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Using *Locusta migratoria* as a Nitrogen Source for the Growth and Development of Microorganisms

Perihan AKBAŞ*[1], Esabi Başaran KURBANOĞLU[2]

Abstract

Characteristics and the use as culture media of protein hydrolysate from *Locusta migratoria* were determined in comparison with different peptones. After powdering, it was hydrolyzed chemically (acid hydrolysis) and obtained product Locust Peptone (LP). The contents of nitrogen, protein, fat, ash, total sugars, minerals and amino acids of LP were determined and it was seen that it has both organic and inorganic materials enough to use as a component of the medium. The effects of different concentrations added 20g/l glucose of LP on the growth of four test bacteria (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas putida* and *Escherichia coli*) and test yeasts (*Rhodotorula glutinis, Candida albicans, Saccharomyces cerevisiae*) were investigated and it was found that the optimal concentration for bacteria and yeast are %0,6 and LP was compared with bacteriological peptone (BP), fish peptone (FP) and meat peptone (MP) .The obtained results with surface streaking and shaking culture procedures showed that LP yielded a little higher or equal FP and BP in both normal bacteria, but these values were lower than values obtained from MP. The results show that LP performed similar to or even better than commercial peptones as nitrogen sources for microorganisms growth. A new peptone has been developed from locust for microbiological media in the present study.

Keywords: Culture media, *Locusta migratoria*, Locust peptone, nitrogen source, peptone, protein hydrolysate

1. INTRODUCTION

A large number of microorganisms require organic nitrogen sources, which are incorporated into the commercial culture medium in the form of peptones, or protein hydrolysates, in varying degrees of hydrolysis [1-3]. There are many articles comparing various media to support the greatest number of bacterial growth from environmental samples [4].

Peptones are defined as protein hydrolysates that cannot be precipitated with heat, alkalis or ammonium sulfate, which are readily soluble in water. It is one of the important components of the bacterial culture medium. The biological properties of each peptone are different and no

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component can meet the needs of all microorganisms or cells in the culture medium. Many different sources of animal and plant origin are used for the production of valuable and expensive peptones [4-7].

Most part of microorganisms can not use proteins as a source of nitrogen. Therefore, nitrogen compounds with proteins are needed to convert more useful protein hydrolysate. For this reason peptones are obtained by breaking up with peptide bond of protein macromolecules consist of the chain of amino acids, either enzymatic way (pepsin, trypsin, papain ie.) or chemical hydrolysis using acid, alkali. [8, 9]. Peptone can be used as a medium without additives. Many bacteria can grow media having very low peptone rate, such as 1% [10-12]. Peptones have many macroelements and microelements, so it is used source of mineral nutrients [9, 10].

The Migratory Locust is a large insect, the Migratory Locust is mainly graminivorous cover near the ground occupying the grass. Sea banks, lake, river and with plantings of sedges and reeds. *Locusta migratoria* continue to occur on all continents except Antarctica, Solitary hoppers and adults can damage rice, cotton, various vegetable crops as well as plantations of volatile oil bearing plants in Tajikistan. During years of mass increases/outbreaks, crops are severely damaged as well as hayfields and pastures [13].

Locusta migratoria is a locust both cultured and sold as pet food in Turkey. In this study a peptone prepared from Migratory Locust by acid hydrolysis and it has been tested for the enumeration of aerobic bacteria and compared with standard peptones. Locust peptone (LP) has not been extensively investigated as a source of bacterial nitrogen not widely used for microbial production studies.

2. MATERIALS AND METHODS

The chemicals, culture media and test peptones employed in the present study were analytical grades and purchased from Oxoid (Basingstoke, UK), Merck (Darmstadt, Germany) and Difco (Detroit, MI, USA).

Locusta migratoria were bought from MIRA farm alive animal and insect Agricultural Tourism Company Antalya in Turkey. Bacteria: *Escherichia coli, Bacillus subtilis, Pseudomonas putida* and *Staphylococcus aureus*, yeast; *Rhodotorula glutinis, Candida albicans, Saccharomyces cerevisiae* obtained from Atatürk University Science Faculty Biology Department Microbiology Laboratory collection.

2.1. 1. Production of LP

Locusts were stored -20 degree in freezer and later dried in an furnace at 105 ºC. Dried locusts were cut into small pieces and then ground with a blender (Waring Products Corp., New York City, NY, USA). This material was termed as locust flour (LF). For the preparation of Locust Peptone were used an acid hydrolize method [10, 14, 15] and the production scheme of LP is shown in Figure 1.

