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Farmed salmon rest raw materials as a source of peptones for industrial fermentation media

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ABSTRACT

Twelve marine peptones prepared from rest raw materials (RRM) from farmed salmon have been evaluated as nutrient sources in growth media for industrial microorganisms. The peptones were prepared from head and backbones, or from head, backbones and viscera, using different proteases and one or two-step hydrolysis. Growth was determined as optical density using a high-throughput robotic system, allowing for testing of a large number of peptones and microorganisms. For two *Lactobacillus*-strains tested, the peptones were the only nitrogen source, while for four aerobic bacteria and yeasts, the peptones were assessed as a source of growth factors, with inorganic nitrogen in excess in the media.

The peptones containing viscera resulted in higher cell yields than those without, and high growth rates were maintained to higher cell densities. The viscera-containing peptones were better than meat-based peptones, and equally good as yeast extract. The differences between the performance of peptones with and without viscera could be explained by the mineral content and the degree of hydrolysis. Since peptones based on farmed salmon RRM can be provided in large quantities with a stable quality, they should be further explored as a nutrient source for the fermentation industry.

1. Introduction

Hydrolysed proteins and nutrient-rich extracts are common ingredients in growth media for microorganisms. While some microbes can utilize inorganic nitrogen such as ammonium, others require amino acids, vitamins, and other organic growth factors. These growth factors can be supplied by peptones, which are the water-soluble fraction of partly hydrolysed proteins of animal or vegetable origin. The most commonly used peptones are of animal origin, like meat peptones produced from animal rest raw materials and casein. Search for alternatives to the meat-based peptone sources was triggered by the BSE occurrences in the 1990'ies. For food applications, eg production of lactic acid bacteria starter cultures, also kosher requirements exclude use of meat-based products. More recently, the trends towards reduced meat consumption may represent an additional demand for alternatives to meat-based products. Also important is that independent of the source, cheap, but high-quality peptones are demanded, since organic nitrogen is often the most expensive component of microbial growth media.

The use of fish-based peptones is surprisingly scarce, although that these have been reported to have good properties [1–4], and that the fish processing industry generates large amounts of rest raw materials (RRM). One reason may be the availability and logistics related to raw material supply. Wild-caught fish is often brought to small processing facilities in the coastal areas, and in many cases gutted and the viscera discarded, on the sea. Also fileting may be carried out at sea in 'factory trawlers'. The rest raw materials available on shore thus represent small quantities, geographically scattered. Farmed fish represents another situation, where for salmon, the slaughtering and gutting occurs in large, centralised facilities on shore. This not only simplifies the logistics, but also ensures a fresh raw material of high, and food-grade quality. Norway produces 1.3 mill. tonnes salmon annually, generating more than 400 000 tonnes rest raw materials [5]. Traditionally, RRM from fish has been used for products of low economic value, such as silage or meal for animal feed. Better utilization of the rest raw materials by conversion into products of higher value, such as peptones, therefore has a large economic potential.

Fish peptones have been most extensively studied as nutrients for lactic acid bacteria [4,6–10] but has also been used for marine bacteria

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[2,4,11] and several others. Screens of a spectre of strains have been used as a tool to evaluate the peptones [1,3]. The raw materials have included different RRM-fractions from a wide range of fish species, such as cod viscera [1,2,6,8,9], tuna heads [3,10], whole mackerel and herring [7] and filet trimmings from silver carp [12]. For hydrolysis, either endogenous or commercial proteases, such as Alcalase®, papain, Neutrase® and Protamex® have been applied. The only report on peptones based on salmon RRM as microbial growth substrates is a recent study comparing peptones from various farmed fish species [4]. However, salmon RRM hydrolysates have been studied for other applications, such as animal feed, and hydrolysis conditions, yields and product compositions have been reported for frames without head, using Alcalase, Neutrase and pepsin [13], and Protamex [14,15], and for viscera using Flavourzyme® [16]. Hydrolysis of salmon viscera and mixed RRM by commercial proteases, with and without heat-treatment of the viscera, showed that the endogenous enzymes had a significant contribution to the degree of hydrolysis obtained [17].

The different RRM-fractions, such as heads/frames and viscera will have different amino acid content and different mineral and vitamin composition. Use of enzymes with different specificities may generate hydrolysates with different degree of hydrolysis and amino acid composition. No systematic comparisons of these factors have been reported. In the present study, peptones generated from different fractions of salmon rest raw materials have been compared as nutrient source in growth media for industrial microorganisms. The peptones were produced from head and backbones only, and from head, backbones and viscera, applying proteases with different specificities. Use of microplate fermentations and high-throughput screening facilities for growth measurements enabled screening of a high number of peptones and microorganisms, in total twelve peptones and six different microorganisms.

