- og luktfrie fiskeprotein ingredienser . Forprosjekt.

Altavida AS, August 2011.

Innhold

Bakgrunn

På oppdrag fra Rubin har Altavida tidligere gjennomført en kartlegging av potensialet for fiskeproteiner i det amerikanske helse- og ernæringsmarkedet.

Prosjektet identifiserte et stort potensial for fiskeprotein som et alternativ til melke- og soyabaserte proteiner med betydelig bedre priser enn det som oppnås for dagens fiskeprotein ingredienser (mel og hydrolysater).

I diskusjon og samtaler med potensielle brukere av fiskeproteiner er det definert et absolutt krav om ingen lukt/smak av fisk eller lukt/smak som følge av oksidasjon av fiskefettsyrer i ferdige applikasjoner (i ferdigproduktets holdbarhetstid på 18-24 mnd).

Det er i dag ingen norske produsenter som kan levere fiskeprotein ingredienser som vil tilfredsstille disse kravene.

For at norske produsenter skal kunne etablere en posisjon inn mot det internasjonale protein ingrediensmarkedet, er det derfor behov for å utvikle eller forbedre prosesser for produksjon av smak og luktfrie fiskeprotein ingrediens produkter.

Mål

Evaluere og komme med anbefaling til de best egnede råvarer, mulige teknologier og prosesser for kommersiell/industriell produksjon av smak og luktfrie fiskeprotein ingredienser for bruk i humane helse og ernæringsprodukter

- Gjennomgang og vurdering av potensielle råvarer fra norsk fiskeri og oppdrett
- Gjennomgang og vurdering av mulige teknologier for ekstraksjon og prosessering av fiskeprotein ingredienser
- Vurdere og evaluere teknologier for å fjerne smak eller forbedre holdbarhet
- Grovt beregne produksjonskostnader og investeringsbehov for de mest aktuelle/anbefalte prosesser/teknologier
- Forslag til videre utvikling og kommersialisering

Planlegging

- Prosjektet engasjerte henholdsvis SINTEF og Chalmers Tekniske Högskola i samarbeid med Wageningen University and Research Centre til uavhengig av hverandre foreta en teoretisk og praktisk vurdering av alternative råvarer, metoder/prosesser for produksjon av smaks- og luktfrie fiskeprotein ingredienser.
    - Råvarer (tilgjengelig fra Norsk råstoff)
    - Ekstraksjon og prosessering av råvare til ingrediens
    - Maskering, viderebearbeiding

- Gjennomgang av teknologier/Investering: Med basis i ovennevnte arbeid samt dialog med utvalgte teknologileverandører er det uarbeidet et grovt estimat av investeringskostnader for prosesstrinn og et industrielt anlegg for produksjon av smaks og luktfrie fiskeprotein ingredienser.

- Estimat produksjonskost og sammenligning med substitutt produkter: Basert på arbeid over er det foretatt grove anslag av produksjonskost (råvare, prosess, applikasjon). Tilslutt er det foretatt sammenligninger av priser for de mest sannsynlige substitutter i markedet for å avsjekke potensiell konkurransedyktighet (kasein, myse konsentrat og isolat, soya isolat).

Organisering og prosjektteam

Prosjektet ble gjennomført av Altavida AS med Bjørn Skjævestad som prosjektleder. Prosjektgruppen bestod dessutom av Robert Wahren, Core Competence AB.

Fremdrift

SINTEF og Chalmers-Wageningen har utført sine oppdrag. Rapporter, se Vedlegg 1 og 2.

Allerede tidlig i diskusjonene med forskningsinstituttene ble det klart at vi ikke kunne oppfylle forprosjektets ambisjon om en vurdering av alle tilgjengelige råvarer, ekstraksjon og prosessering, maskering og viderebearbetning. Til dette var prosjektets økonomiske ramme, 125 000 NOK per prosjekt, for lite. For å få frem optimal informasjon for en mulig videreføring ble følgende prioriteringer gjort:

SINTEF: Råvare lakserygg, går i dag for det meste til ensilasje, god tilgjengelighet, men med høy fettinnhold. Prosessen er relativt enkel: denaturering og vasking. Mulighet for lave investeringer og lønnsomhet med mindre volumer. Tørkeprosessen er ikke studert.

Chalmers – Wageningen: Råvare torskerygg, går i dag for det meste til ensilasje, relativt god tilgjengelighet, lavt fettinnhold. Prosess: pH-shift, standardutførende, og deretter ”pervaporation”, avdamping og en type membranfiltrering for å fjerne smaks-

og lukt komponenter og et eksempel på viderebearbetning for å oppnå smaksneutralitet. pH-shift og ”pervaporation” gir også funksjonelle, ikke denaturerte proteiner, som skulle kunne vurderes og selges på sine funksjonelle egenskaper. Tørkeprosessen ikke studert.

Ved valg av metoder og prosesser har enkelhet og mulighet for lønnsom drift i begrenset skala blitt prioritert, dvs mulighet for videreforedling ved hvert slakteri eller fileteringslinie. Dessuten har de kritiske trinnene blitt studert, rensingen av proteinet, samt videre fjerning av lukt- og smaksstoffer.

Prosjektene gjennomført av Chalmers og SINTEF gir ikke grunnlag for å foreta en nøyaktig produksjons- og investeringskalkyle. Kalkylene er derfor beheftet med stor usikkerhet og må først og fremst sees på som et diskusjonsunderlag for et eventuelt fortsatt arbeid.

Resultat

Uttesting prosesser

Det arbeid som er utført av SINTEF og Chalmers oppsummeres i tabellen nedenfor.

	Prosess	**Utbytte %** [1]	**Lipidinnhold %** [2]	**Produkt**
SINTEF	Vasking høy temperatur, etanol-ekstraksjon.	Ca. 10	14,5	Denaturert protein, svak karakteristisk smak, godt sammenlignbart med soya- og myse isolat (etter 4 mnd. lagring).
Chalmers/ Wageningen	Vasking ved høyt pH, lav temperatur, kombinert med ”pervaporation” (avdamping og membranfiltrering)	Ca. 10	11,7	Ikke denatuert, funksjonelt protein, betydelig smak av ”sjømat”(etter 2 mnd. lagring).

1) Utbytte beregnet som torr produkt for salg i forhållande til råvaran, våt lakserygg eller torskerygg.
Utbytteprosenten i SINTEF-prosessen er basert på at kjøtt separert fra lakseryggene utgjør 46% av ryggene
Utbytteprosenten i Chalmers-prosessen er i hht. muntlig meddelelse

2) Lipidinnhold beräknad på torr produkt for salg.

Proteiner er store molekyler med en volumstruktur, og marine proteiner er ikke noe unntak. Ved oppvarmning til 60 – 70 grader mister eller forandrer mange proteiner sin volumstruktur. Denna prosessen kalles denaturering. Et denaturert protein mister sine biologiske og funksjonelle egenskaper, dvs enzymer inaktiveres, geldannende og vannholdende evne forsvinner. Fra et kommersielt perspektiv er denaturerte proteiner interessante som en kilde til aminosyrer og peptider, nødvendige byggeblokker for mennesker og dyr.

Ikke denaturerte proteiner er kommersielt interessante også for sine funksjoneller egenskaper som geldanning, vannholding, ”texturizers” (viskositetsmodifisiering), skumstabilisering etc.

Råvarer

Mulige råvarer for fremstilling av smaksnøytrale marine proteinerlister listes i tabellen nedenfor.

