UTILIZATION OF WHOLE BOVINE BLOOD AS PROTEIN SUBSTRATE FOR THE OBTAINMENT OF BACTERIOLOGIOCAL PEPTONE

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SUMMARY: Whole bovine blood was employed as protein substrate for the obtainment of bacteriological peptone by using papain as hydrolytic agent at pH 5 and temperature 70 c degree. The characteristics of the peptone resulted as follows: humidity - 5.66%, amino nitrogen - 3.8%, total nitrogen - 12.66%, amino nitrogen/total nitrogen relation - 30.03%, pH (2% water solution after sterilization) - 5.05, hydrolysis degree - 19.76%, chlorides - 9.24%, calcium - 1085 ppm, and satisfactory growth promoting properties for different specimens of microorganisms.

INTRODUCTION: In most of the countries bacteriological peptones are mainly produced by using meat proteins as substrate in the enzymatic hydrolytic process (Orthana, 1985; OXOID, 1982). This protein source is necessary for human nutrition and by this reason the actual trend in culture media manufacturing industry point out to the employment of other protein sources such as blood and blood proteins. In the USSR several methods for the production of whole bovine blood and blood components' protein hydrolysates were developed by carrying out the hydrolysis with acids (Spravochnik, 1973) ore with enzymes such as pepsin and pancreatic enzymes (Azizov, 1988; Ruzal et al.,1982; Spravochnik, 1973; Varashilova et al.,1988). In Cuba preliminary works for the obtainment of blood protein peptone were developed at laboratory scale in the National Center of Animal Health by using blood cuagulates as protein substrate and pepsin as hydrolytic agent, and carrying out the hydrolysis at low pH values (Fuentes, 1984). In the "Carlos J. Finlay" enterprise for production of biologicals some experiments in this field were also developed (Rodriguez, et al., 1988). The target of this work was the obtainment of bacteriological peptone by using whole bovine blood as protein substrate for growing a wide group of microorganisms.

MATERIALS AND METHODS: Blood was collected and inmediatly stabilized with sodium citrate and refrigerated at 8-12 c degree for 24 h. Papain preparate (60000 U) was added as hudrolytic agent. An experimental design model 2x3 was applied for developing experiences in wich the main parameters of the process were varied: ezzyme:substrat relation (4 and 5 g/Kg) (X1), boiling as a method for enzyme inactivation (X2) and the pH value (5 and 6.5) (X3). Different dependent variables were tested at different times (from 1 to 4 h): amino (Na) and total (Nt) nitrogen content; relation between Na and Nt (Na/Nt). Yield (refered to the initial weight of the system) was also calculated

for the best experience. A matricce system was employed to develop the relationship between variables. A student test was applied ($p<0.05$) to evaluate the significance; Fisher test was used to determinate the addecuation of the models and Duncan test ($p<0.05$) for the variance analysis (if media differed). At different times regression equations were calculated for the Na content (López, 1988). Statistical analysis and polynomes were developed with the "MICROSTAT" statistical software in an IBM-PC fully compatible microcomputer in MC-DOS system.

The methods employed in this work for the determination of the characteristics of the products were: amino nitrogen - by potenciometric titration with formaldehyde (Chechatkin et al., 1987), total nitrogen - by Kjeldahl method (Tecator, 1987), aminoacid content in an automatic analyzer from "ITACHI" model KIA-%, hydrolysis degree - as described by Norris (Norris, 1970), humidity/dry weight and chlorides - as described by Matrozova (Matrozova, 1977), pH - with a digital PHM-83 pH meter from "RADIOMETER", biological reactivity (Acethyl-Methyl-Carbinol, Indole and sulphidric gas production and carbohydrate fermentation) with different strains from the " American Type Culture Collection" (ATCC): Escherichia coli 25922, Streptoccus faecalis 19433, Enterobacter aerogenes 13048) and Salmonella typhibismuth 1454 from "GISK Tarasievch Type Culture Collection" (TTCC) by the methods described by Sonnerwirth (Sonnerwith et al., 1983). The biological efficiency of different experimental batches of the peptone were evaluated in 5 experimental batches of MacConkey Agar, Kligler Iron Agar and Violet Red Bile Agar with the fallowing strains: ATCC: Shigella sonnei 25931, Proteus vulgaris 13315, Escherichia coli 25922, Klebsiella pneumoniae 13883, Salmonella typhimurium 14028, Shigella flexneri 12022, Pseudomona aeroginosa 27853; TTCC: Salmonella cholera 1455, Shigella dysenteriae 1458, Salmonella aerogenes 1469, Salmonella typhibismuth 1454. Media were tested in different capital's hospitals and research centers.

