IMPACT OF FISH WASTE PROTEIN ENZYMETIC HYDROLYSATE ON GROWTH OF SOME OF *Lactobacillus sp.* AND *Bifidobacterium sp.*

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ABSTRACT

This study was aimed to investigate the impact of protein enzymatic hydrolysate extracted from waste of common carp (*Cyprinus carpio*) on some bacterial growth. Two type protein concentrates were prepared from defatted carp fish waste powder, the first at pH 2/4.5 and the second at pH 12/4.5. They were hydrolysed by pepsin enzyme lyophilized powder ≥ 2500 units/mg conc.1.5gm/100gm protein concentrates for (30, 60, 90, and 120) minutes. The obtained hydrolysates characterized in term of color, amino acids, minerals elements, degree of hydrolysis, solubility in addition to bacterial growth promotor and biomass production properties. The whiteness of acidic and basic protein concentrates were (68.62 and 67.09) %, respectively, their content of essential amino acids were (35.73 and 34.5)%, respectively, they contained similar concentrations of mineral elements. The highest degree of hydrolysis for the concentrate was acheived after 120 minutes of enzymatic reaction time, being (50.8 and 52.4)%, their solubilities were increased with increasing enzymatic reaction time, and highest values were recorded at pH 2 and 10. Number of colonies and biomass of *Bifidobacterium infantis*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus GG* was in alternative media higher than commercial medium (MRS) .

Key words: Alternative media, *cyprinus carpio*, degree of hydrolysis, Lactic acid bacteria, peptone.

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تأثير المُتحللات الإنزيمية لبروتينات مخلفات الأسماك في نمو بعض انواع كل من بكتريا *Lactobacillus* و *Bifidobacterium*

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المستخلص

هدفتْ هذه الدراسة إلى التَعرف على تأثير المُتحللاتِ البروتينية الإنزيمية المُحضرة من مُخلفاتِ أسماك الكاربِ (*Cyprinus carpio*)في النمو البكتيري. حُضرَ مركزين بروتينين من مسحوقِ مخلفاتِ أسماك الكارب منزوع الدهن، الأول عند الرقم الهيدروجيني 4.5/2 والثاني عند الرقم الهيدروجيني 4.5/12، عُوملَ المُركزين بإنزيم البيبسين لمدة (30، 60، 90، 120) دقيقة، دُرستْ خصائص الببتون الناتج من خلال تَقدير مؤشرات اللون والأحماض الأمينية والعناصر المعدنية ودرجة التحلل المائي والذوبانية والنمو البكتيري و إنتاج الكتلة الحيوية. بلغَتْ درجة بياض المُركز البروتيني الحامضي والقاعدي (68.62 و 67.09) على التوالي، وكان مُحتواها من الأحماض الأمينية الأساسية (35.73 و 34.5)% وبنفس الترتيب، وأحتويا على تراكيز مُتقاربة من العناصر المعدنية، وسُجلت أعلى درجة تَحلل بعد 120 دقيقة عى زمن التفاعل الانزيمي بِواقع (50.8 و 52.4)%، وازدادت الذوبانية مع زيادة زمن التفاعل الأنزيمي، وسُجلتْ أعلى قيمة عند الرقم الهيدروجيني 2 و 12. كان عدد المُستعمرات و أنتاج الكتلة الحيوية *Bifidobacterium infantis* و *Lactobacillus plantarum* و *Lactobacillus rhamnosus GG* في الأوساط البديلة أعلى من الوسط التجاري .

الكلمات المفتاحية: الاوساط البديلة، *Cyprinus carpio* ، درجة التحلل، بكتريا حامض اللاكتيك، الببتون.