2. Materials and methods

2.1. Raw materials and enzymes

Raw materials for the peptones were RRM fractions of fresh Atlantic salmon. The peptones were prepared either from fresh raw materials, or from concentrated commercial hydrolysates (Salmigo®) provided by Biomega Norway AS. Two peptones were prepared from decanter sludge, i.e. the non-soluble fraction after protein hydrolysis in Biomegas industrial process. The enzymes used for hydrolysis were Protamex® (Novozymes, Bagsværd, Denmark), Alcalase® (Novozymes Bagsværd, Denmark), BZ-16 (Barentzymes, Tromsø, Norway), B-Pro-04 (Barentzymes, Tromsø, Norway), Acid Protease A (Amano Enzymes Ltd, Chipping Norton, Oxfordshire, UK), Tail 1 Acid Protease (Tailorzyme, Herlev, Denmark). Raw materials, enzymes and hydrolysis conditions used for preparation of the twelve peptones employed as nutrient sources in growth media are summarized in Table 1.

2.2. Preparation of peptones

2.2.1. Hydrolysis of RRM from fresh salmon and decanter sludge

The raw materials were minced (8 mm), mixed with equal amounts of deionized water and transferred to glass reaction vessels with thermostatic jacket and electrical stirrer. After temperature- and pH adjustment, selected enzymes were added at 0.1 % (v/v) concentration and the hydrolysis allowed to proceed for one hour, except for HBS-AA, 48 h and VS-BT, 2 h (see peptone abbreviations in Table 1). Enzymes were inactivated by heating to above 85 °C in a microwave oven. Press-liquid was separated from press-cake/sludge by straining through nylon screen fabric with mesh-opening 0.4 mm (Monodur PA 400, Clear Edge Filtration, Geldern-Walbeck, Germany).

2.2.2. Preparation of peptones from hydrolysates

The press-liquid from the hydrolysis process, or two-fold diluted commercial salmon hydrolysates, were centrifuged at 4600 G for 20 min. The aqueous phase was isolated by decanting/siphoning and retained, while the bottom solid sediment and top oil and emulsion layers were discarded. The separation process was repeated after a second centrifugation at 16 500 G for 20 min. The resulting slightly turbid aqueous phases, except for peptone HB-P, were micro-filtered in a tangential flow filtration system (Sartorius Vivaflow 200, with 0.2 µm PES membrane) and concentrated by vacuum evaporation in a Buchi Rotavapor R-220 SE (5 L capacity). HB-P were micro-filtered in a pilot scale filtration system (Atech α-Al203/TiO_2, 0.05 µm, 19/3.3 membrane), concentrated in a pilot scale 4-stage falling film evaporator (AVP Anhydro) and finally spray dried (Niro P-6.3).

2.3. Analytical methods

Ash was determined by incineration (ISO 5984) and nitrogen by the

Table 1
Raw materials and enzymes used for preparation of the peptones.

Raw material	Peptone code	Primary hydrolysis				Secondary hydrolysis			
		Alcalase T: 55 °C t: 1h pH na	Protamex T: 55 °C t: 1h pH na	BZ-16 T: 55 °C t: 1h pH na	Tail 1 T: 45 °C t: 1h pH 4.5	B-Pro 04 T: 55 °C t: 1h pH na	Tail 1 T: 45 °C t: 1h pH 4.5	Tail 1 T: 45 °C t: 2h pH 4.5	Acid-P-A T: 55 °C t: 48h pH 2.5
HB	HB-P		X[1]						
	HB-B1			X[1]					
	HB-B2			X[1]					
	HB-BB			X[1]		X[1]			
	HB-T				X[2]				
	HB-BT			X [2]			X[2]		
HBV	HBV-P		X[1]						
	HBV-B			X [2]					
	HBV-T				X[2]				
	HBV-BT			X [2]			X[2]		
H/B Sludge[3]	HBS-AA	X[1]							X[2]
V Sludge[3]	VS-BT			X[1]				X[2]	

1): Industry scale hydrolysis.
2): Laboratory scale hydrolysis.
3): Hydrolysate after primary hydrolysis was separated into a liquid and a solid sludge fraction. The peptone was prepared from the water-soluble fraction after the secondary hydrolysis of the sludge.
HB: Head and backbones; V: Viscera; HBV: Head, backbones and viscera. T: Temperature; t: time. na: not adjusted. HB-P was dried, the other peptones were used as liquid concentrates.

Kjeldahl method (ISO 5983). Minerals (Fe, K, Ca, Cu, Co, Mg, Mn, Mo, Na, Ni, Se, Zn, S, V) were determined by atomic absorption spectrophotometry according to NMKL 161 [18].

Total amino acids were determined by reverse phase HPLC after acid hydrolysis of proteins and derivatization of amino acids by 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) [19,20]. Free amino acids were determined by reverse phase HPLC after derivatization of amino acids by phenylisothiocyanat (PITC) [21].

The degree of protein hydrolysis was measured by the method of Nielsen et al. [22], where free amino-groups formed by peptide-bond cleavage react with *o*-phthaldialdehyd in the presence of thiothreitol, forming a coloured compound that is measured spectrophotometric at 340 nm. Peptide size distribution in the 200 to 20 000 Da range was determined by Size Exclusion Chromatography, using HPLC with a Superdex Peptide 10/30 column (GE Healthcare), eluent 0.1 % TFA in 30 % acetonitrile, and UV-detection [23].