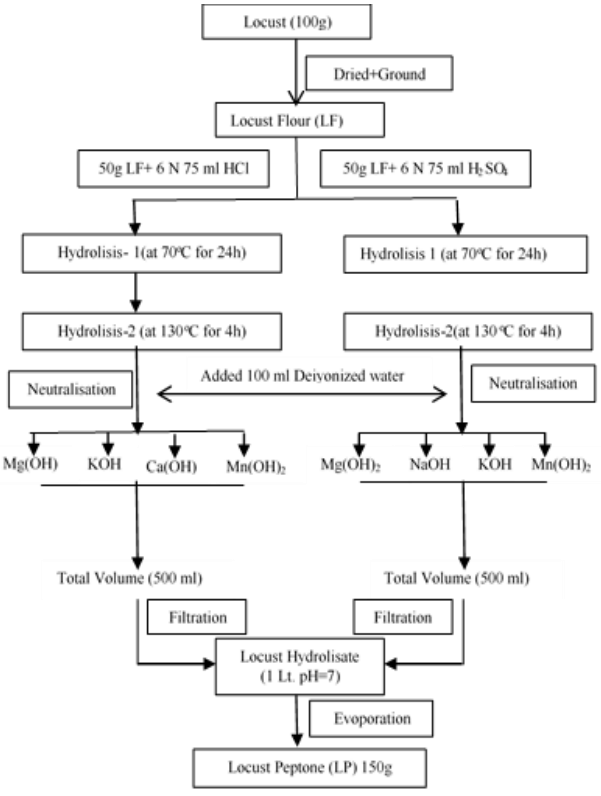

Figure 1 Production scheme of locust peptone

Analysis of LP; Dry matter and ash contents of the peptones were determined according to AOAC methods [16]. Amino acid (HPLC method) and total protein (Kjeldahl method) were estimated in

Duzen Norwest laboratory (Ankara, Turkey). Total sugar contents of LP were determined according to the anthrone method using glucose as a standard [17]. Fat analyses of the peptones were performed by Soxhlet extraction method, Ministry of Agriculture and Rural Affairs Directorate of Erzurum Provincial Control Laboratory [6, 18, 19].

2.2. Determination optimal concentration of peptone and Comparison with other peptones

Broth and agar medium prepared containing different concentrations of LP (%0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 ve 1.0), %2 glucose and pure water and pH adjusted to 7. Test bacteria were incubated in Nutrient broth (NB) at 30 ^{o}C for 48 h and yeasts were incubated in Sabouraud Dextrose Broth (SDB) at 24 ^{o}C for 48 h 200 rev in the min. a shaking incubator (ROSI 1000 Thermolyne), the optical density (600 nm) of the cultures was spectrophotometrically adjusted to 2. 0, 1 ml of each culture was inoculated into each concentration of LP liquid medium. Growths of these microorganisms were investigated by two techniques, dry matter measurements and optical density. For agar culture studies, the inoculation cultures were diluted and 1ml of each dilution inoculated in each concentration of LP. After incubation broth cultures measured by the spectrophotometer and centrifuged at 5000 rpm 20 min and after twice washed distilled water, dried at 60 °C till they came to constant weight. Colonies counted on agar plates [10, 20]. LP was compared with three commercial peptones (fish peptone, bacteriological peptone and meat peptone) at the optimal concentration on agar and broth cultures.

2.3. Statistical analysis

The experiments for all chemical, physical, and microbiological analyses, the determinations were run in triplicate. Data were subjected to analysis of variance (ANOVA). Differences among means were tested for significance ($p < 0.05$). Statistical analysis was carried out using SPSS statistic program (Version 15.0) for Windows [21].

3. RESULTS

Locusta migratoria tested for its quality of ash, protein, fat, and element by Hannover Veterinary Foundation School. It is showed that Table 1. *Locusta migratoria* is an appropriate choice for peptone production.

Table 1 Some characteristics of *Loocusta miratoria*

Components	Original matter g/kg	Dry mater g/kg
Ash	15,2	40,4
Protein	230	612
Fat	0,30	0,92
Calsiyum (Ca)	1,23	3,27
Phosphor (P)	2,52	6,7
Magnesium (Mg)	0,47	1,25
Sodium (Na)	0,61	1,62
Potassium (K)	3,67	9,76
Clor(Cl)	1,81	4,81
Copper(Cu)	127	33,8
Zinco(Zn)	509	135
Manganese(Mn)	5,05	13,4
Iron (Fe)	39,3	105
Selenium (Se)	0,07	19

These data show that LP (Locust Peptone) is rich in organic materials. As an expected result, LP does have no methionine and threonine amino acids, because of the high-temperature application during acid hydrolisation process.

Table 2 Amino acid composition of LP.