Materiale	Egenskaper	Användning idag	Kommentarer
Lakserygg	Kjött og bein, relativt rent, høyt fettinnhold. Tilgang ca. 25 000 ton per år i Norge.	I stort sett til ensilasje. En del fryst til Øst-Europa for videre bearbetning.	Eksport Øst-Europa: Salgspris ca 1 USD per kg, men betydelig kost for frysing, emballasje. Ensilasjepris ca NOK 1-1,30/kg
Torskerygg	Kjött og bein, relativt rent, lavt fettinnhold. Tilgang i Norge 15 000 – 20 000 ton per år.	Lavpris til pelsdyrfor og ensilasje.	Kommer fra saltfisk, klippfisk og mindre del fra filetering. NOK 1-1,30 per kg.
Lakseslo	Blandigsprodukt, bitre komponenter fra galle kan være vanskelig å vaske ut? Emulsioner og ikke enzymatisk frisetning er riskfaktorer. 125 000 tonn per år.	Ensilasje	Lite aktuelt å bruke til humant i første omgang, pga. lukt/smak
Trimmings laks	Sammenlignbart med lakserygg, kanskje litt mer fett. Mulig startmateriale. 10 000 tonn åer år	I størrelsesorden halvparten til konsum, for eksempel i Japan, resten ensilasje.	NOK 15 per kg til konsum
Laksehoder	Mye bein og brusk, lite protein 25 000 tonn per år	Ensilasje, noe til konsum - eksport.	NOK 1-1,30 per kg NOK 5 per kg til konsum

Hvitfisk hoder	Mye bein og brusk, lite protein 100 000 tonn per år	En god del tørkes og eksporteres. Resten lavpris til pelsdyrfor og ensilasje	Lave priser på ubehandlede hoder Rundt NOK 1 per kg?
Pelagisk restråstoff	Som lakseslo Ca. 350 000 tonn per år	Ensilasje og mel/olje	NOK 1,30 per kg

Kalkyler

Forutsetninger for en investeringskalkyle:

- Råvare lakserygger, kapasitet 4 000 ton per år (et filetanlegg, ikke kontinuerlig drift).
- Driftstid 2 000 timer per år.
- Kapasitet 2 ton per time.

Prosess: Denaturering, beinseparasjon (ikke optimert), vaskning, avvanning, ekstraksjon, tørking (ikke undersøkt), pakking.

Infrastruktur: Fabrikkbygning, vann, damp og el, næringsmiddelshygiene på plass.

Prosesstrinn	**Utstyr**	**Investeringskostnad kNOK**
Denaturering	Oppvarmbar tank, 3-4 kubikkmeter, rørverk	200
Beinseparasjon	Ikke optimert, kan være ganske enkel? Sil? Tank for biprodukter	250
Vasking	Vasketank med silbunn, doseringsutstyr	200
Ekstraksjon med etanol	Tank, static mixer, pump, ex-klasset utstyr. Etanol-gjenvinningssystem	800
Tørking	Antatt spraytørke, ikke testet.Ex-klasset utstyr	700
Pakking	Standardutstyr 25 kg sekk.	200
Prosjektering, montasje, rørdragning, el, prosesstyring	20 % av ”hardware” kost	470
Sum investeringskost, estimat med betydelig usikkerhet		2 820

Investeringskostnader for pH-shift prosessen vill være i samme størrelseorden som for SINTEFprosessen. Her inngår flere og større tanker med pH regulering for de ulike vasketrinnene. Beinseparasjon og ekstraksjon med etanol utgår. Eventuell membranfiltrering kommer i tillegg, ”pervaporation” eller annen industriell membranfiltrering, og øker investeringen.

Materialbalanse: Et lakseslakteri slakter ca 60 000 tonn laks per år. Ca 50 % fileteres og ca 10 % av laksen blir lakserygger. Dette gir ca 3 000 ton lakserygger per år. Utbyttet av vått laksekjött fra lakserygger varierer fra 36 til 56 %, se referanse i SINTEFrapporten. Vi antar 46 % og får 1 380 ton vått laksekjött. Utbytte av tørt marint proteinpulver fra vått laksekjött er 20 %, hvilket gir 276 ton produkt for salg. Dessuten fås 3 000 – 1 380 = 1 620 eller ca 1 500 ton restprodukt til ensilasje.

Preliminär produktkalkyl, kNOK, 3 000 ton lakserygg.

Inntekter		
Salg proteinpulver	16 560	276 ton, 50 - 90 NOK per kg ? Her antatt 60 NOK per kg.
Salg restprodukter	1 500	1 500 ton ensilasje, NOK 1,00 per kg.
Sum inntekter	18 060	
Kostnader		
Råvare, lakserygg	3 900	Alternativ ensilasje, NOK 1,30, 3 000 tonn. Et noe lavere pris kan være diskuteres. Evt kan ensilasjekostnaden dras fra.
Drift: Kjemikalier, arbeide, el, rep. og vedlikehold	660	Etanol 20 NOK, el-energi 50NOK, prosessoperatør og pakking av produkt: ett manneår, 500 NOK Vedlikehold 3 % av investering, 90NOK
Salgskostnader	0	Selges av aktuell salgsorganisasjon?
Utvikling	100	Dokumentasjon av produktegenskaper
Administrasjon	50	Avtal, oppföljning, fakturering, delas med resten av bedriften
Avskrivninger	300	10 år avskrivningstid, investering 3 000 kNOK
Sum kostnader	5 010	
Resultat före finans	13 050	
Räntor	90	3 % av investering, 3 000 kNOK
Resultat etter finans	12 960	Vinstmargin 72 %

Kommentarer til SINTEF- og Chalmersprosjektene

En fordel med SINTEFs prosess er at den er relativt enkel, investeringene er ikke store og den benytter ”hyllevarer”. Dessutom virker det som prosessen kan gjøres lønnsom på de volumer som kommer frem i et lakseslakteri med fileteringslinje. En del av utstyret kan også allerede være tilstede i fabrikken.

Produktene som fremstilles har en blek lysegul farve, svag god lukt og smaker etter 4 månaders lagring i rumstemperatur bra, en svag karakteristisk smak, ikke harskt. Lagringstesten fortsätter.

Avgjørende for utbyttet i begge prosessene er mengden kjøtt på lakse- eller torskeryggene, og hvor godt dette kjøtt kan separeres fra beinen.

pH-shift metoden er også relativt enkel, og krever ikke komplisert utstyr. Den er patentert og patenteierne er interessert i å selge lisenser. Utbyttet beregnet på tørt produkt fra torskerygg ble i Chalmersprosjektet av samme størrelseordnen som i SINTEFprosjektet. Den store variasjonen er i begge forsøkene mengden protein i råvaren, lakse- eller torskeryggen.

”Pervaporation” er en ny teknologi for å separere flyktige komponenter fra diverse materialer. ”Pervaporation” kombinerer evaporation (avdunstning) med membran filtrering. Materialet som skal behandles sirkuleres over en spesiell membran. Små molekyler, molekylvekt under 200, deriblant vann, kan passere gjennom membranen. På membranens andre side, vakuumsiden, gjenfinnes de små molekylene i gassfase og kondenseres i en kylfelle. I vårt tilfelle önsker vi å fjerne små molekyler som gir opphov til lukt og smak, og ta vare på et fint og rent protein på membranets ”ovansida”.

Torskeprotein som i Chalmersprosjektet ble renset med pH-shiftmetoden, men ikke behandlet med ”pervaporation”, får en svagt orange farve, lukter tydelig ”sjømat” og smaker tydeligt av fisk (sample 6). Etter behandling med ”pervaporation” forsvinner en del av farven og mye av lukten, men en del fisksmak gjenstår (Samples 1,2). Disse resultatene er etter 2 måneders lagring.

Prøvene fra Chalmersprosjektet bedømmes ha altfor sterk smak for å være kommersielt interessante. Det må til mye optimalieringsarbeid for å få dem i samme klasse som SINTEFprøvene. Til tross for at sammenligningen kan virke lite hensiktsmessig ettersom prosjektene hatt ulike råvaror at starte med, anbefales det allikevel å basere eventuelt fortsatt arbeid på de forsøk som er utført på SINTEF.

Marked:

Det viktigste målet med prosjektet har vært å vurdere og teste ut prosesser for produksjon av marine proteiner med nøytral eller ingen smak og lukt. Gitt at man klarer dette vil det kunne åpne seg et betydelig marked for fiskeprotein ingredienser inn mot det humane helse og ernæringsmarkedet. Altavida har tidligere på oppdrag

fra RUBIN gjennomgått mulighetene for marine proteiner i dette markedet (se RUBIN-rapport 186).

Priser som er mulig å oppnå i disse markedene avhenger blant annet av pris på konkurrende fiskeprotein kilder, pris på substitutter samt dokumentasjon og kvalitet.