2.4. Microorganisms and growth media

The microorganisms used were *Escherichia coli* DH5α, *Corynebacterium glutamicum* ATCC 13032, *Saccharomyces cerevisiae* ATCC 96581, *Pichia jadinii* CBS 4511, *Lactobacillus sakei* Lb790, and *Lactobacillus rhamnosus* GG, NCIMB 6375. The microorganisms were grouped into non-lactic acid bacteria 'non-LAB' (the first four) and lactic acid bacteria 'LAB' (the last two). For the non-LAB, the basis medium contained (g/L): glucose·H_2O, 10.0; $(NH_4)_2SO_4$, 0.7; KH_2PO_4, 0.15; $MgSO_4$·7 H_2O, 0.02; $FeSO_4$·7 H_2O, 0.01 and 2-(N-Morpholino)ethanesulfonic acid hemisodium salt (MES), 20.0. For the LAB, the basis medium contained (g/L): glucose·H_2O, 10.0; KH_2PO_4, 2.0; Na-acetate·3 H_2O, 5.0; $MgSO_4$·7 H_2O, 0.2; $MnSO_4$·4 H_2O, 0.05; Tween 80, 1.0 and MES, 20. The peptones were compared based on nitrogen content, i.e. the same N concentration was used for all peptones. For the non-LAB, the peptones provided 0.05 and 0.1 g/L nitrogen, while inorganic nitrogen ($(NH_4)_2SO_4$, 0.15 g/L N) was kept in excess, with total N concentrations 0.20 and 0.25 g/L, respectively. For LAB, all N was provided from the peptones, and the final N-concentrations in the media were 0.2 and 0.5 g/L.

Yeast extract (Oxoid LP21) was used as reference for non-LAB, and a reduced MRS-medium for LAB. The reduced MRS-medium was prepared from the components of the Oxoid MRS medium (Oxoid CM0359), but with the concentrations of the complex nutrients (yeast extract, meat peptone and meat extract) reduced to give the same N-concentration as for the fish peptones (0.2 and 0.5 g/l). pH was adjusted to 6.8 (non-LAB) and 6.5 (LAB) before filter sterilisation.

In some of the experiments the medium was supplemented with minerals and vitamins. The composition of the vitamin solution was as follows (mg/L): Biotin, 0.12; calcium D-pantothenate, 2.3; nicotinic acid, 2.3; inositol, 57.5; thiamine HCl, 2.3; pyridoxine HCl, 2.3 and 4-aminobenzoic acid, 0.46. The concentrations of vitamins added separately were the same as in the complete solutions. When zinc or manganese were added, $ZnSO_4$·$7H_2O$ 0.92 mg/L and $MnSO_4$·H_2O 0.45 mg/L were used, respectively.

2.5. Cultivation conditions

LABs were pre-cultivated on complete MRS-medium (Oxoid CM0359) in 50 mL plastic tubes (no mixing) containing 5 mL medium. Non-LABs were pre-cultivated in a medium containing (g/L): glucose, 20; Oxoid yeast extract, 5; Oxoid peptone, 10; KH_2PO_4, 0.5, with 50 mL medium in baffled shake flasks (200 rpm). All pre-cultivations were conducted at 30 °C for approximately 16 h until late exponential phase. The cultures were diluted to $OD_{600} = 1–1.5$ in sterile water before inoculation of the main cultures.

Cultivation was performed in 96-well plates (0.2 mL well volume), with four replicates of each medium variant. Each well contained 125 µL medium, including 10 µL inoculum. The non-LABs were cultivated with orbital shaking (900 rpm, 3 mm amplitude), whereas the LABs were grown stationary, but shaken gently before measurement of optical density (OD). Cultivations were performed at 30 °C. Cell growth was determined by OD measurement at 600 nm. OD was measured every two to four hours for 25−35 hours using a Tecan Freedom EVO robotic system (Tecan, Switzerland) with an integrated spectrophotometer (SpectraMax 384 plus, Molecular Devices LLC, USA). The OD-values are presented as average of the four replicate wells. Since the light path depend on the culture volume in the wells, deviations in volume, for instance due to evaporation, will affect the OD-values. The respective reference medium was therefore included on all microwell plates.

3. Results

3.1. Peptone properties

The peptones were produced either from the hydrolysates after a one-step hydrolysis by different enzymes, or after an additional secondary hydrolysis of the hydrolysates. The secondary hydrolysis was carried out using an enzyme with another specificity or property, eg acid protease, where the low pH will dissolve and release more bone minerals. A third variant was produced by a secondary hydrolysis of the decanter sludge after industrial-scale hydrolysis, see Table 1.

Head and backbones (HB) RRM typically contain 35 % protein on dry weight basis, while HBV RRM contain 18 % protein due to a higher content of fat. The protein yields for peptones made from fresh RRM were approximately 70 % using neutral proteases. The main loss occurred in preparation of the hydrolysates, caused by formation of emulsions during processing of the fat-rich raw material. The emulsions were removed to obtain completely water-soluble peptones. When starting with the commercial hydrolysates (Salmigo®), the yields were 84–92 %. Tail 1 Acid protease was less efficient than the neutral protease, with protein yields of 43–50 % when used for primary hydrolysis.