RESULTS AND DISCUSIONS: The analysis of the amino and total nitrogen content and their relation (Table 1) showed that there are significative differences between experiences with or without a boiling stage, but practically we can assume that those differences could be obviated. At the same time boiling allowed the best separation of the residual non degraded protein and the best filtration. The addition of 5 g of papain allowed the obtainment of hydrolyzates with higher values of the tested parameters. By the resulted regression equations we could observe that pH was the parameter that more signifficatively influenced the Na content and Na/Nt relation. These results allowed the stablishment of the values of the main process parameters for further experiences: the addition of 5 g of enzyme per Kg of substrate, the pH of 5 for the hydrolysis and the employment of a boiling stage at the end of the process according to the parameters gived in experience No 4 by which were obtained the higher results.

Table 1.- Influence of the main process parameters on the composition of the products.

Exp. No.	Na, % *	Nt, % *	Na/Nt, % *
1	0.74 a	2.49 a	29.72 b
2	0.76 a	2.44 a	31.15 a
3	0.77 a	2.56 a	30.08 b
4	0.79 a	2.54 a	31.10 a
5	0.51 a	2.28 b	22.37 c
6	0.55 b	2.36 a	23.31 c
7	0.53 b	2.34 ab	22.65 c
8	0.56 b	2.43 a	23.05 c
Std. err.	0.021	0.020	1.06

* - values recalculated to 95% Dw.

Regression equations:

Na $Y = 0.65 + 0.01 X_1 + 0.01 X_2 - 0.11 X_3$

Nt $Y = 2.43 + 0.04 X_2 - 0.08 X_3 - 0.03 X_1 X_3$

Na/Nt $Y = 26.73 - 3.88 X_3$

In table 2 we refered the values of the parameters through the hydrolysis process (t) for experience No 4. at laboratory scale.

Table 2.- Composition and yield of the final product (Exp.No 4)

t, h	Nt, %	Na, %	Na/Nt,%	Dw, %	Yield, %
1	0.16 d	0.69 d	23.18 c	4.8	2.4
2	0.18 c	0.69 d	26.09 b	5.0	3.0
3	0.20 b	0.77 c	27.27 b	5.2	3.1
4	0.23 a	0.79 b	29.11 a	5.5	3.2
5	0.23 a	0.82 b	29.27 a	5.4	3.2
Std. err.	0.001	0.003	0.2		

Regression equations:

$$Na = 0.145 + 0.018*t$$

$$Nt = 0.644 + 0.036*t$$

$$Na/Nt = 23.08 + 1.139*t$$

We could appreciate that after 3 h of hydrolysis the reaction velocity decreased. The yield resulted high even at the first hour of the process. The Na and Nt level resulted also high at this time and elevated at five hour.
At industrial scale the products obtained in five batches presented a characteristic composition for bacteriological peptone (Table 3).

Table 3.- Physico-chemical characteristics of the peptone obtained at laboratory scale.

