African Journal of Biotechnology Vol. 8 (3), pp. 451-457, 4 February, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Production of lipase and biomass by *Staphylococcus simulans* grown on sardinella (*Sardinella aurita*) hydrolysates and peptone

Nabil Souissi[1]*, Ali Bougatef[2], Yosra Triki-ellouz[2] and Moncef Nasri[2]

[1]Laboratoire de Biodiversité et Bitechnologie Marine, Institut National des Sciences et Technologies de la Mer, Tunisia.
[2]Laboratoire de Génie Enzymatique et de Microbiologie, Ecole Nationale d'Ingénieurs de Sfax, Tunisia.

Accepted 24 July, 2008

Fish protein hydrolysates from low cost fish species (*Sardinella aurita*) were prepared and tested as carbon and nitrogen sources for microbial growth and lipase production by *Staphylococcus simulans*. When cultivated in media containing Whole Sardinella Hydrolysate (WSH) or Meat Sardinella Hydrolysate (MSH), lipase production was significantly low, 5 and 10 U/ml, respectively. However, lipase production was strongly enhanced when partially defatted sardinella hydrolysate was used and highest lipase production was obtained with Partially Defatted Meat sardinella Hydrolysate (PDMSH) (55 U/ml), higher than that obtained with tryptone as nitrogen source. Peptone from sardinella was also prepared and compared with a tryptone from casein and other fish peptones. The results revealed that sardinella peptone is a good substrate for the growth of *S. simulans*.

Key words: *Staphylococcus simulans*, culture media, *Sardinella aurita*, sardinella protein hydrolsates, sardinella peptone, lipase.

INTRODUCTION

Due to its high protein content, low-cost fish species and fish by-products represents a potential source of industrial peptones. Peptones from fish species have appeared in media catalogues only in the last decade. Particularly, fish protein hydrolysates have been reported as growth substrate for bacteria (Dufossé et al., 1997).

In Tunisia, sardinella (*Sardinella aurita*) is abundant (about 13,000 tones in 2004). It has been exploited as raw material for canning industries, but large part of its capture is still underutilized. For better management of sardinella catches it is therefore a challenge to utilize the valuable protein fraction from the by-product to convert them into more marketable and acceptable forms as fish protein hydrolysate or peptones. Organic nitrogen substrates, such as protein hydrolysates and peptones, are widely used in many biological and biotechnological applications, such as microbial biomass production (Singh et al., 1995), and metabolite biosynthesis including enzymes (Haltrich et al., 1994; Rapp, 1995). At present, peptones are obtained from casein, soy protein, gelatin and meat (Reissbrodt et al., 1995). The use of fish protein hydrolysates for maintaining the growth of different microorganisms has received a great amount of attention (Clausen et al., 1985; Gildberg et al., 1989; De la Broise et al., 1998; Dufossé et al., 2001), but only limited number of reports have been published about the application of this substrate to metabolite production (Coello et al., 2000). To upgrade protein by-products, many proteases were used to produce fish protein hydrolysates and peptones. Alcalase® is described to be the enzyme giving the best results with fish proteins (Benjakul and Morrissey, 1997).

Lipases are one of the most important groups of industrial enzymes and they are widely used for biotechnological applications in the food and dairy industries (Reed, 1975; Gandhi, 1997), leather processing (Iwai and Tsujusaka, 1984), detergents (Saad, 1995) and pharmaceuticals. Many strains are used for the industrial production of lipases such as *Rhizopus delemar*, *Aspergillus niger*, *Geotrichum candidum*, *Candida rugosa* and *Chromobacterium viscosum* (Gandhi, 1997).

*Corresponding author. E-mail: Nabil.souissi@gmail.com. Tel: +216 22 95 08 54. Fax: +216 74 49 79 89.

Ghorbel et al. (2005) have reported the production of lipase by *Rhyzopus oryzae* in medium containing glucose and fish protein hydrolysate as nitrogen source. The strain exhibited a greater lipase production than that obtained with casein peptone.

In this work, we investigate the preparation of sardinella hydrolysates and peptone and report the production of biomass and lipase by *Staphylococcus simulans* grown on sardinella substrates.

MATERIALS AND METHODS

Sardinella aurita

Sardinella (*S. aurita*) was obtained from a local supplier. It was washed twice with water, then separated into 2 lots and kept in plastic bags at -20°C until use. The first lot served for the preparation of whole sardinella hydrolysates. For the second lot, heads and viscera were eliminated in order to obtain sardinella meat which served for the preparation of meat sardinella hydrolysate.