Andre fiskeprotein produkter:

BlueWave Marine Ingredients er et selskap basert i Peru som hevder å selge fiskeprotein isolater til USD 5/kg som substitutt til egg og myseprotein. Prøver er bestilt men ikke mottatt, men man bør ved en evnt videreføring av prosjektet sammenligne kvalitet med andre alternative fiskeprotein kilder.

Substitutter:

Prisdannelsen på fiskeprotein ingredienser vil først og fremst avhenge av prisene på myse, kasein og eggproteiner. Prisene varierer i forhold til renhet og innhold av proteiner.

Prisene som oppnås for Protein konsentrater fra myse er ca USD 5-9/kg og for Protein isolat (mer enn 90% protein) er fra USD 10-15.

Dokumentasjon av helseeffekt:

Dokumentasjon av biotilgjengelighet og positive helseeffekter vs andre protein kilder vil ha stor betydning for etterspørsel og prisdannelse. Hvis man skal unngå å måtte prise fiskeprotein ingredienser lavere enn eksisterende produkter, bør man gjennomføre min 2 ”double blind” studier som dokumenterer ”helseeffekt.”

Fortsatt arbeid

De utförda projekten og kalkylene indikerer at konseptet å fremstille høyverdige marine proteiner fra norske ferske restprodukter er verdt et fortsatt studium. En enkel holdbarhetstest på produktene fra de to forsøken pågår, og vil bli rapportert under hösten. Velger Stiftelsen RUBIN å fortsette prosjektet i en fase to bør følgende arbeidsområder prioriteres.

- SINTEFprosessen: Separasjon av kjött og bein må studeres og optimeres. Her finnes en stor mulighet i at det förste trinnet, varmedenatureringen, kan kjöres direkte på lakseryggene. Deretter blir separasjonen av kjött og restmateriale mye enklere og billigere. En standard kjött-beinseparator trenger ikke brukes.

- Tørking av marine proteiner: Uansett primærprosess må vi ha en tørkemetode som ikke forstyrrer de gode resultater som er oppnådd i de förste trinnen. Å finne en god törkemetod er et viktigt trinn i et fortsatt arbeide. Spraytörking er komplisert da produktene innholder fast materiale. Tørking med varmluft kan lede til lipidoksidasjon. I laboratorieforsöken i begge prosjektene som nå er kjört har frysetörking blitt brukt. Denne metoden er skonsam, men dyr og er vanskelig å skalere opp til industriell skala.

- Fettinnhold: Sluttproduktene fra begge prosjektene innholder høye andeler lipider, 12 – 15 %, hovedsakelig fosfolipider. Dette er antageligvis for høyt for å gi et godt ”shelf life” til produktene.

- Fortsatt arbeide kan med fordel drives i samarbeide med et lakseslakteri med fileteringslinie.

Vedlegg 1: Rapport fra SINTEF

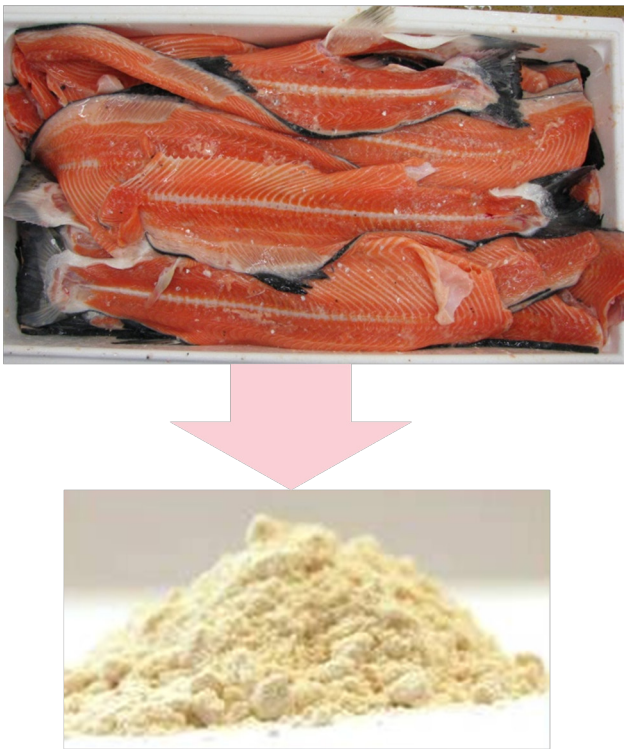
SINTEF

F19825 - Fortrolig

Rapport

Produksjon av smaksnøytralt fiskeprotein fra biråstoff av laks

Forfatter(e)
Rasa Slizyte'
Ivar Storrø



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2011-06-27

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Rapport

Produksjon av smaksnøytralt fiskeprotein fra biråstoff av laks

Undertittel

EMNEORD:
smaksnøytralt
fiskeprotein,
biråstoff,
laks

VERSJON
Versjonsnummer

DATO
2011-06-27

FORFATTER(E)
Rasa Slizyte'
Ivar Storrø

OPPDRAGSGIVER(E)
ALTAVIDA: Bjørn Skjevestad

OPPDRAGSGIVERS REF.

PROSJEKTNR
850369

ANTALL SIDER OG VEDLEGG:
16

SAMMENDRAG

Overskrift sammendrag

The main goal of this project was to evaluate the possibility to remove taste components from salmon meat obtained from backbones. It was found that initial heat denaturing of proteins following by several steps of washing by water gives higher yield of the final product compared to cold washing of native proteins. Extraction by ethanol removed significant part of lipids and pigments soluble in lipids (astaxanthin). Final yield of dried salmon powder based on of dry weight salmon meat was 70% and on wet weight basis 21%. Based on the yields and composition data of the final salmon powder we can conclude that by the performed washing by water and following by ethanol extraction 11% of the proteins, 67% of the lipids and 94% of the ash were removed. Thus this technology makes is possible to wash out small molecular weights components influencing the taste of the material (proteins), significant part of lipids (67%) and almost all ash. Sensory tests indicated that the produced salmon powders were in the same line as all the three tested and commercially available powders. By other words the final products was a powder of concentrated proteins, whitish color and acceptable taste. Economical evaluation of the raw material cost and yield indicate that 1 kg of dried salmon protein powder would cost 8 USD. In addition expenses for washing (water) and extraction (ethanol) should be taken in to account. However it should me mentioned that this process is not optimized, neither with respect on volumes nor process technology. Furthermore washing with ethanol will extract phospholipids and asthaxanthin which could be a valuable by-product in the process.

UTARBEIDET AV
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APPENDIXES

1 Design of the project

The project goal was by a washing procedure remove taste components from salmon meat obtaned from salmon backbones.

This project was divided into three parts:

1. Preliminary test: evaluate different technologies to obtain taste neutral proteins from salmon rest raw material.
2. Main test: by the chosen technology (from the first part) produce a test amount of taste neutral proteins.
3. Sensory evaluation of the final product: to evaluate produced fish proteins in comparison with reference proteins on taste basis.

2 Raw material

Based on 2005 data Norway produced approx. 11 000 tonnes of salmon backbones per year (Østvik et al., 2006). However "Value adding analysis" for year 2010 (RUBIN, 2011) shows that during last 5 years the salmon production increased two times compared to year 2006. This let us make the assumption that today it could be produced approx. 22 000 tonnes of salmon backbones per year in Norway. Amount and quality of scraped muscle from salmon bones depends on filleting technology, scraping equipment and can vary from 36 up till 56% (based on backbones mass and the efficiency of the filleting machinery).(Østvik et al., 2006, Østvik and Grimsmo, 2010).

Backbones from a filleting line (SalMar ASA, Kverva – Norway) were used to obtain raw material for the test. Remaining muscle from the backbones were removed by spoon scraping and homogenised at food processor (Siemens Marche with 21 cm bowl equipped with cutting blade 17 cm in diameter, at lowest possible speed: 1) to get a more homogenous mixture (Picture 1).

Picture 1. Raw material for the test: backbone after filleting (picture at left), scraped muscles (picture at right).