The nitrogen content of the peptones was between 11 and 16 % of dry weight (dw) (Fig. 1a), in the same range as animal-based peptones from meat and casein, and higher than yeast extract and the vegetable-based soya peptone (Fig. 1b). Total amino acids constituted 53–79 % dw (Supplementary data, Table S1), well in agreement with the total nitrogen content. The high ash content of the two peptones made from decanter sludge was mainly due to the depletion of protein in the previous processing (hydrolysis and removal of protein), but the acidification before the secondary hydrolysis and following neutralisation with NaOH also contributed.

The degree of hydrolysis was highest for peptones produced from raw materials that included viscera (HBV and VS), using the enzyme BZ16 for the primary hydrolysis (Fig. 2). The presence of viscera had a higher impact on the degree of hydrolysis than use of two hydrolysis steps. The two-step hydrolysis increased the fraction of free amino acids and peptides with molecular mass below 500 Da from 27 to 41 % for peptones based on head and backbones (HB) and from 56 to 64 % for peptones also including viscera (HBV) (Supplementary Data, Fig. S1).

3.2. Peptones as growth factors, role of viscera

Peptones prepared from head and backbone (HB) and peptones prepared from head, backbone and viscera (HBV), see Table 1, were compared in cultivation studies using all six strains. Ammonium was the main nitrogen-source in the media for the aerobic, non-LAB. The peptones were thus a source of organic nutrients. The peptones that contained viscera, promoted higher growth rates and cell densities for most of the organisms, as illustrated for the yeast *P. jadinii* in Fig. 3a. The peptone HBS-AA, which were produced from the decanter sludge after a primary hydrolysis with Alcalase, performed similarly to those with viscera. The peptones that included viscera, were also the best for the lactic acid bacterium *L. rhamnosus* (Fig. 3b). Comparison of the two peptone levels showed an extended period with exponential growth for the highest levels.

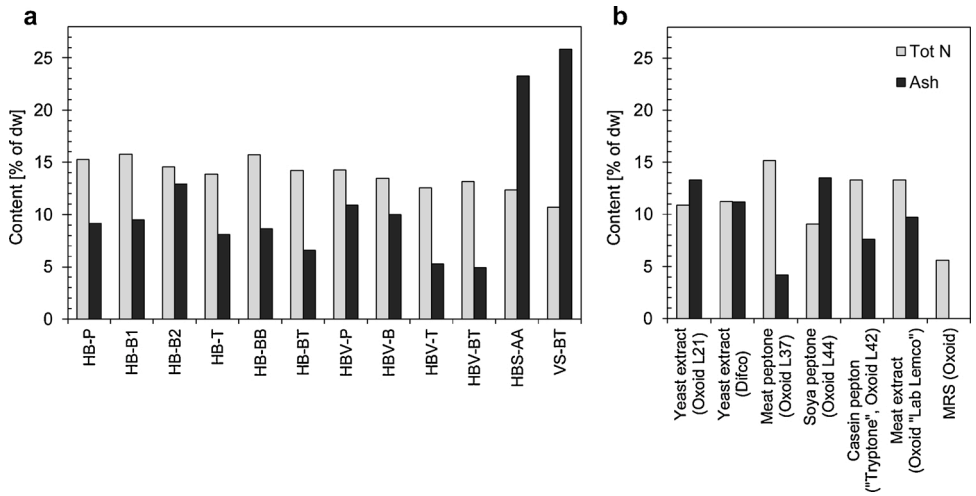

Fig. 1. Content of nitrogen and ash in the salmon-based peptones (a) and some commercial peptones and extracts (b). Salmon peptone designations: See Table 1. MRS: Ready mixed growth medium for lactic acid bacteria.

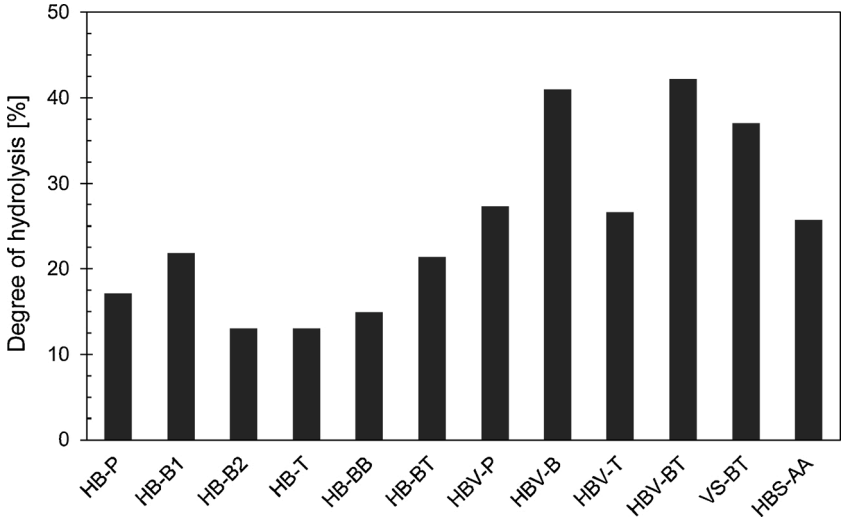

Fig. 2. Degree of hydrolysis of the peptones, determined as the proportion of cleaved peptide bonds. Peptone designations: See Table 1.

