UTILIZATION OF BEEF HEART FOR THE OBTAINMENT OF PEPTONE FOR BACTERIOLOGY

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SUMMARY: Minced beef heart - a residual from the beef heart infusion's production was used as protein substrate for the obtainment of bacteriological peptone by its hydrolysis with a papainic preparate. The peptone has satisfactory growth promoting characteristics, an addecuate composition (humidity - 3.13%. amino nitrogen - 2.45%, total nitrogen - 13.58%, amino/total nitrogen relation - 18%, chlorides - 3.6%, pH - 5.5) and a characteristic for this kind of product buffering capacity (for acids - 7.14, for alkalis - 9.16).

INTRODUCTION: Beef heart have been often used in microbiology for the preparation of beef heart infusion (Hottinguer, 1913; Organotechnie, 1984) and seldom used as protein substrate for the peptone's obtainment (Berger et al., 1987; Camacho, 1985). According to the existing methods raw beef heart is minced and hydrolyzed with pancreatic enzymes at 42-47 cent. deg. The hydrolysate is used in fermentation media and media for growing several specimens of microrganism, In Cuba, a signifficant amount of minced boiled beef heart resulted from the industrial production of beef heart infusion is available and recently was decided to develop a new product for increasing the production of papainic hydrolyzates from this by-product. For these reasons we considered appropiate to do a serie of experiments with the porpuse of recovering this by-product and employing it as protein substrate for the obtainment of bacteriological peptone.

MATERIALS AND METHODS: As was described, the minced and boiled beef heart was summited to hydrolysis in order to obtain the correspondent hydrolysate. Papain was used as hydrolytic agent (60 000 U.). It was applied a statisticall fully randomized design model 3x2 for the development of the experiences. Two independent variables were choosed: temperature (70, 75 and 80 c degree) (X1) and pH (5, 6.5 and 8) (X2); and as dependent variables were calculated the amino nitrogen (Na) and total nitrogen (Nt) content,their relation (Na/Nt) and dry weight (Dw). The duration of the hydrolsys' process was varied from 1 to 4 hour and depend variables were calculated at every hour. Coefficients of the polynomes were founded according to a matricce system and their signifficance for $p<0.05$ by a Student test. Fisher test was applied to determinate the addecuation of the model. A Duncan test for $p<0.05$ was applied for the variance analysis in cases when media differed. Characteristic regression equations were calculated for the Na content though the time

(López, 1988). Polynomes and statistical analysismof the results and regression equations were calculated with the "MICROSTAT" statistical software in an IBM-PC fully compatible microcomputer in MC-DOS system.

The following methods were employed for the determination of the composition and characteristics of the products: amino nitrogen - as described by Chechetkin (Chechetkin et al., 1984); total nitrogen - with the KJELTEC SYSTEM (Tecator, 1987); humidity or dry weight as well as chlorides as described by Matrozova (Matrozova, 1977); pH - with a digital PHM 83 pH-meter from RADIOMETER.

Biological assays comprised: the evaluation of the biological reactivity (Acethyl-Methyl-Carbinol, Indole and sulphidric gas production and carbohydrate fermentation) with strains from the American Type Culture Collection (ATCC) (Escherichia coli 25922, Streptococcus faecalis 19433, Enterobacter aerogenes 13048 and Salmonella thyphibismuth 1454 from "GISK Tarasievich Type Culture Collection" (TTCC) (Sonnerwirth et al., 1983); the evaluation of the growth of different strains of microorganism from the ATCC (Salmonella typhimurium 14028, E. aerogenes 13048, E. coli 25922, Klebsiella pneumoniae 13883 and Proteus vulgaris 13315 in different culture media (Urea Agar Base and Heart Broth Medium). Control media from OXOID ltd. were also tested for the best analysis of the results. Media were prepared as described by OXOID (OXOID, 1982) as well as microbiological evaluation was carried out as recomended by this firm.

RESULTS AND DISCUSIONS: As it was expected the larger the time of hydrolysis the higher the amino nitrogen content of the hydrolysate (Table 1). Between all the experiences, exp. No 8, 7 and 2 showed the greater Na content, but the first two of them had a low content of solids (Dw = 3.5 and 5 respectively). For the final product (Table 2) experiences No 1 and 4 had the highest Na content. The Nt content in all experiences except No 3 and 9 was the same or closely one. The best results for the Na/Nt relation were obtained for experiences No 1 and 4. In spite of the fact that the highest values for the tested parameters were obtained for other experiences different from No 2, it was considerated that the Na, Nt, Dw content and Na/Nt relation values were satissfactory for this experience.