3 Chemical analyses

The moisture in the samples (raw material and sediments) was determined gravimetrically after drying at 104 °C for 12 h. Ash content was estimated by charring in a crucible at 550°C until the ash had a white appearance (AOAC, 1990). Total nitrogen (N) was determined by CHNSO elemental combustion analyzer (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies, Inc) and crude protein was estimated by multiplying total N by 6.25. These measurements were performed in triplicate. The extraction of lipids from raw material and sediment was performed according to the method of Bligh and Dyer (Bligh and Dyer, 1959). The total fat content was determined and expressed as gram lipid pr gram sample material (wet weight). The measurements were performed in duplicates.

4 Preliminary test

The aim on the first part of the project was to evaluate how much of the taste (fish taste) it was possible to remove by a simple washing of salmon backbone meat by hot (›90°C) and cold (approx 10°C) water followed by drying and a ethanol extraction to remove phospholipids and colour components (Figure 1). At the same time it was important to estimate a possible yield from the final proteinaceous fractions as the yield is a key factor for successful industrial implementation of the process.

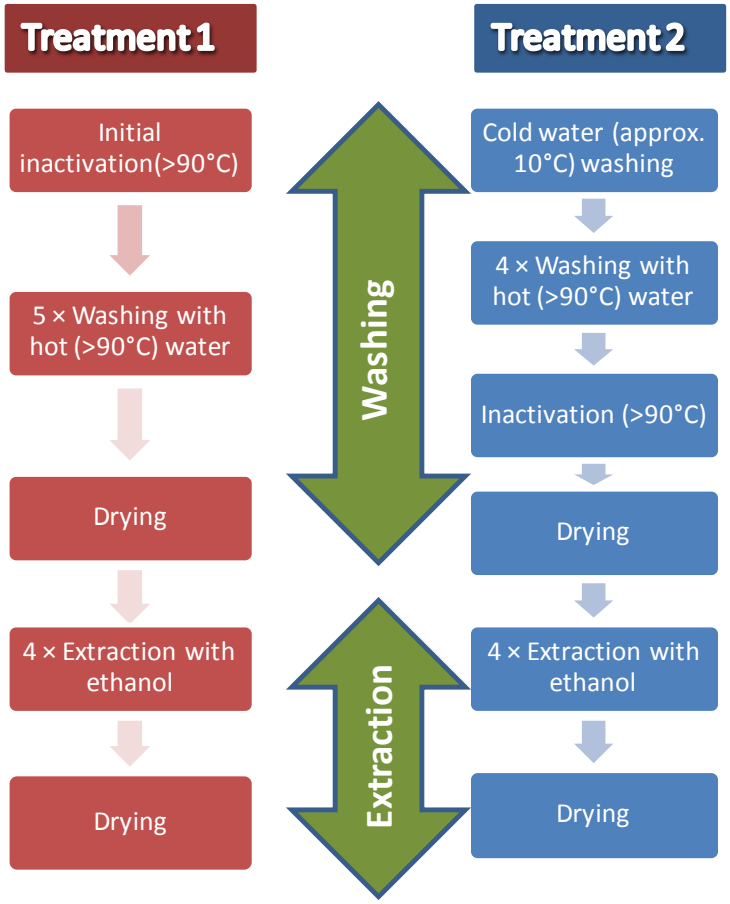


Figure 1. Experimental design.

In treatment 1 the salmon meat was heated to 90 °C during the first washing step to inactivate structure proteins and endogenous enzymes. As expected, initial heating gave higher dry material yield after centrifugation compared to the treatment 2 where the salmon meat was washed with cold water (approx. 10 °C). The higher yield is due to the denaturation of soluble proteins which became insoluble and ended in the sediments part. The same tendency was observed during all washing steps. In Treamtment 2 the subsequent steps consisted of washing with hot water (›90 °C). To ensure inactivation of endogenous enzymes in the final product of Treatment 2, an extra step of heating the whole mass to 90 °C was performed. At the last washing step all the soluble proteins were already washed away. Amount of oil separated during the washing steps (calculated amount) were very similar, that indicates that final yield was influenced by amount of washed proteins.

Yield of dried final powders (%) based on of dry material in raw material gave 61% and 43% for treatments 1 (washing by hot water) and 2 (washing by cold water) respectively (Table 1). Final yield of dried final powders (%) based on wet raw material gave 18% and 13% for treatments 1 and 2 respectively. As can be seen from Table 2, the ethanol extraction removes almost the same amount of lipids and lipids soluble components from both sediments indicating that the first washing part (washing by water with different temperatures) is a critical step for final yield. The initial treatment of the rest raw material with high temperature should be advisable based on the yield on the final products.

Table 1. Yield of the products (after washing with water (part 1) and after extraction with ethanol (part 2)) based on the dry material.

	Heat denatured protein Treatment 1 (>90°C)	Native protein Treatment 2 (10°C)
After washing with water	67%	48%
After extraction with ethanol	61%	43%

Table 2. Amount of lipids (and lipid soluble components) extracted by ethanol during the 4 steps extracting procedure (% of dried sediments after washing).

	Treatment 1 (%)	Treatment 2 (%)
Raw material: dried sediments from part 1		
1 EtOH extraction	3,5	3,7
2 EtOH extraction	2,4	2,6
3 EtOH extraction	1,3	1,3
4 EtOH extraction	2,1	1,8
Extracted oil,% of dried sediments from part 1	9,36	9,43

Based on the higher yield in treatment 1 (with initial heat denaturing of proteins) this procedure were chosen for the production of the test amount of the final product which were the second part of the project.

5 Main test

The aim of this part was, by using the chosen technology (from the first part), produce a test (20-50g) amount of taste neutral proteins.

Raw material. Backbones from filleting line (SalMar ASA, Kverva – Norway) were used to obtain raw material for the test. Remaining muscle from the backbones were removed by spoon scraping and homogenized at food processor (Siemens Marche with 21 cm bowl equipped with cutting blade 17 cm in diameter, at lowest possible speed: 1 (Picture 1). Raw material contained 29.5±0.3% dry material, of which 66.2±4.8% were proteins, 30.9±1.1% lipids and 3.4±0.1% ash. Mass balance of the analyzed composition: 100,5 %. For the first part of the washing 750 g of homogenised rest raw material were used (150g per each paralel: Table 3).

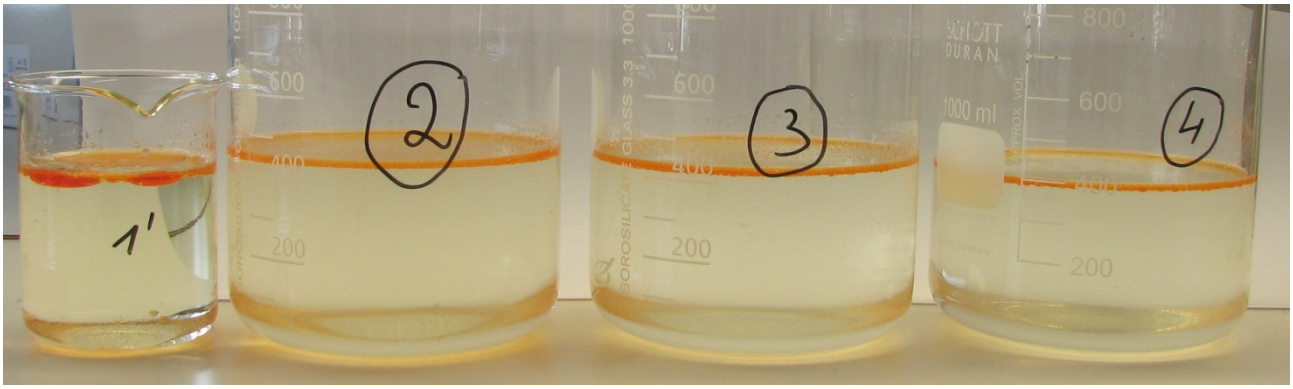
Table 3. Design of the experiments to study the washing of salmon meat with hot water.