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INTRODUCTION

Lactic acid bacteria include a group of microorganisms that produce lactic acid as an end product of carbohydrate fermentation, fastidious nutritional requirements, no spore forming rods and cocci, Gram-positive, anaerobic, but aerotolerant bacteria (10, 32, 36). The role of lactic acid bacteria in foods lies in three main aspects, they are single or mixed starter cultures for the production of fermented foods, biopreservatives, and one of the main components of foods that contain probiotics (13, 22, 31). The three aspects can be utilized together by using starter cultures that produce antimicrobial agents in production of probiotic foods (21, 47). The cultural media of lactic acid bacteria contain growth factors and nutrients (28, 45). Lactic acid bacteria need complex nutritional requirements such as peptides, amino acids, vitamins, nucleic acid, fatty acid esters, salts, and carbohydrates, these needs are met when culture medium contains fermentable sugars, yeast extract, and peptone meat (34, 43, 49). It is preferable that organic nitrogen be in form of peptides or amino acids in culture media designated for lactobacilli, amino acids such as Glutamic acid, Valine, and Isoleucine are considered essential growth factors that bacteria are unable to synthesize they must be available in culture media as they require many types of Lactic acid bacteria, presence of acetate, manganese, and Tween 80, which contains Oleic acid esters (15). Complex commercial media are used to grow lactic acid bacteria such as De Man Rogosa and Sharpe (MRS), Sodium Lactate (NaLa), Brain Heart Infusion (BHI), Trypticase Soy Broth Yeast Extract (TSBYE) and M17, these media are designed for specific strains, such as MRS for lactobacilli and M17 for lactococci (1). MRS used to grow lactic acid bacteria in the laboratory, are considered to be very expensive because they contain expensive nitrogen sources, such as yeast and meat extract, they do not support growth of all types of lactic acid bacteria (40). Many studies indicate possibility of replacing expensive commercial growing media with alternative media that contain agricultural or industrial waste at low or medium cost (4). Anggraini et al (12) stated that natural media containing tofu liquid waste at a concentration of 100% and media containing 15% palm sugar are the best for cultivation of *Pedioccocus acidilacti* bacteria and production of Gamma-aminobutyric acid. Malikil et al (26) found that the best ratio of bacteria and components of alternative culture medium to produce highest biomass and lower pH was 1:1:1:2:1 for *Lactobacillus parabuchneri*, *L. buchneri*, *L. harbinensis*, *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner*. The best alternative medium was medium containing coconut water, cassava flour, and fish waste flour at a concentration of (90, 5 and 5) %, respectively, as the biomass reached 22.47 mg/ml and pH decreased to 2.84. The common carp (***Cyprinus carpio***) is a freshwater fish (8), it is an omnivorous fish that feeds on various plant and animal sources (44), it grows quickly and reaches maturity after two years (9, 50), therefore, it is of great economic importance in some countries and a riverine resource in others (15, 19, 46). Carp fish waste contains varying percentages of protein, as it constitutes 22.1% of scales, 13.9% of fins, and 25.9% of bones (25, 30). This study was aimed to exploit the waste of common carp fish polluting the environment in preparation of an inexpensive peptone for growth of some types of bacteria.

MATERIALS AND METHOS

Microorganisms: *Bifidobacterium anmals Bb12, Sandoz. Bifidobacterium infantis, Biocodex. Lactobacillus plantarum, Swanson. Lactobacillus rhamnosus GG, Lacto 66. Lactobacillus acidophilus, Nature's bounty.*

Cultural medium

DeMan, Rogosa and Sharpe broth, Diagnostici Liofilchem, Italy.

Preparation of protein concentrates

The acidic and basic protein concentrates were obtained as described in our a previous study by the same researcher.

Color measurement

The color of the defatted carp fish waste powder, and the acidic and basic concentrations prepared from defatted carp fish waste powder, were measured following method of (41) using a Chroma meter, to obtain values of L*, a*, and b*, the whiteness values were calculated from the following equation:

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Where: L*: lightness, a*: greenness to redness, and b*: blueness and yellowness.

Amino acids analysis

Amino acid composition for both concenters (42) using a High-Performance Liquid Chromatography (HPLC) Shimadzu Corportion model LC-2010A HT), column (Shim-packTM XR-ODSII, flow rate 10 ml/min, at 40°C.

Determination of mineral content

Mineral contents in acidic and basic concentrates were estimated according to method mentioned in (3) using atomic absorption method and a Spectrophotometer (AA-6800).

Preparation of protein hydrolysastes

Protein hydrolysate were prepared according to (38) by mixing acidic and basic concentrates with distilled water in a ratio of 1:4, pH was adjusted to 2 by hydrochloric acid 2N, heated in a water bath at 85°C for 15 minutes, lyophilized pepsin (≥ 2500 units/mg protein) was added, at a concentration of 1.5 g/100 g of protein concentrate in a shaking incubator at 37°C, the hydrolysate were withdrawn after (30, 60, 90 and 120) minutes for both concentrate, heated in a water bath at 85°C for 15 minutes, lyophilized and stored at 4°C.