Strain

S. simulans was isolated in Laboratoire de Biochimie et de Génie Enzymatique des Lipases, Ecole Nationale d'Ingénieurs de Sfax, Tunisia. It's a Gram positive coccus having lipolytic activity.

Peptones

Salmon Fish Liquid S490 (SFL) was purchased from PRIMEX, France. Tryptone (T), and Fish Peptone n°:1 (FP) were purchased from DIFCO, U.S.A.

Cultivation media

Preculture and culture media for *S. simulans* were prepared as described by Sayari et al. (2001). Preculture medium was composed of the following (g) in 1 L: yeast extract (Biorad), 2; pepsic meat peptone (Biorad), 5; meat extract (Biorad), 1; NaCl, 5. pH was adjusted to 7.5 Preculture was done in rotated shaker for 16 h at 37°C with an agitation of 150 rpm.

Culture medium was composed of (g/l): tryptone, 20; glucose, 2.5; NaCl, 5; K_2HPO_4, 2.5. pH was adjusted to 7.0. Medium was sterilized 20 min at 120°C. Inoculation from the preculture was done in order to have an initial OD in the culture measured at 600 nm of 0.2 to 0.3. Cultures were run in rotated shaker for 25 h at 37°C with an agitation of 150 rpm.

When media based on sardinella hydrolysate were used, only tryptone from the culture medium described above was replaced and this substitution was calculated in order to keep the same nitrogen content in all compared media.

All experiments were carried out in duplicate and repeated at least twice.

Preparation of sardinella powders and sardinella protein hydrolysates

As shown in Figure 1, to obtain fish-meat powders, heads and viscera were first eliminated. The raw material was washed then heated till boiling. The bones were removed and the cooked meat was pressed to remove water and fat. The resulting pressed product was minced in a meat grinder, and then dried at 80°C for 24 - 48 h. The dried fish preparation was minced again to obtain a fine powder, and then stored in glass bottles at room temperature. In order to obtain whole sardinella powder, raw material was cooked, pressed, minced and then dried.

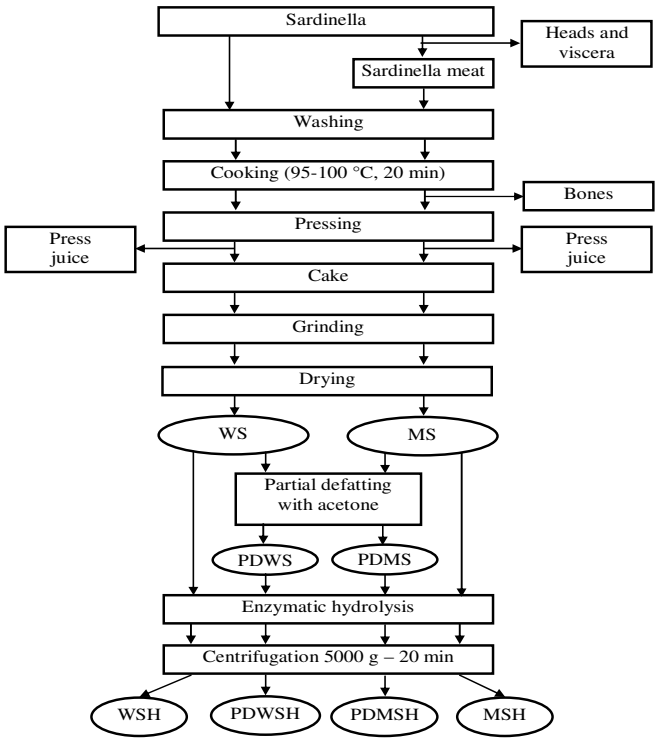

Figure 1. Preparation of different sardinella powders and hydrolysates. WS: whole sardinella, MS: Meat sardinella, PDWS: Partially Defatted Whole sardinella, PDMS: Partially Defatted Meat sardinella, WSH: Whole sardinella Hydrolysate, PDWSH: Partially Defatted Whole sardinella Hydrolysate, MSH: Meat sardinella Hydrolysate, PDMSH: Partially Defatted Meat sardinella Hydrolysate.