	Homogenised raw material 150 g	
	Amount of added water	Temperature of water
1st washing	90 ml	90°C
Microwave till ›90 °C	+	
Centrifugation	10 min at 8066*g	
2nd washing	90 ml	90°C
Centrifugation	10 min at 8066*g	
3rd washing	90 ml	90°C
Centrifugation	10 min at 8066*g	
4th washing	90 ml	90°C
Centrifugation	10 min at 8066*g	
Washed wet sediments	103 g	

Homogenized rest raw material was heated during the first washing step to inactivate endogenous enzymes. The first washing step removed a significant part water soluble material together with a part of oil (Table 4). The following washing steps also removed some soluble material and oil from the raw material (Picture 2), but the amount were significant lower (Table 4). The amount of oil separated during the washing steps was about 12 % (of dry material). In general, during the 5 washing steps 23.5% (100-76.5%) of dry material were washed away, which yielded 76.5% of the dry material in the sediments fraction (Table 4). For washing of 100g wet rest raw material 240 ml hot (›90°C) water was used in this experiment. The sediment fraction had less intense red colour compared to the raw material before washing. The dried sediment powders (Picture 3) were used for the second part of the test.

Table 4. Dry material balance based on washing of 100 g wet weight minced salmon muscle.

	g	%
Raw material	29,5	100
1 washing	2.2	7.4
2 washing	0.7	2.4
3 washing	0.3	1.1
4 washing	0.2	0.5
Sediments	22.6	76.5
SUM (washed material+sediments)	26.0	87.9
Oil (?) calculated	3.6	12.1
Washed material	6.9	23.5
Sediments yield, %		76.5



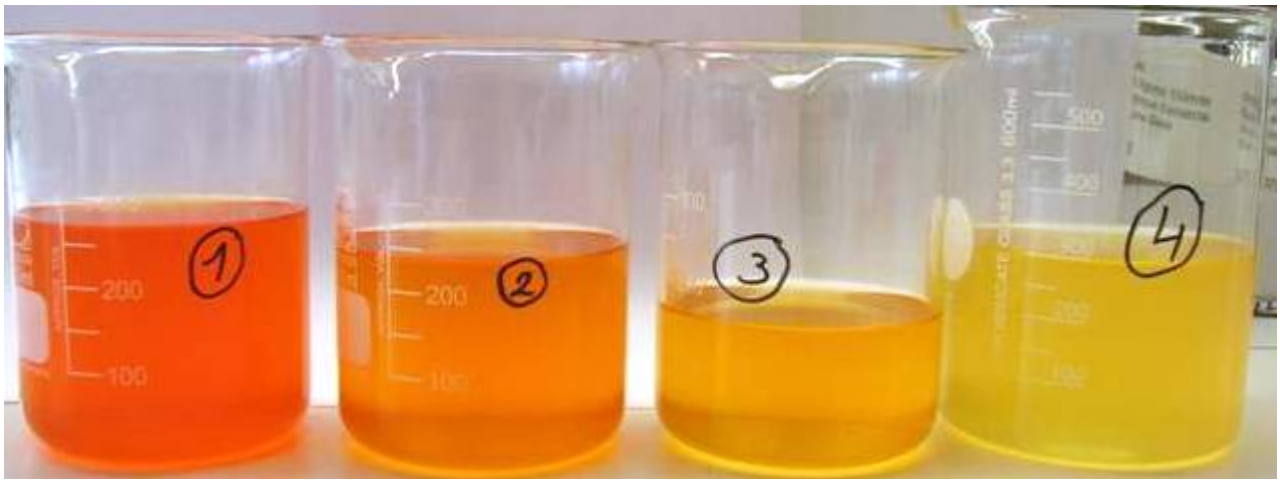
Picture 2. Liquids obtained after each washing and centrifugation steps.

Picture 3. Freeze-dried sediments after 4th washing with water.

During the second part of the test, the dried sediments form the first part was washed with ethanol. Design of the second part of the test is presented in Table 5. Each extracting step yield ethanol with intense colour (Picture 4) indicated the removal of lipids and pigments soluble in the lipid phase (astaxanthin). Totally by 4 ethanol extraction steps it is was washed off about 9,2% (based on dried sediments mass) of lipids and lipid soluble components (Table 6). For extracting of 100g dry sediments from first part of the procedure 1400 ml ethanol was used in this experiment.

Table 5. Design of the second part of the test (extraction with *ethanol*).

	Extracted and dried sediments 20 g
1st extraction (added ethanol)	100 ml
Centrifugation	10 min at 8066*g
2nd extraction (added ethanol)	60 ml
Centrifugation	10 min at 8066*g
3rd extraction (added ethanol)	40 ml
Centrifugation	10 min at 8066*g
4th extraction (added ethanol)	80 ml
Centrifugation	10 min at 8066*g
Final products (wet)	**Final powder (wet)**



Picture 4. Ethanol fractions after each of 4 steps of ethanol extraction of the dried washed sediments.

Ethanol extraction removed almost all color components and yielded the white final material, which after final freeze-drying have a grayish-white powder. Final freeze-dried powder (Picture 5) had a slightly yellow and grayish-white color. The final powder was made of 85.1±1.4% proteins, 14.5±0.3%lipids and 0.3±0.1% ash.

Table 6. Amount of lipids (and lipid soluble components) extracted by ethanol during the 4 steps extraction procedure (%, based on dried sediments from part 1).

	%
Raw material: dried sediments from part 1	
1 EtOH extraction	4.4
2 EtOH extraction	2.5
3 EtOH extraction	0.7
4 EtOH extraction	2.1
Extracetdoil,% of dried sediments from part 1	9.2

Picture 5. Final dried powders obtained after water washing and ethanol extraction.

The overall mass balance for dry material and proteins during the two steps washing technology is presented in Table 7.

Table 7. Dry material and protein balance over whole technological process based on 100 g wet weight minced salmon muscle.

	Dry material, g	Proteins, g
Raw material	29,5	19,5
Washed sediments	22,6	
Extracted powder	20,5	17,4

From the amount of soluble compound washed out during the successive washing steps it might be possible to reduce the washing to two steps, and thereby increasing the yield. When it comes to extraction with ethanol it is more difficult to say from these experiments what might be an optimal extraction procedure. Both washing with water and extraction with ethanol has to be optimized with regards to cost and quality of the finished product.

One interesting product from this process could be astaxanthin from the ethanol extraction. Astaxanthin can most probably be separated from the ethanol by solvent resistant membranes in a cross flow filtration process Or the ethanol can be recovered by distillation, leaving a astaxanthin rich residue. Astaxanthin production can improve the economy of the production of salmon powder.

We expect the ethanol solution to contain salmon phospholipids which would be valuable components and would increase the value of an ethanol residue after ethanol evaporation.

6 Sensory evaluation of the final product

The aim of this part of the projects was to evaluate the produced salmon powder in comparison with reference protein powders on taste basis. For this purpose "Triangle test" and "Ranking test" were performed. The followed reference proteins were used:

Soya protein isolate – SPI, (WDF-930M, from Norfoods Sweden AB),
Milk protein isolate – MPI, (from Milk Specialties Global, USA),
Whey protein isolate – WPI, (from Milk Specialties Global, USA).

All three reference samples were kindly provided by Core Competence, Sweden.
The structure and the design of the sensory tests are presented in the Appendix.

6.1 Triangle test

Samples were evaluated by two groups of panelist. The first group (10 sensory evaluators) compared salmon powders against milk protein isolate (MPI), while the second group, (9 sensory evaluators) compared salmon powders against soya protein isolat (SPI). All tested powders were dissolved in water (Imsdal, pure Norwegian water without carbondioxide, bought in supermarket) to have a 1% final concentration. Solutions were filtered trough glass wool in order to ensure that small particle will not disturb the sensory evaluation. Both evaluating groups could distinguish salmon powder from MPI and salmon powder from SPI solutions. As triangle test is design to indicate a detectable difference between two samples we can conclude that salmon powder was found different from both MPI and SPI.

In the second part of the triangle test (the second group of evaluators) sensory evaluators were asked to evaluate the intensity of the taste of salmon powder solution and two identical solutions of SPI (1% final concentration) in the intensity scale from 1 (least intense) to 5 (most intense). Results from this part of evaluation indicate that even sensory evaluators that could distinguish the different samples in the first part, but there were not significant intensity difference among three samples (Figure 2).

From these results we can conclude that there where not the intensity of the taste that was the reason for the discrimination between soy protein isolate in the first triangle test.