L. sakei, a very fastidious lactic acid bacterium, did not grow on the peptone without viscera (Fig. 4). *E. coli*, which does not require organic growth factors, had the same growth rates and maximum cell densities on medium without peptones or other complex nutrient sources. This also applies to *C. glutamicum*, where the peptone supplementation only had a marginal effect. *S. cerevisae* (Fig. 4) behaved like *P. jadinii* (Fig. 3), where the supplementation with peptones increased the growth rate and cell densities, and with a clearly higher effect for the peptones with viscera than those without. In general, the viscera-based peptones performed similarly to yeast extract, and considerably better than meat peptone (Supplementary data, Fig. S2) for the aerobic strains, and considerably better than MRS for the two *Lactobacillus*-strains (Figs. 3 and 4).

3.3. One- or two-step hydrolysis

The majority of the hydrolysates were prepared by one hydrolysis step. A secondary hydrolysis was carried out for some peptones, using acid proteases, see Table 1. In the secondary hydrolysis, large peptides resulting from the primary hydrolysis will be further degraded to shorter peptides. Low pH will in addition release minerals from the bones. One of the enzymes used for secondary hydrolysis (Tail 1, "T") was also tested for primary hydrolysis, in order to be able to distinguish between the effects of each of the hydrolysis steps. The peptones prepared in the two-step hydrolysis (adjustment of pH before the second step), gave variable results. Only for *L. sakei*, and peptones without viscera, the two-step hydrolysis provided better results than by use of BZ16 only (Fig. 5). For the peptone HB-BB, produced by use of the enzyme B-Pro-04 for the secondary hydrolysis, the growth rates and maximum cell densities were the same as on HB-B1, produced by BZ16 only (data not shown).

3.4. Limiting factors in peptones without viscera

As an attempt to identify the limiting factors in the peptones produced without viscera, vitamin and trace mineral solutions were added to the growth media. Zn-supplement generally improved growth in all media based on peptones without viscera (Table 2). This was in accordance with the analytical data, which showed that the Zn-content was highest in HBV-P, lowest in HB-P (Supplementary Data, Table S1). The effect of Mn-supplement varied and was strain-dependent. Vitamins had small effects but improved the growth yields for *L. rhamnosus*. For *L. sakei*, the most fastidious of the tested organisms, neither vitamins, nor Zn, could compensate for lacking viscera. For this strain, the degree of hydrolysis seemed to be the most critical factor, which was highest for

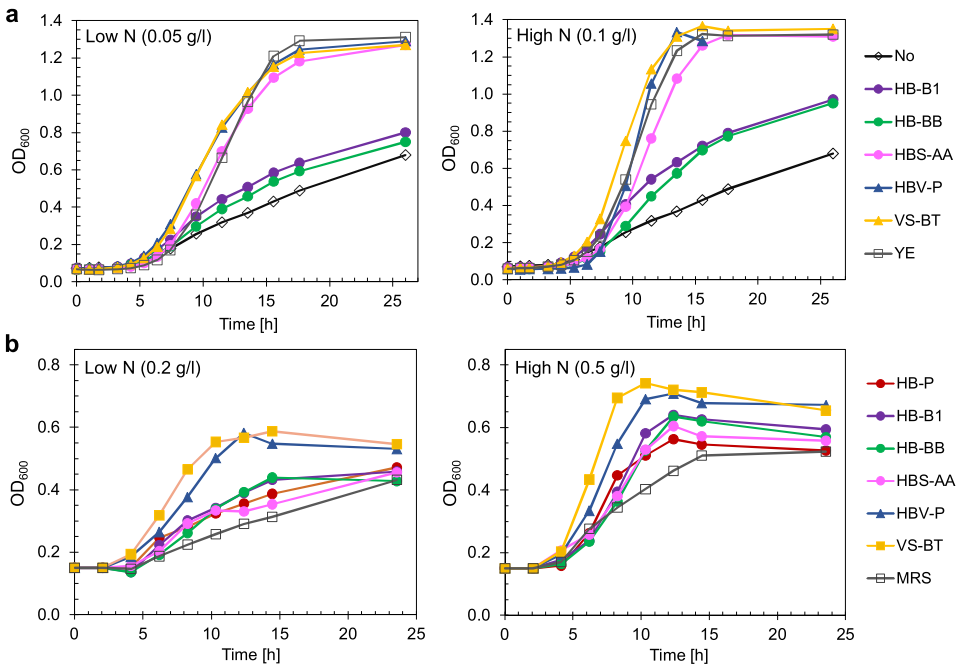

Fig. 3. Growth of *P. jadinii* (a) and *L. rhamnosus* (b) on selected peptones.
For *P. jadinii,* 0.05 and 0.1 g/L N were provided by the peptones, with ammonium in excess. For *L. rhamnosus* all N was provided by the peptones. Peptone designations: See Table 1. No: Basis medium without peptone; YE: Yeast extract; MRS: Reduced MRS-medium. For *P. jadinii*, glucose was exhausted at OD ~1.3.