In order to prepare sardinella protein hydrolysate, sardinella powders, obtained as described above, were subjected to enzymatic hydrolysis with Alcalase® (purchased from Novozymes®-Denmark). For the preparation of partially defatted sardinella protein hydrolysates, sardinella powders were first partially defatted by extraction with acetone and then hydrolyzed by the same enzyme. Hydrolysis of fish proteins was carried out in a pH-stat at pH 8.0 and 50°C. Defatted or non-defatted fish substrate was suspended in water at a concentration of 8% (w/v). The pH was adjusted to 8.0 by 4 N NaOH, and then the enzyme was added. The digestion mixture was incubated for about 3 h. Once the hydrolysis had been completed (the hydrolysis degree remained constant) the mixture was heated at 80°C in a water bath for 15 min to inactivate the enzyme. The hydrolysates were stored in glass bottles at 4°C. The degree of hydrolysis (DH) was used as an indicator for cleavage of peptide bond. Percent DH is defined as percent of total peptide bond cleaved. The degree of hydrolysis was calculated according to Adler-Nissen (1986).

Preparation of sardinella peptone

The preparation of sardinella peptone is described in Figure 2. Meat sardinella was first minced then cooked to inactivate endogenous

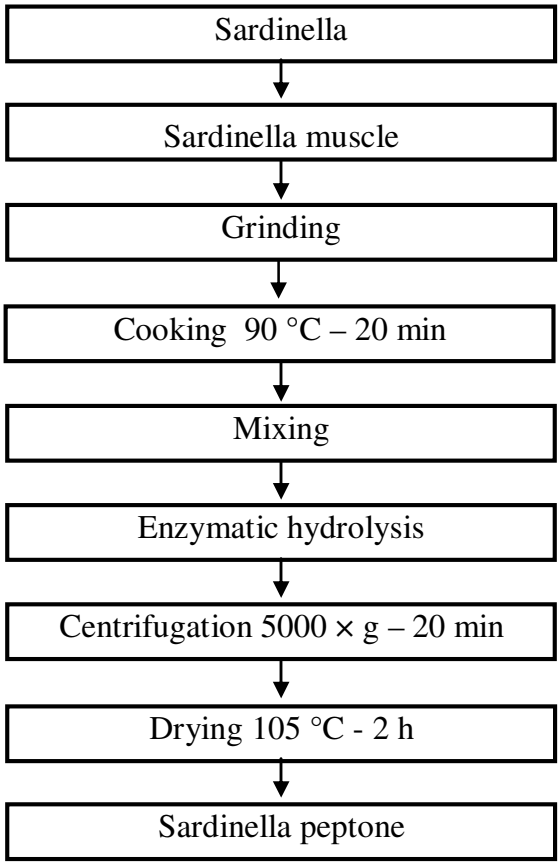

Figure 2. Scheme for the sardinella peptone preparation process.

enzymes. The cooked sample was mixed with an equal amount of distilled water and homogenized in Moulinex® blender for about 2 min. The pH of the mixture was adjusted to the optimum activity value for the enzyme. The hydrolysis was performed under the following conditions: pH 8; temperature of 50 °C and enzyme units of 363.63 x 10^3 UL^{-1}.

The pH of the mixture was kept constant at 8 during the enzymatic reaction by continuous addition of a 4 N NaOH solution to the reaction mixture. The reaction was stopped by heating at 80°C for 20 min to inactivate the enzyme. The hydrolysate was centrifuged at 5000 g for 20 min to separate insoluble and soluble fractions and than dried at 105°C for 2 h.

Chemical analysis

The moisture and ash content was determined according to the AOAC (1995). Total nitrogen content of sardinella preparations was determined by the Kjeldahl method (AOAC 981.10). Crude protein was estimated by multiplying total nitrogen content by the factor of 6.25. Lipids were determined gravimetrically after Soxhlet extraction of dried sample with hexane. All measurements were performed in triplicate.

Analysis of peptones by high pressure liquid chromatography

Chromatographic profiles of peptones were obtained using a liquid chromatograph Waters ®600 with a Superdex Peptide HR 10/30 column (Pharmacia) having a total volume of 24 ml. Liquid phase is composed by 30% solvent A (acetonitrile) and 70% solvent B (1% ultra pure aqueous solution of trifloroacetic acid). This mixture is known to be an excellent solvent for peptides of low molecular weight and having a poor absorbance in Ultra Violet light. Sample was solubilized at a final concentration of 2% in the mobile phase then filtered on a 0.2 µm filter. All solutions were also filtered on 0.4 µm filter. UV-absorbance was detected at 220 nm. The protein standard used which were purchased from SIGMA were: Substance P (4-11), 966.20 Da; Substance P, 1347.60 Da; Neurotensine, 1673.00 Da; Insuline chain B, 3496.00 Da; Aprotinine, 6500.00 Da; Cytochrome C, 12500.00 Da. The analysis of chromatogram was done with Millennium® software provided by Waters®.