It can be taken into account that these values are characteristics for commercial preparates, moreover, for this experience the temperature value is the least (70 c degree) and its means that at industrial scale the energy consumption would be lesser than for other experiences. By the other hand the pH value of the boiled minced beef heart is closely to 6.5 and the employment of alkalis or acids for the pH adjustment practically is not necessary and this fact allows the obtainment of a product with a low salt content. By all these reasons the fallowing parameters were selected for the industrial scale production of this peptone: pH - 6.5; temperature - 70 c degree.

Table 1.- Evaluation of the amino nitrogen content during the hydrolytic process

Exp. No.	Na, % x h					Std. err.	DW, %
	0	1	2	3	4		
1	0.013 a	0.051 f	0.072 e	0.088 g	0.110 d	0.00039	3.0
2	0.016 a	0.086 c	0.125 a	0.167 b	0.180 b	0.00273	8.1
3	0.016 a	0.100 b	0.129 a	0.132 d	0.154 b	0.00175	7.7
4	0.016 a	0.059 e	0.079 d	0.100 f	0.115 d	0.00080	3.2
5	0.016 a	0.085 c	0.125 a	0.143 c	0.155 b	0.00094	5.1
6	0.017 a	0.087 c	0.111 c	0.118 e	0.144 bc	0.02120	4.5
7	0.016 a	0.071 cd	0.116 b	0.144 c	0.196 b	0.02550	2.2
8	0.015 a	0.132 a	0.139 a	0.163 b	0.175 b	0.00318	7.7
9	0.016 a	0.101 b	0.151 a	0.187 a	0.233 a	0.00054	3.5
Std. err.	0.00058	0.00067	0.00187	0.00158	0.00457		

Regression equations. Dependence of the Na content from the studied parameters:

$$Na(1\ th\ h)\ Y=0.091+0.017X_1+0.010X_2-0.022X_1^2+0.014X_2^2-0.003X_1X_2$$

$$Na(2\ nd\ h)\ Y=0.118+0.021X_1+0.013X_2-0.020X_1^2+0.017X_2^2-0.005X_1X_2$$

$$Na(3\ th\ h)\ Y=0.140+0.017X_1+0.018X_2-0.030X_1^2+0.027X_2^2$$

$$Na(4\ th\ h)\ Y=0.157+0.035X_1+0.043X_2-0.028X_1^2+0.020X_2^2-0.027X_1X_2$$

Regression equations. Dependence of the Na content from the time of hydrolysis:

exp. No

1 Na= 0.032 + 0.019*t

2 Na= 0.059 + 0.032*t

3 Na= 0.086 + 0.017*t

4 Na= 0.041 + 0.019*t

5 Na= 0.070 + 0.023*t

6 Na= 0.071 + 0.018*t

7 Na= 0.031 + 0.040*t

8 Na= 0.114 + 0.015*t

9 Na= 0.060 + 0.043*t

Table 2.- Composition of the peptone obtained at laboratory scale

Exp. No.	Composition, %			
	Nt *	Na *	Na/Nt *	Dw
1	12.96 ab	3.07 a	23.88 a	3.30 g
2	13.01 ab	2.37 c	18.33 c	8.42 b
3	10.80 d	1.52 f	14.07 c	10.00 a
4	13.32 a	3.12 a	23.42 a	3.35 g
5	12.95 ab	2.33 d	17.99 c	5.69 d
6	12.69 ab	1.78 e	14.03 e	4.79 e
7	13.06 a	2.77 b	21.21 b	2.40 h
8	13.26 a	2.33 d	17.60 cd	8.00 c
9	12.16 c	1.60 e	13.19 e	3.75 f
Std. err.	0.0936	0.0156	0.1578	0.0008

* - values recalculated to 95% Dw.