Batch No	Humidity, %	Na, %	Nt, %	Na/Nt, %	Chlorides, %	Calcium, ppm	pH	Hydrol degree %
1	3.40	3.78	13.10	28.85	8.04	990	5.05	19.6
2	2.75	3.78	12.69	29.79	7.31	1290	4.89	19.6
3	4.54	3.78	12.43	30.41	10.09	1165	5.11	19.6
4	4.80	4.06	12.67	32.04	9.80	990	5.21	21.7
5	2.81	3.61	12.43	29.04	10.97	990	5.03	18.3
Media	5.66	3.80	12.66	30.03	9.24	1085	5.05	19.76
Std err	0.01	0.003	0.01	1.06	0.10	13.31	0.002	0.01
Peptone OXOID	4.4	2.5	14.3	17.5	-	-	-	-

The content of some aminoacids of the peptone obtained at industrial scale (Table 4) showed that in general, the level of the aminoacids in the experimental product is within the range described in the bibliography (Orthana, 1985; OXOID, 1982; OXOID, 1986; MERCK, 1987) for products elaborated by using meat and meat by-products as protein substrates with the exception of methionine and isoleucine wich presented a lower level in experimental product, and phenylalanine and valine wich showed a higher level.

Table 4.- Aminoacid content of the peptone.

Aminoacids	Aminoacid content, g/100 g of the product	
	Experimental peptone	Report from bibliog.
Aspartic acid	7.10	5.5 - 9.0
Threonine	3.13	1.6 - 3.5
Serine	2.63	0.7 - 2.7
Glutamic acid	8.00	6.6 - 13.0
Proline	4.60	4.5 - 12.3
Glycine	2.88	3.0 - 9.3
Alanine	6.55	3.5 - 8.5
Valine	5.66	1.5 - 4.6
Methionine	0.34	0.2 - 3.2
Isoleucine	0.37	1.4 - 4.0
Leucine	5.35	3.2 - 6.1
Tyrosine	1.81	0.7 - 2.4
Phenylalanine	3.80	2.0 - 3.3

The organoleptic characteristics of the product resulted as follow: colour of the powder - light cream, colour of the 2% water solution - light yellow, apparience of the powder - fine powder without grumes.
The evaluation of the biological reactivity of the product demonstrated the satisfactory composition and properties of the peptone as nutrient base (Table 5)

Table 5.- Biological reactivity of the developed peptone.

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

The results of the evaluation of the growth promoting properties of the developed product in MacConkey Agar and Kligler Iron Agar media confirmed the addecuate quality of this nutrient base (Tables 6 and 7)

Table 6.- Biological evaluation of the experimental peptone in MacConkey Agar medium

Microorganism	Media	
	Experimental	OXOID
E. coli	red, non mucoid	red, non mucoid
P. aeroginosa	green, brown	green, brown
P. vulgaris	colouress	colouress

Table 7.- Biological evaluation of the experimental and control peptones in Kligler Iron Agar medium.

Microorganism	Media					
	Experimental			OXOID		
	Butt	Slope	H_2S	Butt	Slope	H_2S
S. sonnei	A	NC	-	A	NC	-
S. cholera	AG	ALK	-	AG	ALK	-
S. dysenteriae	A	NC	-	A	NC	-
S. paratyphi A	AG	ALK	-	AG	ALK	-
S. paratyphi B	AG	ALK	+	AG	ALK	+
Providencia	AG	ALK	-	AG	ALK	-
S. typhimurium	AG	ALK	+	AG	ALK	+
P. vulgaris	AG	NC	-	AG	NC	-
K. pneumoniae	AG	A	-	AG	A	-
E. coli	AG	A	-	AG	A	-
E. aerogenes	AG	A	-	AG	A	-

AG: acid (yellow) and gas formation; A: acid (yellow); ALK: alkaline (red); NC: no change; +: hydrogen sulphide (black); -: no hydrogen sulphide (no black)

CONCLUTIONS: It was obtained a bacteriological peptone from whole bovine blood by enzymatic hydrolysis with papain enzyme (5 g per Kg of substrate), at pH = 5 and constant stirring for 3 h, the further separation of the protein residual by boiling at certain pH value, the filtration of the supernatant, it's concentration, spray drying and pH adjustment. The peptone resulted with an addecuated composition and characteristics: humidity - 5.66%, total nitrogen - 12.66%, amino nitrogen - 3.8%, pH (2% water solution after sterilization) - 5.05, hydrolysis degree - 19.76%, chlorides - 9.24% and calcium - 1085 ppm.

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