Assay of lipolytic activity

Lipase activity was determined according to Gargouri et al. (1986) with olive oil as substrate, by continuous titration with NaOH 100 mM at 37°C, pH 8.2 in a pH-stat. The enzymatic reaction was performed in a reaction medium containing: 250 µl bovine serum albumin, 20 ml distilled water and 10 ml of a 10% (v/v) emulsion of olive oil. The olive oil was emulsified by sonication of 10 ml of olive oil with 90 ml gum arabic (10%). 10 µl of culture medium supernatant containing lipase were added to the medium and the enzyme activity was determined.

One unit of lipase activity was defined as the amount of enzyme that released 1 µmol of free fatty acid per minute under assay conditions.

Growth kinetics, modeling growth curves: Gompertz model

A lot of mathematical models can be used to describe the lag phase (λ), maximum growth rate (μ_{max}) and maximum biomass at the stationary phase (A) (Zwietering et al., 1990). Gompertz model (1), well suited for such purpose, was applied to the growth curves obtained on peptones.

$$\ln\frac{N}{N_0} = A\times\exp\left(-\exp\left(\frac{\mu_{max}\times e\times(\lambda - t)}{A}+1\right)\right) \quad (1)$$

N_0 = initial population; N = population at instant t

Calculation was run on EXCEL (Microsoft®) by the least square method to adjust the model to the data and the correlation coefficient (r) to estimate the fitness of this adjustment was also reported.

Statistical analysis

Statistical analysis was performed with Statgraphics® Ver. 5.1 professional edition (Manugistics®, corp.) using ANOVA analysis. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Preparation of sardinella powders and hydrolysates

As shown in Figure 1, a great variety of sardinella substrates can be prepared and used as nitrogen source: meat sardinella powders (MS), whole sardinella powders (WS), partially defatted meat sardinella powder (PDMS), partially defatted whole sardinella powder (PDWS), meat

Table 1. Chemical composition of sardinella powders (g/100 g product).

Sardinella powder	Water	Protein	Lipids	Ash
Whole sardinella (WS)	1.3 ± 0.1	64.3 ± 1.2	11.4±1.1	11.7±0.9
partially defatted whole sardinella (PDWS)	1.5±0.4	70.5±1.8	4.5±0.6	12±1.1
Meat sardinella (MS)	1.3±0.2	80.0±2.0	8.0±0.9	4.4±0.4
partially defatted meat sardinella (PDMS)	2.0±0.5	84.5±2.1	3.5±0.4	4.3±0.5

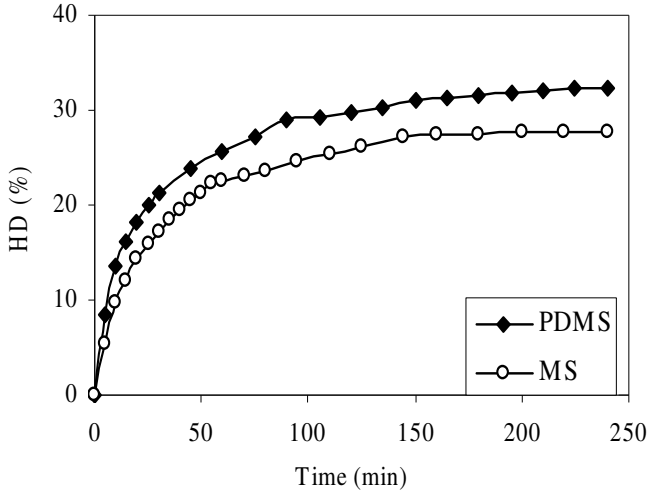

Figure 3. Hydrolysis of MS and PDMS with Alcalase®.