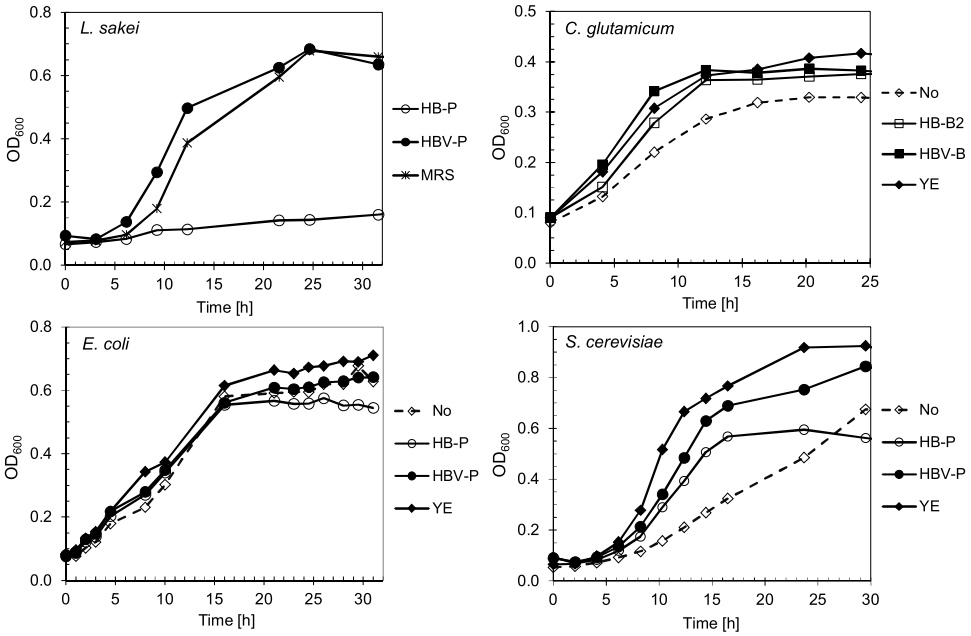

Fig. 4. Growth on peptones prepared by the same production process from head and backbones (HB-), and head and backbones + viscera (HBV-). Low concentrations (0.05 g/L) of complex N.
Peptone designations: See Table 1. No: Basis medium without peptone; YE: Yeast extract; MRS: Reduced MRS-medium.

the peptones that contain viscera.

4. Discussion

Our studies were carried out in microwell plates, using a liquid handling robot for plate filling and inoculation, and automated growth measurements. This enabled testing of a high number of combinations of raw materials, enzymes and microbial strains. However, although very efficient for screening of a high number of variables, the microwell format has some disadvantages, such as no control of pH or dissolved oxygen. Therefore, the media were diluted to avoid oxygen limitation for the aerobic organisms, and additional buffer was added to limit the pH-reduction caused by the acid production by the lactic acid bacteria. Use of diluted media has the additional advantage that differences between the media better can be detected when operating at the low cell densities obtained in microwell plates. However, to document the potential of the peptones in industrial production processes, cell and product yields and productivities should be determined. For this,