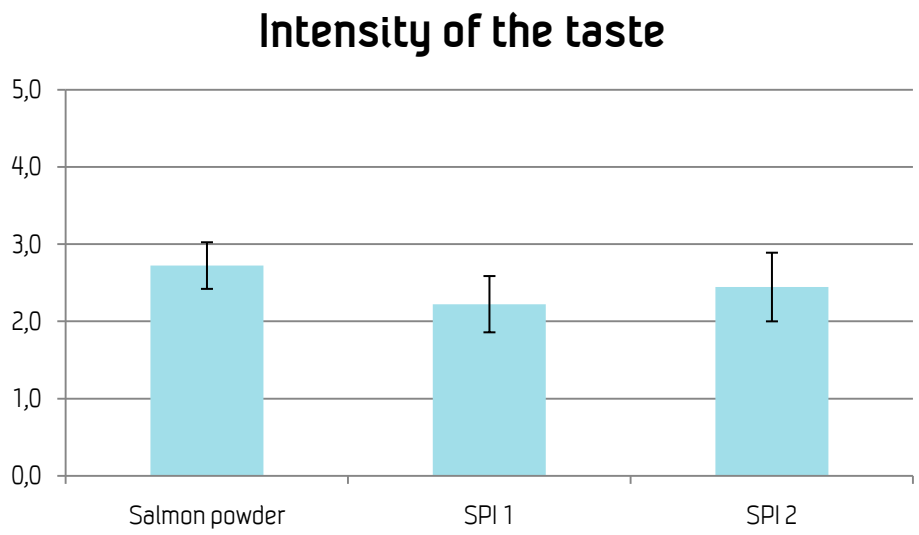


Figure 2. Intensity of the taste of three 1% solutions (salmon powder, two identical soya protein isolate (SPI1and SPI2) evaluated in the scale from 1 to 5 (values presented as average ± standard error of mean).

6.2 Ranking test

For the "Ranking test" the sensory evaluators were asked to arrange the samples according to their intensity of taste. For this part four coded solutions (1% concentration) were presented for the sensory evaluators :

- Soya protein isolate – SPI,
- Milk protein isolate – MPI,
- Whey protein isolate – WPI
- Salmon protein powder.

For this test the taste intensity of the samples were evaluated by using a scale from 0 (least intense) to 3 (most intense). Similarly to the intensity evaluation in the Triangle test sensory panel did not indicate significant differences between the salmon powder and the soya protein isolate (Figure 2). However these two samples (salmon powder and SPI) had significantly higher taste intensity compared to MPI and WPI. These results lead us to the conclusion that our produced salmon powder has the same taste intensity as SPI and are higher compared to milk and whey protein isolates. The discrimination between milk protein isolate and salmon protein isolate in the first triangle test can in fact be due to taste intensity, but this is only a possibility and is not confirmed.

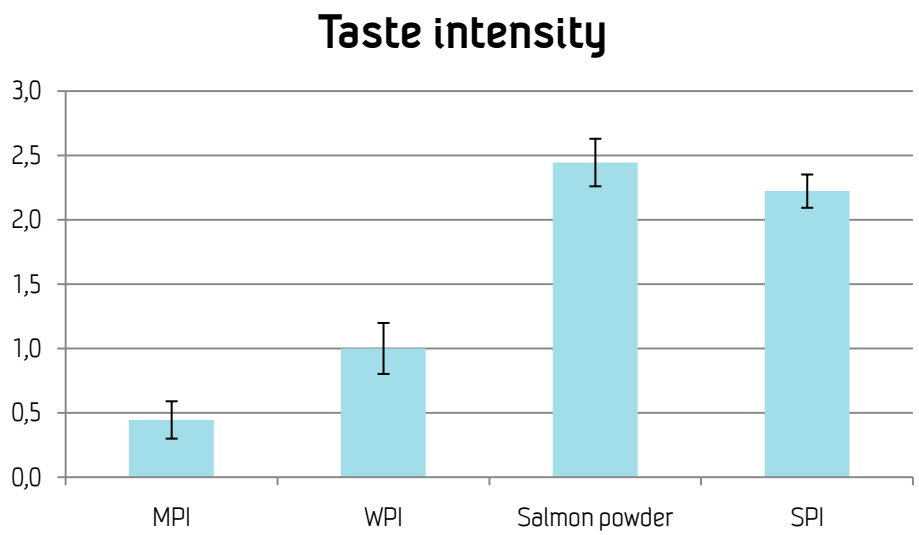


Figure 3. Taste intensity of four 1% solutions (Milk protein isolate – MPI, Whey protein isolate – WPI, salmon powder and Soya protein isolate – SPI) , soya protein isolate (SPI) evaluated in the scale from 1 to 5 (values presented as average ± SDOM).

Both Triangle and Ranking test include the part where panelists were asked to describe the taste of the coded samples. The Table 8 presents the collected description of all powders used in the tests.

Table 8. Description of the coded samples described by the sensory evaluators.

Sample	Description of the taste
Salmon powder	Light fish taste, rancid, dry, weak rancid, fresh, citrus, fish taste, fish taste, a little bit bitter, salty, fish taste, a little acid, fish taste, rancid, fish, salty, taste like fish, salty acid, rancid, fish, taste a little bit fish, bitter, fish taste, bitter, sweet, fish taste.
Soya protein isolate (SPI)	Sweet, vegetable, bitter, bad, milk taste, sweet, meat taste, salty, acid, milk taste, very weak fish taste, most bitter taste, salty, almond, not good, rancid, bitter, sweet, bitter, neutral, sweet
Milk protein isolate (MPI)	Metallic, salty, rice taste, fish, fermentation taste, aftertaste, milk, sweet, little spicy, milk
Whey protein isolate (WPI)	Rancid, acidic, sweet, sweet, sweet, fish, fermentation taste, sweet, little taste, lakris, fermentation, powder taste, neutral taste, sweet, neutral, bitter, caramel, slightly sweet

Description used by the sensory evaluators shows that all tested powder was described by using both positive and negative words. This let us make the conclusion that the produced salmon powders were in the same line as all the three tested and commercially available powders.

7 Yields and economy of the process

The yield on the final products (based on both washing and extraction parts by water and ethanol) was calculated as the yield of the final products is an important key for successful implementation of technology.

Final yield of dried salmon powder based on of dry weight salmon meat was 70% and on wet weight basis - 21%. Based on the yields and composition data of the final salmon powder we can conclude that by the performed washing and extraction 11% of the proteins, 67% of the lipids and 94% of the ash were removed. By this technology it is possible to wash out small molecular weights components influencing the taste of the material (proteins), significant part of lipids (67%) and almost all ash. By other words the final products was a powder of concentrated proteins whitish in color and with acceptable taste.

By the use of available info one kg of salmon backbones costs 0.8 USD (personal communications with SalMar ASA, Kverva – Norway, price not negotiated) and yields approx 0.5 kg salmon meat (1.6 USD per 1 kg salmon meat from backbones). One kg salmon meat from backbones would yield 200 g dried washed and extracted salmon powder (0.2 kg) giving 8 USD per 1 kg of dried salmon protein powder. In addition expenses for washing and extraction of rest raw materials should be taken in to account. In order to produce 1 kg salmon protein powder, in this first not optimized process, it was used approx. 12l water and 16l ethanol. The amount of water and ethanol used can most probably be reduced as indicated earlier in this report. The ethanol can also be recovered. However it should me mentioned that this process is not optimised, neither with respect on volumes or process technology. It should also be mentioned that the freeze-drying was used for the test. This is a common drying technique used in the lab practise, but is quite expensive (and energy demanding) and other drying methods should be considered for industrial implementation. Furthermore washing with ethanol will extract phospholipids and asthaxanthin which could be a valuable by-product in the process.

8 Recommendations and future steps

Evaluation of the produced salmon powder indicated that the main goal of the project was successfully achieved. The final salmon powders gave 89% protein yield and the taste was in the same line as all the three tested and commercially available powders.