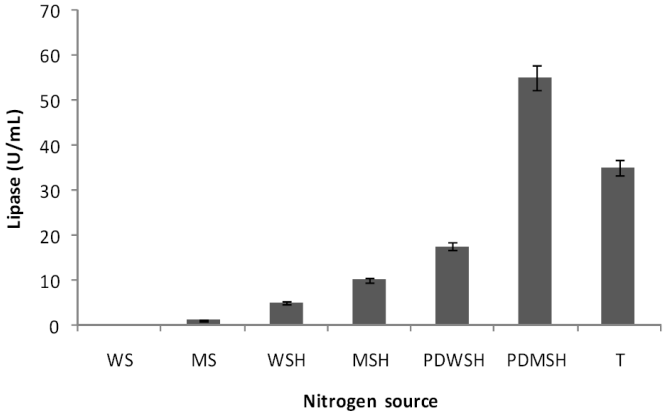

Figure 4. Effect of different sardinella preparations on lipase production by *S. simulans*. Cultures were conducted in medium containing 20 g/l of sardinella preparation, 2.5 g/l of glucose, 5 g/l of NaCl and 2.5 g/l of K_2HPO_4. WS: whole sardinella, MS: Meat sardinella, WSH: Whole sardinella Hydrolysate, PDWSH: Partially Defatted Whole sardinella Hydrolysate, MSH: Meat sardinella Hydrolysate, PDMSH: Partially Defatted Meat sardinella Hydrolysate, T: tryptone.

sardinella hydrolysate (MSH), whole sardinella hydrolysate (WSH), partially defatted meat sardinella hydrolysate (PDMSH), and partially defatted whole sardinella hydrolysate (PDWSH). Table 1 shows the chemical composition of the partially defatted and non-defatted sardinella powders, meat sardinella contained higher protein content (80±2%) but lower ash and lipid content than whole sardinella powder. Elimination of heads, viscera and bones resulted in an increase in the protein content because of the high lipid and ash contents in heads and viscera. The residual fat content of the meat material after treatment with acetone was about 3.5±0.4% of PDMS and 4.5±0.6% of PDWS. To obtain partially defatted sardinella protein hydrolysates, sardinella powders, obtained as shown in Figure 1, were first treated with acetone then subjected to hydrolysis with Alcalase®.

Hydrolysis reaction

The curve of hydrolysis of MS and PDMS with Alcalase® is shown in Figure 3. Fish protein hydrolysate obtained from PDMS showed an appreciably higher degree of hydrolysis (32%) than that obtained from MS (27%). These results indicate that high lipid content may inhibit Alcalase® by creating heterogeneous media by interacting with different powder constituents.

Evidence of lipase production by *S. simulans* grown on different sardinella preparations

Six culture media were tested for lipase production by *S. simulans*, WS, MS, WSH, MSH, PDWSH, PDMSH and peptone. The medium based on tryptone as nitrogen source was reported by Sayari et al. (2001) as the best culture media for *S. simulans* so we did not test other commercial media for lipase production by this strain. As shown in Figure 4, when cultivated on WS, the strain did not show any lipase activity and only 2 U/mL were obtained on MS. Lipase activity was slightly increased when whole sardinella powder and meat sardinella powder were used as the nitrogen source but the enzyme production was still poor (about 5 and 10 U/mL for WSH and MSH, respectively), compared to that obtained with commercial peptone medium based on tryptone as nitrogen source (35 U/mL) (Figure 4).

The lower lipase induction obtained in medium containing non-defatted MS, could be due to the existence of a compound in lipid fraction that could repress enzyme synthesis. In order to study such hypothesis, PDWSH and PDMSH were tested for lipase production. The lipid content in the initial powders was reduced by approxi-