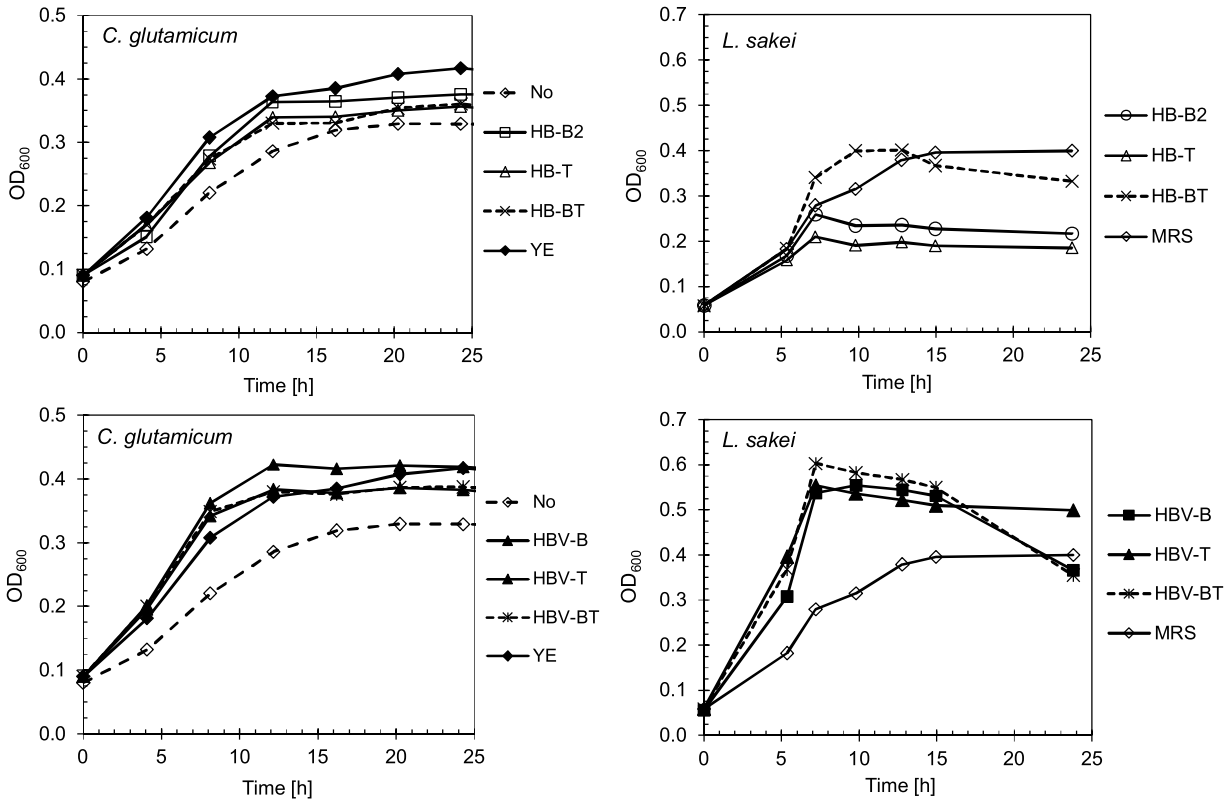

Fig. 5. Growth on peptones produced using different enzymes for hydrolysis – comparison of one-step hydrolysis with the enzymes BZ16 (-B2 or -B) and Tail 1 (-T), or a two-step, sequential hydrolysis using both enzymes (-BT), see Table 1.
High N-concentration (0.5 g/L) for *L. sakei*, low concentrations of complex N (0.05 g/L) for *C. glutamicum*. "No": Basis medium without peptone; "YE": Yeast extract.

Table 2
Relative increase in maximum cell density by mineral or vitamin supplementation to growth media based on peptones without viscera, and the corresponding relative increase by using peptones including viscera. For concentrations of minerals and vitamins, see Materials and methods.

Peptone	Added minerals or vitamins	Maximum OD with addition / Maximum OD without addition					
		E. coli	*C. glutamicum*	*P. jadinii*	*S. cerevisiae*	*L. sakei*	*L. rhamnosus*
HB-B1	Mn	1.04	1.18	1.26	1.06		
	Zn	1.16	1.05	1.10	1.96		
	Thiamine	1.16	1.05	1.26	1.22		
	Mn, Zn, thiamine	1.22	1.22	1.02	1.68		
	Vitamins					0.99	1.14
	Mn, Zn					1.01	1.02
	Mn, Zn, vitamins					0.96	1.17
HB-BB	Thiamine, biotin	1.09	0.95	1.01	1.21		
	Mn, Zn	0.93	1.38	1.50	1.30		
	Thiamine, biotin, Mn, Zn	1.05	1.35	1.45	1.39		
	Vitamins					0.96	1.37
	Mn, Zn					1.30	1.05
	Mn, Zn, vitamins					0.91	1.36
HB-P	Viscera (HBV-P)	1.15	0.92	1.23	1.26	2.37	1.24

bioreactor fermentations with control of pH and dissolved oxygen are needed, enabling growth to higher cell densities and analyses of dry weight, substrate consumption and product formation.

Since many microorganisms can grow well on inorganic nitrogen, which is a cheaper N-source than peptones, the peptones were compared as a source of organic nutrients, not as an N-source. The peptone concentrations used for the aerobically grown "non-LAB" provided 0.05 and 0.1 g/L N, which correspond to 0.4−0.8 g/l of a peptone with 12 % nitrogen. Glucose was the limiting component of the media (10 g/L) and would provide maximum 5 g/L dry weight. For an industrial fermentation with 100 g/L cell mass, the corresponding peptone concentrations would be 10 and 20 g/L. For the lactic acid bacteria, which cannot utilise inorganic N, the peptones were the only N-source and provided 0.2−0.5 g/L N, corresponding to 1.7-4 g/L peptone, if 12 % N. Commercial media, like the MRS-medium, contain 22 g/L complex nutrients, including peptones, yeast extract and meat extract (Oxoid CM0359).

The peptones that were produced from rest raw materials containing viscera, in general performed similarly to the most used commercial products; yeast extract for the aerobic organisms and MRS-medium for the lactic acid bacteria. Even the peptones produced from head and backbones without viscera, performed better than a meat-based peptone. No comparable studies have been reported, but an extensive work on peptones from cod viscera also showed better performance for the fish peptones than commercial meat and vegetable-based peptones [1,6,8,9]. In a recent work comparing peptones from aquaculture fish species, no differences between the fish peptones and commercial

peptones were observed [4]. However, these results are not directly comparable to ours, since the peptones either were the only medium component in addition to glucose, or were added in too high concentrations to be able to detect differences.