Detrmation of dgree of hydrolysis (DH)

Degree of hydrolysis was estimated according to (33) by mixing 2 ml of protein hydrolysate 50 mg/ml with 2 ml of TCA solution (20% w/v), leaving the mixture for 30 minutes, then centrifuging at 5000 x g for 30 minutes, the protein in mixture and supernatant fraction was estimated using Kjeldahl method. Degree of hydrolysis were calculated according to following equation:

$$\text{DH \%} = \frac{10\%\ TCA\ soluble\ nitrogen\ in\ the\ sample}{Total\ nitrogen\ in\ the\ sample} \times 100$$

Solubility: The solubility of the obtained hydolusates were estimated according to method mentioned in (41). pH of 50 mg/ml hydrolysates solution were adjusted using hydrochloric acid 0.1 N and sodium hydroxide solution 0.1 N to 2, 4, 6, 8, and 10. Solutions were mixed for 30 minutes at 150 rpm, centrifuged at 8000 x g for 20 minutes, the supernatant was collected, protein was estimated using Kjeldahl method, and the solubility was calculated from following equation:

$$\text{Solubility \%} = \frac{protein\ content\ in\ the\ supernatant}{total\ protein\ content\ in\ the\ sample} \times 100$$

Activation of bacteria

Lactic acid bacteria were activated in MRS broth media prepared according to company's instructions and autoclaved at 121°C and 15 lb/in². The media with bacteria was incubated at 37°C for 72 hours. Activation was repeated three times under the same conditions.

Preparation of alternative media

Two types of culture media were prepared as an alternative to commercial MRS medium, the first medium was without yeast extract, contained 25 g of hydrolysate, while the second medium was with yeast extract, which contained 20 g of hydrolysate and 5 g of yeast extract. The commercial medium contained (Peptospecial 10g, Beef extract 10g and Yeast extract 5g), all media contained glucose, Triammonium citrate, Sodium acetate, Magnesium sulphate, Manganese sulphate, Di-potassium phosphate, agar, and Tween 80 (20, 2, 5, 0.2, 0.05, 2, 15 and 1g) respectively. Autoclaved at 121°C,15 lb/in².

Growth of lactic acid bacteria

Lactic acid bacteria were grown by pouring plates and decimal dilutions were prepared according to the method in (45), plates were incubated at 37°C for 72 h. Coefficient of efficiency were calculated according to following equation:

$$\text{Coefficient of efficiency} = \frac{N1}{N0}$$

Where: N1: the sum of colonies on alternative media, N0: the sum of colonies on MRS.

Biomass: ss production was determined according to the (36) at 24 h of growth. 25 ml of broth culture was centrifuged at 5000 xg for 20 minutes. The precipitate was separated then added 5 mL of 0.85% NaCl (w/v), centrifuged at same condition and dried at 105°C for 24 h.

RESULTS AND DISCUSSION

Color Parameter: Table (1) shows color parameter of defatted carp fish waste powder and the acidic and basic concentrates under study. The whiteness of acidic protein concentrate was higher than basic protein concentrate. Alkaline treatments contribute to dissolution of many of dyes naturally present in fish waste. Klompong et al (24) found that the protein hydrolysate prepared from yellow

stripe trevally by Flavozyme enzyme was darker than Bacto Peptone. Setijawati et al (41) found that L*, a* and b* for peptone prepared from mackerel (*Scomber japonicas*) heads were (85.11, 0.04 and 19.04), respectively.

Table 1. The color parameter of defatted carp fish waste powder, acidic and basic protein concentrates

Parameter	defatted carp fish waste powder	Acidic protein concentrate	Basic protein concentrate
Lightness (L*)	52	7.02	68
Green-red (a*)	10.3	- 1.5	- 2.5
Blue-yellow (b*)	7.2	9.7	7.3
Whiteness	50.38	68.62	67.09

Determination of amino acids

Table (2) shows the amino acid content of both acidic and basic protein concentrates prepared from defatted carp fish waste powder, the percentage of Aspartic, Glutamic, Cysteine, Proline, Hydroxyproline, Threonine, Valine, Methionine, Isoleucine, Leucine, and Phenylalanine were higher in acidic concentrate than in basic concentrate, while the percentage of Serine, Alanine, Glycine, Tyrosine, Arginine, Histidine, and Lysine were highest in basic concentration. Despite closeness of total percentages of essential amino acids in both concentrate, the content of the acidic concentrate of essential amino acids were higher, and both concentrate contained all essential amino acids except tryptophan.