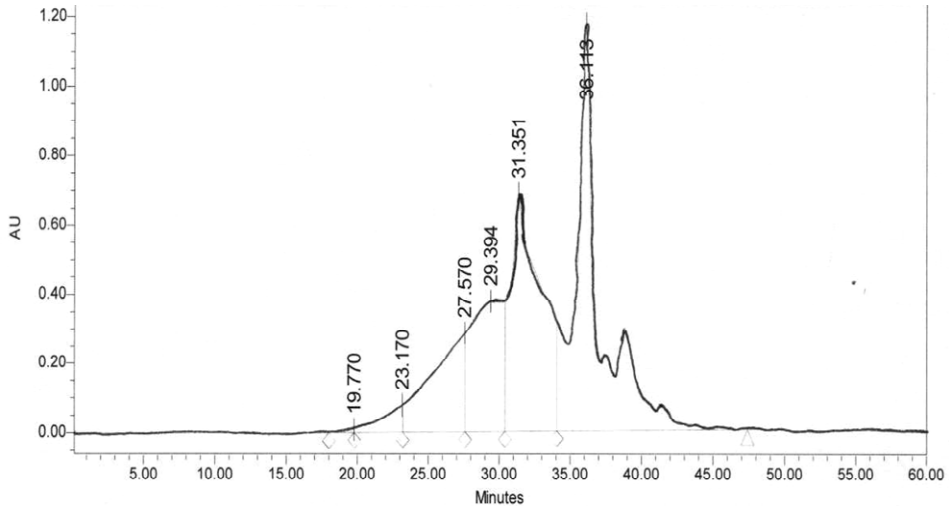

Figure 5. Peptidic profile of sardinella peptone (SP).

Table 2. Chemical composition of sardinella peptone and commercial peptones.

Peptone	Moisture (%)	Lipids (%)	Ash (%)	Protein (%)
Sardinella Peptone (SP)	1.88 ± 0.6	3.31 ± 0.14	16.98 ± 1.23	81.51 ± 1.16
Fish Peptone (FP)	12.89 ± 0.18	2.21 ± 0.24	34.8 ± 0.14	66.25 ± 0.21
Salmon Fish Liquid (SFL)	35.00 ± 0.30	0.40 ± 0.12	15.8 ± 0.21	65.62 ± 0.18
Tryptone (T)	5.77 ± 0.24	Trace	17.01 ± 0.29	83.06 ± 0.32

mately the same factor, 2.53 in the case of PDWSH (from 11.4% in WS to 4.5 in PDWS) and 2.28 in the case of PDMSH (from 8% in MS to 3.5 in PDMS). Figure 4 shows that lipase synthesis is strongly enhanced when sardinella hydrolysates were used as nitrogen source. The lipase production reached 19 U/mL for PDWSH and 55 U/mL for PDMSH and with these hydrolysates, the enzyme activity is slightly greater than that obtained with commercial peptones as nitrogen source (35 U/ml). These results clearly indicated that lipase synthesis is affected by the composition of the medium, especially by lipid content.

The difference shown between PDMSH and PDWSH can be attributed to the fact that there are some compounds in heads and viscera that can inhibit lipase production, those results are the same found previously by Ghorbel et al. (2005) with *R. oryzae* as lipolytic strain.

Sardinella peptone as new medium for biomass production by *S. simulans*

Sardinella peptone (SP) prepared as described in Figure 2 was characterized chemically and either by peptidic profile and compared to 3 commercial peptones used as control: SFL, T, and FP. Chemical composition of peptones shown in Table 2 indicates that SP has a comparative composition to the commercial ones; in fact SP has a good protein fraction (about 81%), and comparative ash fraction to SFL and T (about 17%). The high ash content in FP is probably due to the process by which this peptone is obtained; it consists of an acidic hydrolysis and the step of neutralisation which takes place in the end of the process contributes to obtain salts (HCl + NaOH → NaCl + H_2O) that increases the ash content in peptone (Gildberg et al., 1989).

The molecular size distribution determined by high pressure liquid chromatography is shown in Table 3 and Figure 5. SP and T have approximately the same protein molecular weight distribution with major part of protein between 1000 and 3500 Da. FP and SFL have the major part of peptide size lower than 1000 Da indicating that those peptones are much hydrolysed. This can be explained by the fact that they were acid hydrolysed.

In order to test the microbiological performance of our peptone, we investigated the growth parameters of *S. simulans* by performing culture kinetics using different peptones. To estimate growth parameters Gompert z → Gompertz model was used. This model was first described by Zwietering et al. (1990) as the best one permitting good mathematical interpretation in the case of studying bacterial growth (Cheroute-Vialette and Lebert, 2000 ; Dufossé et al., 2001; Guérard et al., 2001; Wei and Fang, 2001; Cenkowski et al., 2002; Tomas et al., 2002). Table 4 represents the different growth parameters obtained with Gompertz model which is applied in order

Table 3. Molecular weight distribution of peptones used in this work.

Range of molecular weight (Da)	Sardinella peptone (SP)	Fish peptone (FP)	Salmon fish liquid (SFL)	Tryptone (T)
> 12500	0.10	1.21	0.15	0.08
12500- 3500	2.36	11.06	1.57	4.25
3500 - 1000	58.99	-	14.84	60.61
< 1000	38.36	87.73	83.45	35.05

Table 4. Growth parameters of *S. simulans* grown on sardinella and commercial peptones.