This was a preliminary test in order to evaluate how by several simple washing steps it is possible to remove undesirable taste components and at the same time to keep the yield of the final product at a reasonable level. However for the successful industrial implementation of the tested technology the following issues should be considered:

- Influence of rest raw material (back-bones) composition and quality on end product yield and properties should be studied;
- Amount of water and ethanol like the number of washing steps used should be optimized with the respect to process costs;
- Different technology for extraction, separation and dewatering should be evaluated in order to find the most cost- and quality effective;
- Amount, composition and quality of other valuable components like phospholipids and asthaxanthin should be evaluated in order to find if or how much these components could increase the profitability of the process;
- Stabilizing of the produced salmon protein should be investigated;
- To check the quality and taste changes during the prolonged storage of the final products (18-24 months);
- Content of (the remaining) n-3 fatty acids in the product, and if these acids could be advantageous, should be examined.

9 References

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APPENDEXES

Templates used for sensory evolution of final powders.

Navn _____ Dato _____

TRIANGLE TEST

You are presented with three coded samples of proteins. Two of these samples are identical while the third is odd or different. Taste each sample. *Indicate the code of odd sample by placing an **X** mark across the code.*

Sample code	Odd/different sample
Comments	

Indicate the intensity of the each sample:

weak		medium		Very strong
1	2	3	4	5

Describe the difference:

Spicy

Salty

Sweet

Acid

Bitter

Rancid

Other

SINTEF

RANKING TEST

Taste neutral proteins

Arrange the samples in order according their intensity of taste. In the case of observed taste describe the taste

Sample	Taste intensity	Describe the taste /comments
	0	
	1	
	2	
	3	

Vedlegg 2: Rapport fra Chalmers-Wageningen

pH-shift processing as a way to add value to back bones from white fish

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Background

Fish frames can carry up to 20% edible flesh, with ~20% proteins. Using mechanic techniques like pressing, roughly 3-4% flesh can be squeezed out. Minces resulting from pressing are often heavily coloured due to blood and other pigments. It may also contain other unwanted substances like skin, small bones and cartilage, since it is necessary to use a high belt pressure to maximize yields.

In this pre-project, one of the aims was to apply an alternative strategy, the pH-shift process, to add value to cod frames through the production of a functional cod muscle protein isolate. The acid and alkaline versions of the pH-shift process was originally invented and patented at the UMass marine station by Herbert Hultin, Stephen Kelleher and co-workers (1999; 2000; 2001; 2002; 2004; 2007). A clear advantage with this process is that the meat must not be pre-separated mechanically. Instead, the proteins are dissolved away from the bones by acidifying (pH 3) or alkalizing (pH 11) a homogenate of ground raw materials in water. Oil, potentially oil-soluble contaminants and insoluble materials like skin, bones, and, under favourable circumstances, even cellular membranes can then be separated from the solubilized proteins by centrifugation/filtration. The soluble proteins, forming a supernatant between the floating oil layer and the sediment, are precipitated at pH 5.5 and then recovered by a second centrifugation/filtration. The process in conducted under cold conditions so that the proteins maintain their capacity to form a gel. This is thus a significant difference from protein hydrolysates where the proteins are cleaved down to small peptides. The protein isolate can be dried and used e.g. for a high quality surimi, a gel-enhancing ingredient in seafood products, a coating for fried products, or in seafood marinades. It should be stressed though that drying process must be selected with great care to not induce protein denaturation or cause oxidation of trace lipids.

In a BSc project at Chalmers University of Technology (Ekholm et al., 2008), a protein yield of 50% was found during pH-shift processing of whole frames from white fish (back bones and head). The ash content here decreased by 97% illustrating a highly efficient removal of bones, cartilage etc. Using whole gutted herring and whole herring, 60-70% protein yields

have been reached (Marmon & Undeland, 2010; Marmon *et al*., 2009). Here 88% ash was removed. Although the fish research group at Chalmers have focused a lot on optimizing e.g. removal of ash, lipids, lipid soluble contaminants (dioxins and polychlorinated biphenyls) and DSP toxins during pH-shift processing of seafood, only few evaluations have been done on the sensory properties of pH-shift-produced protein isolates. The work that has been done in this area has been focused on herring, and primarily on development lipid oxidation-derived odours during the actual pH-shift processing and during ice storage of the final protein isolates (e.g. Undeland et al., 2005). In the study by Undeland et al. (2005), it was found that the initial odour notes that could be found in different kinds of freshly made herring protein isolates were "ocean", "mineral" "fishy", and "painty". Isolates made with antioxidants had more mineral odour and less painty odours compared to reference samples.

In Norway, it has been identified that there is a large market for dried fish proteins as an alternative ingredient to soy and milk proteins. However, with currently evaluated techniques, the price of fish proteins is higher, and it has been recognized that the odours/taste of fish proteins are more intense than for soy/milk-based isolates. That proteins are virtually free of odour and taste are indeed a crucial factor when introducing them into non-seafood products like sport drinks.

To optimize the removal of volatiles affecting odour and taste of cod protein isolates, e.g. aldehydes, ketones and amines such as trimethyl amine (TMA) and dimethylamine (DMA), this project has combined two innovative techniques; pH-shift processing and PerVaporation. PerVaporation is combining evaporation with membrane permeation. The product to be treated is circulated over a dense membrane. Due to interactions with the specific top-layer of the membrane, smaller molecules like volatiles (Mw $\leq \pm 200$) and water are able to pass through the membrane. On the other (vacuum) side of the membrane, these smaller molecules - in a gaseous phase - are condensated by means of a cold trap.

Research studies have been carried out to study pervaporation as a process for recovery and enrichment of aroma compounds from a dilute aqueous solution, such as from fruit juices, and other beverages (Olsson & Trädgårdh, 1999; Rajagopalan & Cheryan,1995; Sampranpiboon et al., 2000). In the study of Martínez et al., (2011), the pervaporation technique was also investigated to separate dilute solutions of volatile compounds from a brown crab effluent in order to obtain a valuable food flavoring fraction. PerVaporation has also been mentioned as a part of analytical methods, e.g. for analysis of Trimethylamine (TMA) in fish (Garcia-Garrido, 1997). However, to our knowledge, no studies have earlier been conducted where pervaporation has been applied in order to create a low-odour fish product.

Aim

To produce a protein isolate low in odour and taste from cod back bones using pH-shift processing in connection with PerVaporation.

Materials and methods

pH-shift processing

Cod caught April 8th 2011 was filleted on April 11th by Leröy Allt i Fisk AB, Göteborg. The backbones were minced within 4h storage on ice and then vacuum packed and frozen at -40°C. Prior to processing, the frozen cod back bone mince was thawed under cold running water. The pH-shift process was then applied in a similar manner according what has been previously described by Marmon et al. (2009). Two hundred grams of cod back bone mince was homogenized with 9 volumes of water after which the pH of the slurry was adjusted to either pH 11.3 or 2.3 wing NaOH and HCl, respectively. The homogenate was then centrifuged at 10 000g for 30 min. The supernatant was adjusted to pH 5.5 and precipitated proteins were recovered by a second centrifugation at the same conditions as above. Samples for protein analyses were taken at different steps of the process for calculations of protein yield. The isolates were then frozen at -80°C and characterized for proteins, lipids and moisture content.

PerVaporation

PerVaporation was applied at different stages of the pH-shift process; during solubilization and precipitation. The set-up of the PerVaporation is showed in **Figures 1 & 2**. The selected membrane for these tests was a SolSep PV 030705F PDMS (polydimethylsiloxane) flat sheet membrane of 95 cm^2. A radial feed flow was set at 180 liter/hour and circulated over the membrane out of a jacketed feed vessel. Vacuum was set to 5-10 mbar. Temperature of the fish homogenate circulating over the membrane module was ± 7°C.