Table 2. Amino acid analyses % of the acidic and basic protein concentrates using HPLC

Non-essential amino acids			Essential amino acids		
AA. %	Acidic protein concentrate	Basic protein concentrate	AA. %	Acidic protein concentrate	Basic protein concentrate
Asp	10.31	8.56	Thr	2.5	1.98
Ser	5.23	6.34	Val	4.17	3.54
Glu	12.25	10.23	Met	3.22	2.8
Ala	7.55	9.76	Ile	3.38	2.56
Gly	10.22	12.43	Leu	8.12	7.06
Cys	0.52	0.22	Phe	4.2	3.67
Tyr	2.92	3.56	His	2.87	3.24
Arg	6.55	8.34	Lys	7.27	9.65
Pro	5.22	3.46	Total	35.73	34.5
Hyp	3.5	2.6			
Total	64.27	65.5			

Jogeir et al (23) studied the amino acids in the bones of eight different types of fish, Glutamic acid and glycine were highest among rest amino acids and aromatic amino acids were lowest. Peter et al (35) found that essential amino acids in the body, head, skin, meat, and viscera of catfish was (35.46, 29.76, 22.13, 38.45, 38.13)%, respectively. Rosmawati et al (39) studying the amino acids in skin and bones of snakehead fish (*Channa striata*), the concentration of amino acids in skin was higher than that in bones, and that Glycine was higher than rest, followed by Proline, Glutamate, and Alanine, respectively. Vignesh et al (48) showed that fish farming systems have an effect on amino acid content in the powder prepared from bones and heads of Tilapia fish.

Determination of mineral content: Table (3) shows the concentration of mineral elements in acidic and basic protein concentrates prepared from defatted powdered waste of carp fish. The concentrations of phosphorus, potassium, magnesium, calcium, manganese, iron, copper, and zinc in acidic protein concentrate were (143, 80, 12, 79, 0.43, 0.30, 0.12, and 0.4) mg/100 g respectively and the basic protein concentrate contained (98.6, 65, 32, 68, 0.32, 0.37, 0.14, and 0.27) mg/100 g in the same order. The content of phosphorus, potassium, and calcium, manganese, and zinc were higher in the acidic protein concentrate than basic protein concentrate, while the concentration of magnesium, iron, and copper was higher in basic protein concentrate. Treatment with acid and alkali, centrifugation process and washing with water led to removal of a large amount of mineral elements. Chan et al (16) found when studying the mineral elements present in the fins of the hybrid grouper resulting from the

breeding of *Epinephelus lanceolatus* and *Epinephelus fuscoguttatus* that they contain potassium, magnesium, and calcium. Zinc, iron, and copper in concentrations of (1038.6, 193.2, 6881.5, 7.4, 1.8, and 0.8) mg/100 g based on dry weight.

Table 3. Concentrations of protein and minerals in the acidic and basic protein concentrates

minerals mg/100g	Acidic protein concentrate	Basic protein concentrate
P	143	98.6
K	80	65
Mg	12	32
Ca	79	68
Mn	0.43	0.32
Fe	0.30	0.37
Cu	0.12	0.14
Zn	0.34	0.27
Protein %	85.6	84.3

Degree of hydrolysis: Table (4) shows the degree of hydrolysis of acidic and basic protein concentrates prepared from defatted carp fish waste powder by pepsin enzyme. The degree of hydrolysis of acidic protein concentrate were (15.6, 33.4, 49.4, and 50.8) % after (30, 60, 90, and 120) min of reaction time respectively and the degree of hydrolysis of basic protein concentrate was (17.6, 38.2, 51.1, and 52.4) %, in same order. The degree of hydrolysis increased with enzymatic reaction progress and became close after (90 and 120) min, which indicates the end of enzymatic reaction. Enzymatic reactions are affected by many factors, such as time of hydrolysis, type and concentration of enzyme and substrate, pH, temperature, and absence or presence of inhibitory agents (11, 20, 33). Cheng et al (18) tested the ability of three enzymes Alcalase (2.4 L 2500 U/mg), Papain (10,000 U/mg), and Bromelain (1250 U/mg)in the hydrolysis of Eel fish (*Anguilla marmorata*) proteins. Alcalase enzyme gave the highest degree of hydrolysis after 8 h of enzymatic reaction time at a concentration of 1%.