Growth parameter	Sardinella peptone (SP)	Fish peptone (FP)	Salmon fish liquid (SFL)	Tryptone (T)
A	3.351 ± 0.032	3.254 ± 0.018	3.911 ± 0.017	3.512 ± 0.053
μ_{max} (h^{-1})	0.682 ± 0.058	1.393 ± 0.106	1.452 ± 0.081	1.300 ± 0.165
λ (h)	0.914 ± 0.231	1.534 ± 0.097	1.724 ± 0.082	1.391 ± 0.186
r	0.989	0.995	0.997	0.986

A: maximum biomass at the stationary phase; λ: lag phase; μ_{max}: maximum growth rate; r: correlation coefficient.

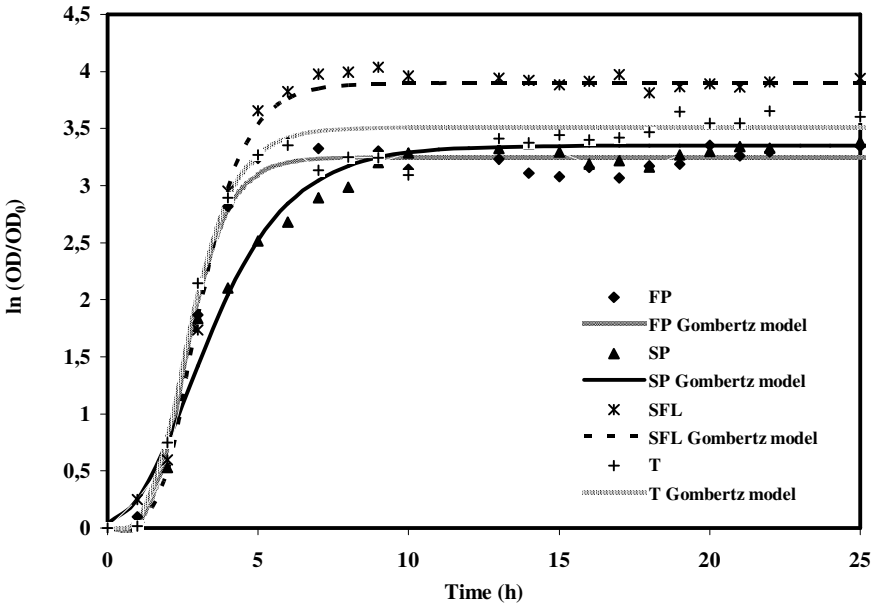

Figure 6. Gompertz modelling of growth curves of *S. simulans*. FP: Fish peptone n°:1; SP: sardinella peptone; SFL: Salmon fish liquid S490; T: Tryptone.

to estimate growth parameters of *S. Simulans*, from which we obtain a correlation factor (r) of at least 0.986. From kinetic curves shown in Figure 6 and growth parameters mentioned in Table 4, it can be concluded that maximum biomass produced in stationary growth phase decrease when peptone used was changed in this order SFL, T, SP, and FP. The SP leads to reduction of the lag phase but it contributes to decrease of the maximum growth rate.

The results obtained with the mathematical model combined to the chromatographic analysis of peptones indicate that the DH is not the only parameters affecting bacterial growth. For example, FP is the most hydrolysed peptone but it produces the minimum biomass.

Conclusion

In conclusion, by using defatted sardinella meat hydrolysed with Alcalase®, it was possible to increase lipase

production by *S. simulans*. In addition, the production of biomass on sardinella peptone prepared by a low cost process was better than a commercial fish peptone. The high lipase activity obtained with cheap fish meal and the good biomass production on sardinella peptone clearly indicated that these substrates could be used in industrial fermentation processes.

REFERENCES

Adler-Nissen J (1986). Enzymic Hydrolysis of Food Proteins. Barking, UK: Elsevier Applied Science Publishers. ISBN 0-85334386-1.

Association of Official Analytical Chemists (AOAC) (1995). Official methods of analysis. 16th ed., Arlington, Va.: AOAC.

Benjakul S, Morrissey MT (1997). Protein hydrolysates from Pacific Whiting solid wastes. J. Agric. Food Chem., 45: 3423-3430

Cenkowski S, Blank G, Chung-Lewis M (2002). Modeling of *Listeria monocytogenes* growth in pre-sterilized ground beef as affected by fat content, temperature and atmosphere. Can. Biosyst. Eng., 44: 11-16.