Polynomes' equations

$$Nt: \quad Y = 13.37 - 0.61X_1$$

$$Na: \quad Y = 2.43 - 0.68X_1 - 0.13X_2 + 0.10X_1X_2$$

$$Na/Nt: \quad Y = 18.21 - 4.44X_1$$

$$Dw: \quad Y = 6.46 + 1.58X_1 - 1.26X_2 - 2.77X_1^2 + 1.37X_2^2 - 1.34X_1X_2$$

At industrial scale it was defined the following method for the peptone production: conformation of the boiled and minced beef heart suspension- pH and temperature adjustment- addition of the enzyme preparate- pH and temperature control through the hydrolytic process- pH adjustment and boiling of the system - separation of the residual- concentration- filtration- spray drying- pH adjustment.

It were produced 3 industrial experimental batches of the product wich composition (mean values) and physico-chemical characteristics resultad as follow: humidity - 3.13%, amino nitrogen - 2.45%, total nitrogen - 13.58%, amino/total nitrogen relation - 18%, chlorides - 3.6%, buffering capacity for acids - 7.14, for alkalis - 9.16, pH (2% water solution after sterilization) - 5.5, colour of the powder - light cream, colour of the 2% water solution - light yellow.

The biological reactivity evaluation of each of the 3 industrial batches of the experimentals and control media showed characteristic reaction for all the tested strains of microorganism (Table 3). The microbiological evaluation in Urea Agar Base medium (Table 4) showed characteristic reactions for all the tested microorganisms.

Table 3.- Biological reactivity of the experimental industrial batches of bacteriological peptone

Test	Microorganism			
	E. coli	S. faecalis	E. aerogenes	S. typhi-bismuth
Acethyl-Methyl-Carbinol	-		+	
Indole	+		-	
H_2S				-
Carbohydrate fermentation	-	-		

Table 4.- Evaluation of the experimental and control peptones in Urea Agar Base medium (n+: urea hydrolysis, n-: no hydrolysis)

Media: Dilution	Microorganism				
	P. vulgaris	K. pneumoniae	S. typhimurium	E. aerogenes	E. coli
OXOID:					
10^{0}	3 +	3 +	3 -	3 -	3 -
10^{-1}	3 +	3 +	3 -	3 -	3 -
10^{-2}	3 +	3 +	3 -	3 -	3 -
10^{-3}	3 +	2 +	3 -	3 -	3 -
10^{-4}	3 +	3 -	3 -	3 -	3 -
Experimental No 1:					
10^{0}	3 +	3 +	3 -	3 -	3 -
10^{-1}	3 +	3 +	3 -	3 -	3 -
10^{-2}	3 +	3 +	3 -	3 -	3 -
10^{-3}	3 +	3 +	3 -	3 -	3 -
10^{-4}	3 +	3 -	3 -	3 -	3 -
Experimental No 2					
10^{0}	3 +	3 +	3 -	3 -	3 -
10^{-1}	3 +	3 +	3 -	3 -	3 -
10^{-2}	3 +	3 +	3 -	3 -	3 -
10^{-3}	3 +	3 +	3 -	3 -	3 -
10^{-4}	3 +	3 -	3 -	3 -	3 -
Experimental No 1:					
10^{0}	3 +	3 +	3 -	3 -	3 -
10^{-1}	3 +	3 +	3 -	3 -	3 -
10^{-2}	3 +	3 +	3 -	3 -	3 -
10^{-3}	3 +	3 +	3 -	3 -	3 -
10^{-4}	3 +	3 -	3 -	3 -	3 -

These results allowed to observe similar urea degrading reactions for P. vulgaris and K. pneumoniae in both, experimental and OXOID's media at different dilutions (from 10 to 10(-4) after incubation at 37 c degree. The other microorganisms as it was expected showed a no ureal hydrolytic characteristics. In different capital's hospitals the experimental products were tested in Heart Broth Medium with satissfactory results for growing different wild strains of microorganisms isolated from patients.

CONCLUTIONS: It was developed a method for obtaining bacteriological peptone by using the minced and boiled beef heart as protein substrate and carrying out the hydrolysis at pH 6.5 and temperature 70 c degree. At industrial scale three experimental batches of the product were produced with an addecuated composition and characteristics and satissfactory growth promoting properties for microorganisms. All these results suggest that the developed product could be successfully included in culture medis formulation as a nutrient nitrogen source.

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