Table 4. The degree of hydrolysis of the acidic and basic protein concentrate using pepsin (conc.1.5gm/100gm protein concentrates)

Time (min)	Degree of Hydrolysis %	
	Acidic protein concentrate	Basic protein concentrate
30	15.6	17.6
60	33.4	38.2
90	49.4	51.1
120	50.8	52.4

Solubility: Table (5) shows the solubility of hydrolysate prepared from acidic and basic protein concentrates by pepsin enzyme after (30, 60, and 90) min of enzymatic hydrolysis at pH (2, 4, 6, 8, and 10). It reaehed has been noticed that the solubility increased with increasing enzymatic reaction time, it was the highest value after 90 minutes (95and 94)% in acidic protein concentrate hydrolysaste at pH (2 and 10), respectively, and (96 and 95)% in basic protein concentrate hydrolysates, at pH(2, 8 and 10). The highest solubility was recorded at pH 2 and 10, and the lowest solubility was recorded at pH 4. Ramezani et al(38) showed the effect of both pH and hydrolysis time on hydrolysis of ponyfish (*Photopectoralis bindus*). Solubility of protein hydrolysates after one hour were (94, 92, 94, and 96)% and after four hours (98, 95, 98, and 99)% at pH 3, 5, 7, and 9, respectively. Alahmad et al (5) found that solubility increases with increasing degree of hydrolysis for protein hydrolysates from Bighead Carp (*Hypophthalmichthys nobilis*) by Ficin enzyme (610 MCU/mg).

Table 5. Solubility (%) of the hydrolysates prepared from acidic and basic protein concentrates by pepsin enzyme after (30, 60, and 90) minutes at deffernt pH values

Protein hydrolysates	pH				
	2	4	6	8	10
Acidic hydrolysate 30 min	87	16	80	87	93
Acidic hydrolysate 60 min	91	18	84	91	93
Acidic hydrolysate 90 min	95	20	85	93	94
Basic hydrolysate 30 min	86	13	79	86	91
Basic hydrolysate 60 min	92	16	83	93	92
Basic hydrolysate 90 min	96	19	85	95	95