Cheroute-Vialette M, Lebert A (2000). Growth of *Listeria monocytogenes* as a function of dynamic environment at 10 °C and accuracy of growth predictions with available models. Food Microbiol., 17: 83-92.

Clausen E, Gildberg A, Raa J (1985). Preparation and testing of an autolysate of fish viscera as growth substrate for bacteria. Appl. Environ. Microbiol., 50: 1556-1557.

Coello N, Brito L, Nonus M (2000). Biosynthesis of L-lysine by *Corynebacterium glutamicum* grown on fish silage. Bioresour. Technol. 73: 221-225.

De la Broise D, Dauer G, Gildberg A, Guérard F (1998). Evidence of positive effect of peptone hydrolysis rate on *Escherichia coli* culture kinetics. J. Mar. Biotechnol. 6: 111-115.

Dufossé L, De la Broise D, Guérard F (1997). Fish protein hydrolysates as nitrogen sources for microbial growth and metabolite production. Recent Res. Dev. Microbiol. 1: 365-381.

Dufossé L, De la Broise D, Guérard F (2001). Evaluation of nitrogenous substrates such as peptones from .sh: a new method based on Gompertz modeling of microbial growth. Curr. Microbiol. 42: 32-38.

Gandhi NN (1997). Applications of lipase. J. Am. Oil Chem. Soc. 74: 534-621.

Gargouri Y, Pierroni G, Rivière C, Saunière JF, Lowe PA, Sarda L, Verger R (1986). Kinetik assay of human gastric lipase with short and long chain triacylglycerol emulsion. Gastroenterology, 91: 919-925.

Ghorbel S, Souissi N, Triki-Ellouz Y, Dufossé L, Guérard F, Nasri M (2005). Preparation and testing of sardinella protein hydrolysates as nitrogen source for extracellular lipase production by *Rhizopus oryzae*. World J. Microbiol. Biotechnol. 21: 33-38.

Gildberg A, Batista I, Strom E (1989). Preparation and characterization of peptones obtained by two-step enzymatic hydrolysis of whole fish. Biotechnol. Appl. Biochem. 11: 413-423.

Guérard F, Dufossé L, De la Broise D, Binet A (2001). Enzymatic hydrolysis of protein from yellowfin tuna (*Thunnus albacares*) wastes using Alcalase®. J. Mol. Catal. 11: 1051-1059.

Haltrich D, Laussamayer B, Steiner W (1994). Xylanase formation by *Sclerotium rolfsii*: effect of growth substrates and development of a culture medium using statistically designed experiments. Appl. Microbiol. Biotechnol. 42: 522-530.

Iwai M, Tsujisaka Y (1984). Fungal lipases. In Lipases, eds. Borgstrom, B, Brockman HL (pp. 443–449) Amsterdam: Elsevier. ISBN 0-44480526-5.

Rapp P (1995). Production, regulation, and some properties of lipase activity from *Fusarium oxysporum* f. sp. *Vasinfectum*. Enzyme Microb. Technol. 17: 832-838.

Reed G (1975). Microbial lipases. In Enzymes in Food Processing. ed. Reed G, New York: Academic Press. ISBN 0-12-584852-8. pp. 208–214.

Reissbrodt R, Beer W, Muller R, Claus H (1995). Characterization of casein peptones by HPLC profiles and microbiological growth parameters. Acta Biotechnol. 15: 223-231.

Sayari A, Agrebi N, Jaoua S, Gargouri Y (2001). Biochemical and molecular characterization of *Staphylococcu simulans* lipase. Biochemistry, 83: 863-871.

Saad RR (1995). Production of lipase from *Aspergillus tamarii* and its compatibility with commercial detergents. Folia Microbiol. 40: 263-266.

Singh G, Kothari RM, Sharma RK, Ramamurthy V (1995). Enhancement of Spirulina biomass productivity by a protein hydrolysate. Appl. Biochem. Biotechnol. 50: 285-290.

Tomas MSJ, de Labanda EB, de Ruiz Holgado AP, Nader-Macias ME (2002). Estimation of vaginal probiotic *lactobacilli* growth parameters with the application of Gompertz model. Can. J. Microbiol. 48: 82-92.

Wei Q, Fang TJ (2001). Validation of growth models for *Listeria monocytogenes* and *Yersinia enterolitica* in cooked chicken meat. J. Food Drug Anal. 9: 153-159.

Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K (1990). Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 56: 1875-1881.