It would be natural to believe that the positive effect of viscera was caused by a higher content of organic growth factors originating from guts, liver and other organs, or the higher degree of hydrolysis (DH), but other factors seem to have been more critical. Zinc was identified as the most limiting component in peptones prepared without viscera. The peptones produced from heads and backbones performed well by addition of zinc for all the "non-LAB". Since zinc is a cheap mineral, it can easily be supplied in the required concentrations directly to the growth medium, or added by a commercial producer of the peptone. However, for the most demanding strain tested, the lactic acid bacterium *L. sakei*, the addition of zinc could not compensate for the lack of viscera. For this strain, DH seemed to be essential. In a direct comparison of the same raw materials hydrolysed in a one- and two-step hydrolysis (DH 17.3 and 24.4 %, respectively), the growth rates and -yields were higher after the two-step hydrolysis, while addition of zinc to the peptone from one-step-hydrolysis had no effect. The importance of DH for this strain is in accordance with previous studies on peptones from cod, which showed that the enzyme yielding the highest DH and fraction of small peptides (<500 Da) was the best for *L. sakei* [6]. The reason for a more extensive protein hydrolysis when including viscera is most likely the action of endogenous enzymes from the gut. These enzymes will be active after fish slaughtering until processing, and during the primary hydrolysis until inactivation by heat treatment. A higher DH in hydrolysates obtained from salmon viscera was shown by Opheim et al. [17], who compared different enzymes for hydrolysis of viscera. The DH obtained was 48–50 %, but only 35 % when the viscera was heat-treated before the hydrolysis.

An interesting observation was that a peptone based on head and backbones, which was produced by a secondary hydrolysis of the decanter sludge after a primary hydrolysis by the enzyme Alcalase (HBS-AA), performed similar to those based on viscera. Alcalase has been shown to be one of the most efficient enzymes for hydrolysis of fish rest raw materials in reported comparison of enzymes [13,24]. In addition, the secondary hydrolysis would further increase the degree of hydrolysis, and the low pH (pH 2.5) releases more minerals from the bone residues. This was confirmed by the analytical data, which showed a DH in the same range as for the viscera-containing peptones, and considerably higher zinc-content than after a one-step hydrolysis at natural pH.

The inclusion of viscera in commercial peptones from fish could possibly provide challenges related to batch-to-batch variations. Gut content is dependent on fish diets, which for wild fish have geographical as well as seasonal variations. This is, however, omitted for farmed salmon, since they are starved before slaughtering. On the other hand, it has been shown that season had no systematic influence on the performance of peptones produced from cod viscera, although batch variations were observed [9].

This study has only considered growth. In most industrial fermentation processes, an intra- or extracellular metabolite or polymer is the product, not the cell mass. However, for all growth-associated products, such as organic acids, amino acids, many heterologous proteins etc, a high growth rate will also imply high productivities. For non-growth associated products, a rapid initial growth to a high cell mass will normally be advantageous, but the subsequent production phase depends on a growth-limitation. In such cases, slow-metabolizing nutrients may be favourable. In our study, a peptone where small peptides had been removed by membrane filtration, was tested for some organisms. This peptone provided relatively low growth yields but performed excellent for an antibiotic-producing bacterial strain, where it increased the antibiotic yields (data not shown).

In summary, the peptones based on heads, backbones and viscera from farmed salmon performed similarly to yeast extract for *P. jadinii* and *S. cerevisae,* and to MRS-medium for the lactic acid bacteria *L. rhamnosus* and *L. sakei*. For *E. coli* and *C. glutamicum*, the peptones had only marginal effect, since these grow well on inorganic N. Also, peptones that were based on head and backbones without viscera, performed well for the aerobic organisms if zinc was added. As farmed salmon RRM are available in large quantities with a stable quality, peptones based on this resource should be further explored as a nutrient source for the fermentation industry, and in particular for nutrient-demanding organism such as some lactic acid bacteria. For organisms which does not require organic N, but where the peptones increase the growth rate and/or cell yields, the use of peptones will be a cost issue, where the gain in productivity should justify increased medium costs.

Author contributions

IMA, HS and GB designed the microbial growth studies, and GB carried out the experiments and processed the data. HN and KS designed the peptone preparations, and HN produced and characterised the peptones. GB, HN and IMA wrote the manuscript. All authors reviewed, edited, and approved the final manuscript.

CRediT authorship contribution statement

Gunn Broli: Conceptualization, Methodology, Investigation, Writing - original draft. **Halvor Nygaard:** Conceptualization, Methodology, Investigation, Writing - original draft. **Håvard Sletta:** Conceptualization, Methodology, Writing - review & editing. **Kjartan Sandnes:** Conceptualization, Writing - review & editing. **Inga Marie Aasen:** Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

Kjartan Sandnes is Chief Scientific Officer of Biomega Group AS, the company providing some of the protein hydrolysates used for preparation of the peptones. The other authors declare no competing interests.

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Appendix A. Supplementary data

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