Growth of lactic acid bacteria on MRS medium and alternative media: Tables (6, 7, 8, 9, and 10) show the effect of replacing organic nitrogen sources in commercial MRS medium with hydrolysates prepared from acidic and basic protein concentrates digestion by pepsin for (30, 60 and 90) minutes on the growth of *Bifidobacterium animalis*, *Bifidobacterium infantis*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus GG* and *Lactobacillus acidophilus*. Acidic hydrolysate was more effective than basic hydrolysate in the growth of *Bifidobacterium animalis* . Number of colonies increased with increasing enzymatic reaction time and with presence addition of yeast extract. Number of colonies in alternative media containing acidic hydrolysate 90 minutes (A.H.90) was (134 and 152) $\times 10^4$ cfu/mL in absence and presence of yeast extract, respectively, while number of colonies in commercial MRS medium was 134 $\times 10^4$ cfu/mL. Regarding biomass production it is also increased with increasing enzymatic reaction time and reached (520 and 490) mg/100 ml in alternative media containing (A.H.90) and basic hydrolysate 90 min (B.H.90), respectively, in absence of yeast extract, while in the presence of yeast extract the Biomass reached to (620 and 530) mg/100 ml respectively.The number of *Bifidobacterium infantis* colonies in alternative media was higher than the commercial media, and the efficiency factor reached (1.231 and 1.246) in alternative media containing A.H.90 and B.H.90, respectively, in absence of yeast extract, and it increased to (1.306 and 1.266) in the presence of yeast extract. Highest biomass production was recorded in alternative media containing B.H.90 in presence of yeast extract, which was 863 mg/100 mL. Number of *Lactobacillus plantarum* colonies in alternative media was higher than commercial media in the presence and absence of yeast extract. The efficiency coefficients were (1.088, 1.235, 1.302, 1.057, 1.253, and 1.275) in alternative media containing acidic hydrolysate 30 min(A.H.30), acidic hydrolysate 60 min (A.H.60), A.H.90, basic hydrolysate 30 min(B.H.30), basic hydrolysate 60 min (B.H.60) and B.H. 90 respectively in absence of yeast extract and (1.080, 1.257, 1.324, 1.084, 1.262 and 1.293) after adding yeast extract. Biomass production in alternative media was higher than commercial media, and the highest biomass production was reached in media containing A.H.90 in presence of yeast extract at 642 mg/100 ml. Number of *Lactobacillus rhamnosus GG* colonies in alternative media containing A.H.30, A.H.60, A.H.90, B.H.30, B.H.60 and B.H.90 were (132, 138, 142, 128, 132, and 138) $\times 10^6$ cfu/mL, respectively, in absence of yeast extract and increased to (158, 160, 166, 151, 159, 168) $\times 10^6$ cfu/mL in same order after adding yeast extract. Biomass production in alternative media containing A.H.90 reached (380 and 395) mg/100 mL in absence and presence of yeast extract, while biomass production in commercial media was 358 mg/100 mL. The addition of yeast extract had effect on number of colonies of *Lactobacillus acidophilus*, number of colonies in alternative media containing acidic and basic hydrolysates with and without of yeast extract were less than commercial medium. Biomass production in all alternative media was lower than commercial media. Lactic acid bacteria needs amino acids to meet its need for amino nitrogen, which can be obtained from proteolysis. Proteolysis systems consist of proteinases associated with cell wall, which are responsible for digesting proteins outside the cell and converting them into oligopeptides, and peptide, which transport into the cell, to reduce the amount of energy needed to absorb amino acids, intracellular peptidases are responsible for converting peptides into short peptides and free amino acids. Plasmids encode for these systems, Therefore, there is variation between different strains depending on the presence or absence of plasmids that encode them (37). Related studies showed that size of genome has an impact on the amino acid needs of microorganisms. *L. plantarum*, which has a large genome size, needs only three amino acids and therefore requires a simpler environment from a nutritional standpoint, compared to *L. acidophilus* bacteria, which needs 14 amino acids. There is a relationship between the size of the genome of microorganisms and their enzymatic systems, which makes it more adapted to simple culture media (14). Marie et al (27) found that

Bifidobacterium animalis is able to synthesize all protein amino acids except cysteine, because it is unable to absorb inorganic sulfur, and methionine cannot compensate cysteine absence, it can synthesize methionine from cysteine through direct conversion to homocysteine and then to methionine, but the opposite is not true. *Lactobacillus acidophilus* bacteria do not only need amino acids, they have many requirements, and they need many growth factors that were not sufficient for their growth, as Chen et al (17) showed that different sources of nitrogen have an impact on the growth of *Lactobacillus acidophilus*, the organic nitrogen sources was higher in growth than inorganic nitrogen sources because it contains free amino acids, peptides, glycosides, lipids, and growth factors, it was found that complex nitrogen sources prepared from more than one type of peptone gave higher growth rates than yeast extract alone. Meng et al (29) stated in studying the growth requirements of *Lactobacillus acidophilus LA-5* that the Aspartic and Asparagine were the most consumed amino acids (0.94 mmol/L) followed by Glutamic and Glutamine (0.69 mmol/L), Alanine (0.55 mmol/L), Leucine (0.48 mmol/L) and the least consumed amino acid was Phenylalanine at 0.03 mmol/L. while the rest of the amino acids ranged between 0.06 - 0.44 mmol/L. *L. acidophilus* bacteria lack cytochromes, porphyrins and respiratory enzymes, and as a result they are unable to produce energy by oxidative phosphorylation. Since they use sugars such as glucose, esculin, cellobiose, galactose, lactose, maltose, salicin and sucrose as substrates for fermentation, the energy production is two ATP. Hence, these bacteria must metabolize large amounts of substrate to generate sufficient energy, so their generation time is longer than the rest of the species of lactic acid bacteria, and the absence of one of their nutritional requirements will significantly affect the numbers of growing cells (2, 6, 7).