Parameter	LP Result (g/100 g)	Parameter	LP Result (g/100 g)
Aspartic acid	2,220	Valine	2,571
Glutamic acid	3,742	Methionine	‹0,001
Asparagin	0,361	Tryptophane	0,192
Serine	0,590	Phenylalanine	1,234
Hystidine	0,686	Isoleucine	1,970
Glycine	2,123	Ornithine	1,031
Theronin	‹0,001	Leucine	3,227
Citruline	0,111	Lysine	1,796
Arginine	0,850	Hydroxyproline	1,974
Alanine	4,483	Sarcosine	1,015
Tyrosine	1,180	Proline	2,819
Cystine	0,198	Total aminoacids	33,10

According to Table 2. LP is rich in amino acid content. The essential amino acids are included, among them alanine (4.483 g/100 g) and glutamic acid (3.742 g/100 g) is the highest. Acid hydrolysis is very effective, but since the final

product contains a high amount of acid, the neutralization step is needed [10]. At the same time acidic conditions cause some amino acids to disappear such as tryptophan, basic hydrolysis does not lose amino acids, but the process is slow and sometimes cannot complete [22].

The effect of different concentrations of LP on the number of colonies of test bacteria and yeast growing on agar medium showed Table 3.

The effect of different concentrations of LP on the biomass yield of test bacteria and yeast growing in broth medium showed Table 4.

LP in different concentrations was tested for optimum microorganism production (Table 3, Table 4 According to the results obtained, the optimum LP concentration for bacterial and yeast reprocessing was determined to be 6 g/l, and comparisons between other standard peptones sold commercially and LP were made at this concentration. The results for bacterial growth with different peptones were given in Table 5.

Table 3 The effect of different concentrations of LP on the number of colonies of test bacteria and yeast growing on agar medium

	LP Concentration (g / l) / Number of Colonies (CFU / ml)									
Test Microorganisms	1	2	3	4	5	6	7	8	9	10
Bacteria(3 days 30ºC)										
E. coli	84 e	94 de	115 d	126 c	142 b	158 a	135 b	124 c	84 e	52 f
B. subtilis	31 f	51 e	102 c	115 c	123 bc	142 a	131 b	128 bc	76 d	68 d
P. putida	72 ef	78 e	89 d	105 c	112 b	128 a	104 b	86 de	84 de	36 g
S. aureus	35 g	38 g	49 f	72 e	110 c	146 a	128 b	96 d	84 de	51 f
Yeast (3 days 30ºC)										
S. cerevisieae	15 h	34 f	48 de	50 bc	56 b	64 a	52 bc	42 ef	38 ef	26 g
C. albicans	56 c	57 c	61 c	72 b	78 ab	85 a	77 b	75 b	59 c	53 c
S. boulardii	38 e	42 de	49 c	54 bc	58 ab	63 a	54 bc	51 bc	47 cd	36 e
R. glutinis	33 e	38 e	48 cd	52 cd	58 b	57 a	57 bc	51 cd	46 d	33 e

The difference between the values indicated by the same letters in the same lines is insignificant at $p<0.05$.

Table 4 The effect of different concentrations of LP on the biomass yield of test bacteria and yeast growing in broth medium

Test	LP Concentration (g / l) / Biomass (g / l)									
Microorganisms	1	2	3	4	5	6	7	8	9	10
Bacteria										
E. coli	1,25 g	1,61 f	2,06 e	2,13 de	2,25 bc	2,53 a	2,36 b	2,3 b	2,26 bc	1,8 g
B. subtilis	1,62 c	1,98 b	2,08 b	2,14 b	2,22 b	2,42 a	2,26 b	2,19 b	1,61 c	1,431 c
P. putida	0,9 e	1,06 d	1,23 cd	1,32 bc	1,34 b	1,57 a	1,44 ab	1,33 bc	1,18 cd	1,09 d
S. aureus	1,33 de	1,86 d	2,18 c	2,24 c	2,35 bc	2,64 a	2,47 b	2,31 bc	2,09 c	1,42 de
Yeast										
S. cerevisieae	1,13 e	1,4 d	1,65 c	1,91 bc	2,03 b	2,13 a	2,01 b	1,82 bc	1,66 c	1,16 e
C. albicans	1,44 f	2,14 e	2,86 d	3,64 c	3,82 c	4,38 a	4,12 b	3,63 c	2,94 d	2,36 e
S. boulardii	1,28 g	1,87 f	2,16 de	2,29 bc	2,40 b	2,68 a	2,24 bc	2,12 de	2,05 e	1,72 f
R. glutinis	1,89 e	2,09 d	2,20 cd	2,26 cd	2,44 b	2,62 a	2,43 b	2,37 bc	2,24 cd	2,22 cd

The difference between the values indicated by the same letters in the same lines is insignificant at $p<0.05$.