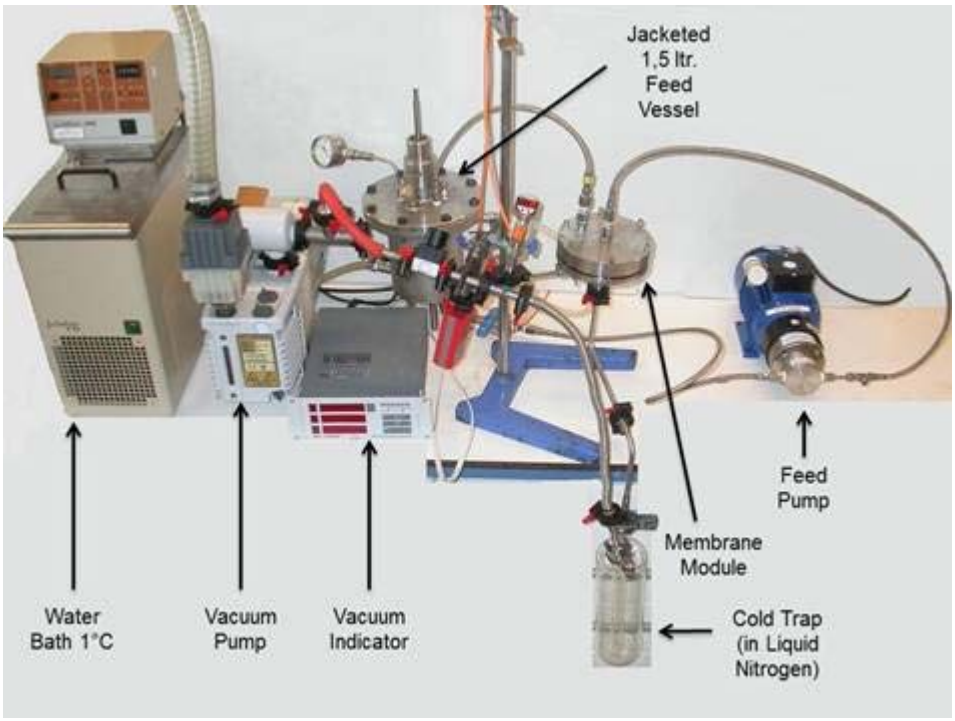

Figure 1: Set up of the PerVaporation process. The Fish extract was pumped over the membrane back to the feed vessel. At the permeate side of the membrane the volatiles were condensated in the cold trap in liquid nitrogen under vacuum.

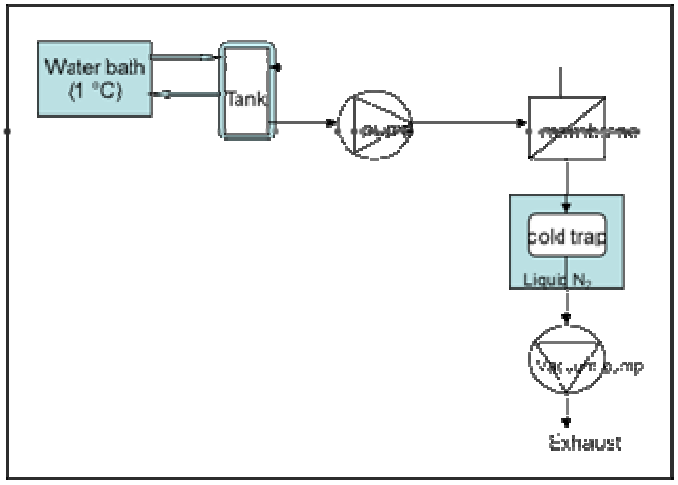

Figure 2: Scheme of the Pervaporation Process

PerVaporation was tested for one or two hours using cod back bone homogenates at the stages when they were adjusted to pH 11.3 and 5.5. It was also tested to combine one hour at pH 11.3 and one hour at pH 5.5. This sample was processed further according to the normal procedure. The finally obtained precipitated protein extract was collected, frozen at -80°C and freeze dried.

Freeze drying

Freeze drying was performed in an Edwards Lyofast S 08 Freeze Drier at a vacuum of 5-10 mbar. Process started at -20°C and ended at 25°C. After freeze drying samples were individually sealed in Aluminum coated plastic bags.

Protein analysis

Protein content of samples taken out during the pH-shift process was determined by the method of Lowry (1951) as modified by Markwell (1978).

The protein solubility in the solubilization step (step 1) and precipitation step (step 2) was calculated as follows:

$$\textit{Protein solubility} = \frac{\textit{Protein concentration of supernatant x 100 \%}}{\textit{Protein concentration of homogenate to be centrifuged}}$$

The protein yield in step 1 and 2 were calculated as:

$$\textit{Protein yield step 1} = \frac{\textit{(Protein concentration in supernatant 1 x Weight of supernatant 1) x 100 \%}}{\textit{(Protein concentration in homogenate x Weight of homogenate)}}$$

$$\textit{Protein yield step 2} = 1-\frac{\textit{(Protein concentration in supernatant 2 x Weight of supernatant 2) x 100 \%}}{\textit{(Protein concentration in Supernatant 1 x Weight of supernatant 1)}}$$

Total yield (%) was calculated as: *Protein yield step 1 x Protein yield step 2*

Moisture determination

Moisture was determined by weighing raw material and protein isolates before and after freeze drying (see above).

Lipid determination

Total lipids were extracted from freeze dried isolates and raw material with chloroform plus methanol and determined gravimetrically using the method of Lee et al. (1996).

Results

Protein solubility and protein yield

Protein solubility and protein yields (step 1, step 2 and total) during the acid and alkaline versions of the pH-shift process are shown in **Table 1.**

Version	Protein solubility step 1	Protein yield step 1	Protein solubility step 2	Protein yield step 2	Total yield
Acid	59%	28%	8.9%	91.8%	25.5%
Alkaline	84%	64%	7.8%	92.7%	59.3%

As can be seen, the yield with the acid process became very low (25.5%), and it was also difficult to reduce the pH due to the presence of bones. Therefore, the focus in further trials of this project was only on the alkaline version of the pH-shift process in which the yield was close to 60%.

Basic composition

In **Table 2** it can be seen that both the lipid content and protein content of the alkali made isolate was higher compared to the freeze dried cod back bone mince. This is since ash is efficiently removed in the pH-shift process (Marmon & Undeland, 2010; Ekholm et al., 2008) making the proteins and lipids more concentrated. The protein analyses have been repeated several times, and at this moment, it cannot e explained why the total sum of proteins and lipids for the isolates goes over 100% on a dry weight basis.

Sample	Moisture (%)	Lipid (% dw basis*)	Protein (% dw basis*)
Cod back bone mince	79,6±0,8	8,1±1,0	61,7 ±2,6%**
Alkali-made isolate	90,0 ±1,5	11,7±1,5	103 ±1,05 %**

*dw=dry weight

**We get too high protein values with these powders, and cannot explain it.

Removal of volatiles using pervaporation

To check the effect of PerVaporation on volatile removal, the freeze dried samples were compared with each other as well as with the non-pervaporated protein isolate and the freeze dried fish. In a small sensorial evaluation of odour, the samples were set in an order from best (almost no smell): position 1, to the product with the most smell (the freeze dried fish, position 7).

1. Pervaporation for 1 hour @ pH 5,5 (2 g): almost no smell
2. Pervaporation for 1 hour @ pH 11,3 (2 g): almost no smell, slightly more than 1
3. Pervaporation for 2 hours @ pH 5,5 (1 g): almost no smell, slightly more than 1 and 2, little acidic
4. Pervaporation for 2 hours @ pH 11,3 (3 g): almost no smell, little fresh fish smelling, slightly more than 1, 2 and 3
5. Pervaporation for 1 hour @ pH 11,3 followed by Pervaporation for 1 hour @ pH 5,5 (9 g in total packed in two different bags): almost no smell, little fresh fish smell, slightly more than 1, 2, 3 and 4
6. pH-shift produced protein isolate (alkaline version) without Pervaporation (6 g): some fresh fish smell, fairly large difference from 1-5.
7. Freeze dried cod back bone mince (16 g): smelled very fishy, large difference from 1-6

Based on these findings, we consider the pH-shift technology a promising technique to isolate muscle components from complex bone structures. The proteins get highly concentrated, and at the same time the odour is significantly reduced. Based on the brief sensory testing, we also consider the pervaporation trials quite successful, but cannot ascertain that new odour-molecules are not forming during storage of the dried protein isolates. There are still trace lipids present that can oxidize, and it is possible that also the proteins can be subjected to oxidative and other reactions.

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