Table 6. Effect of replacing N sources in MRS medium by acidic and basic hydrolysates derived from carp fish waste on *Bifidobacterium animalis* growth

Cultural medium	With out yeast extract			With yeast extract		
	cfu/ml x 10^4	Coefficient of efficiency	Biomass mg/100ml	Cfu/ml x 10^4	Coefficient of efficiency	Biomass mg/100ml
Acidic hydrolysate 30 min	53	0.395	420	63	0.470	455
Acidic hydrolysate 60 min	92	0.686	480	107	0.798	494
Acidic hydrolysate 90 min	134	1	520	152	1.134	620
Basic hydrolysate 30 min	49	0.365	320	62	0.462	410
Basic hydrolysate 60 min	85	0.634	405	94	0.701	487
Basic hydrolysate 90 min	125	0.932	490	132	0.985	530
MRS	134	1	652			

Table 7. Effect of replacing N sources in MRS medium by acidic and basic hydrolysates derived from carp fish waste on *Bifidobacterium infantis* growth

Cultural medium	With out yeast extract			With yeast extract		
	cfu/ml x 10^8	Coefficient of efficiency	Biomass mg/100ml	Cfu/ml x 10^8	Coefficient of efficiency	Biomass mg/100ml
Acidic hydrolysate 30 min	218	1.095	592	225	1.130	675
Acidic hydrolysate 60 min	232	1.165	620	253	1.271	807
Acidic hydrolysate 90 min	245	1.231	640	260	1.306	840
Basic hydrolysate 30 min	210	1.055	490	223	1.120	685
Basic hydrolysate 60 min	230	1.155	620	242	1.216	768
Basic hydrolysate 90 min	248	1.246	652	252	1.266	863
MRS	199	1	689			

Table 8. Effect of replacing N sources in MRS medium by acidic and basic hydrolysates derived from carp fish waste on *Lactobacillus plantarum* growth

Cultural medium	With out yeast extract			With yeast extract		
	cfu/ml x 10^8	Coefficient of efficiency	Biomass mg/100ml	Cfu/ml x 10^8	Coefficient of efficiency	Biomass mg/100ml
Acidic hydrolysate 30 min	245	1.088	552	243	1.080	564
Acidic hydrolysate 60 min	278	1.235	584	283	1.257	597
Acidic hydrolysate 90 min	293	1.302	620	298	1.324	642
Basic hydrolysate 30 min	238	1.057	530	244	1.084	548
Basic hydrolysate 60 min	282	1.253	550	284	1.262	567
Basic hydrolysate 90 min	287	1.275	590	291	1.293	615
MRS	225	1	520			

Table 9. Effect of replacing N sources in MRS medium by acidic and basic hydrolysates derived from carp fish waste on *Lactobacillus rhamnosus GG* growth

Cultural medium	With out yeast extract			With yeast extract		
	cfu/ml x 10^6	Coefficient of efficiency	Biomass mg/100ml	Cfu/ml x 10^6	Coefficient of efficiency	Biomass mg/100ml
Acidic hydrolysate 30 min	132	0.391	355	158	1.067	365
Acidic hydrolysate 60 min	138	0.932	374	160	1.081	380
Acidic hydrolysate 90 min	142	0.959	380	166	1.121	395
Basic hydrolysate 30 min	128	0.864	315	151	1.020	330
Basic hydrolysate 60 min	132	0.891	342	159	1.074	354
Basic hydrolysate 90 min	138	0.932	380	168	1.135	374
MRS	148	1	358			

Table 10. Effect of replacing N sources in MRS medium by acidic and basic hydrolysates derived from carp fish waste on *Lactobacillus acidophilus* growth

Cultural medium	With out yeast extract			With yeast extract		
	cfu/ml x 10^5	Coefficient of efficiency	Biomass mg/100ml	Cfu/ml x 10^5	Coefficient of efficiency	Biomass mg/100ml
Acidic hydrolysate 30 min	120	0.413	240	192	0.662	412
Acidic hydrolysate 30 min	175	0.603	290	210	0.724	450
Acidic hydrolysate 30 min	210	0.724	420	260	0.896	520
Basic hydrolysate 30 min	150	0.517	265	204	0.703	411
Basic hydrolysate 30 min	180	0.620	350	230	0.793	480
Basic hydrolysate 30 min	230	0.793	390	278	0.958	508
MRS	290	1	540			

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