Table 5 The effect of the test bacteria on the biomass yields and colony counts of the cell and other test peptones developed in the liquid culture

Test Bacteria	Test Peptone (6 g/l)	Max. Biomass (g/l)	Max. Absorbace (600nm)	Colony Number (CFU/ml) (10^8)
B. subtilis	LP	2,46 a	6,05	142 ab
	FP	2,36 b	5,31	125 c
	MP	2,48 a	6,12	154 a
	BP	1,78 c	4,42	117 d
E. coli	LP	2,53 a	6,32	158 b
	FP	2,43 b	6,51	115 d
	MP	2,49 ab	6,11	169 a
	BP	2,37 b	6,42	130 c
S. aureus	LP	2,64 a	6,62	146 ab
	FP	2,10 b	5,21	120 c
	MP	2,72 a	6,84	164 a
	BP	2,19 b	5,42	121 c
P. putida	LP	1,46 a	3,67	130 a
	FP	1,32 b	3,40	132 a
	MP	1,41 ab	3,24	134 a
	BP	1,50 a	3,87	111 b

The difference between the values indicated by the same letters between the same columns is insignificant at $p<0.05$.

LP: Locust Peptone, FP: Fish Peptone, MP: Meat Peptone, BP: Bacteriologic Peptone.

The results for yeast growth were shown in Table 6.

Table 6 The effect of on the biomass yield of test bacteria and yeast growing in broth medium and other test peptones developed in the liquid culture.

Test Yeast	Test Peptone (6 g/l)	Max. Biomass (g/l)	Max. Abs. (600nm)	Colony Number (10^8)
S. cerevisiae	LP	2,13 b	5,36	64 b
	FP	2,04 bc	5,68	56 c
	MP	2,19 b	6,02	62 b
	BP	2,35 a	5,42	75 a
C. albicans	LP	4,38 b	10,03	85 a
	FP	4,13 c	11,52	78 ab
	MP	3,60 d	6,71	66 c
	BP	4,47 a	7,84	86 a
R. glutinis	LP	2,64 ab	6,75	68 b
	FP	2,33 d	5,94	57 c
	MP	2,55 c	6,43	61 bc
	BP	2,76 a	7,02	79 a

The difference between the values indicated by the same letters between the same columns is insignificant at $p<0.05$.

LP: Locust Peptone, FP: Fish Peptone, MP: Meat Peptone, BP: Bacteriologic Peptone.

The difference between the values indicated by the same letters between the same columns is insignificant at $p<0.05$.

According to tables 5 and 6, it is seen that the locust peptone has the potential to produce equivalent to bacteriological peptone and fish peptone.

4. DISCUSSION

Causes cause large-scale damage in agricultural areas with the reason that they are crowded. Grasshopper invasion is a difficult situation to avoid. There are also records at the end of summer that adult locusts have died in flocks and dead locusts have caused diseases [13]. In this case the dead locust can be regarded as a waste. Up to now for peptone production purposes has been benefited from different animal and plant sources or wastes [8, 23-25]. In this study, the use of peptone for microorganisms in the locus was investigated. In our work peptone has been produced from a locust and has been reported to be suitable for bacteria and yeast as a substrate for the produced peptone.

The media prepared with waste peptones and standard peptones are enriched with different carbon sources, especially glucose [6, 10, 20]. Addition of 20 g glucose per liter in addition to the standard nitrogen source on a medium resulted in a 67% increase in the amount of biomass [26, 27]. For this reason, in the medium 20 g of glucose, which is preferred primarily by most microorganisms as carbon source, is added.

All of the test yeasts were reproducible on all media. The highest biomass yield in LP was shown by C. albicans. This can be explained as the optimum level of carbon, nitrogen or minerals required for the development of a causative human pathogen, C. albicans. All the yeasts were best produced in BP after this LP. The number of colonies for the LP test bacteria is biodegradable and is suitable for use as a nutrient. Especially biomass yield of S. aureus was found to be bigger than other test peptones in LP. The maximum

number of colonies was E. coli while the lowest number of colonies was P. putida. Considering all these data, it is considered that LP is a suitable nutrient in terms of rich mineral content and nitrogen amount.

The presence of minerals, vitamins, amino acids, and amount of them in each peptone is a factor that affects the reproduction of microorganisms. The findings of this study show that when the selected raw materials were converted into hydrolyzed products, they were high yielding. In addition, all selected raw materials could be converted into peptones, which can strongly promote bacterial growth. This study showed that LP is suitable for microbiological peptone production when appropriate hydrolysis methods are used due to its high mineral and amino acid content. As a result, the use of these raw materials is an inexpensive and effective way to obtain a nitrogen source for microbial growth.

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The Declaration of Conflict of Interest/ Common Interest

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Authors' Contribution

This publication was produced from Perihan Akbaş's doctoral thesis. The authors contributed equally to the study.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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