# LIVER DIGEST FOR MICROBIOLOGICAL USE

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SUMMARY: It was developed a method for production of ox liver digest for its use in microbiology. The method allows the obtainment of a product with good growth promoting characteristics for several specimens of microorganisms and with the following composition: humidity - 7%, amino nitrogen - 4.4%, total nitrogen - 12%, amino nitrogen/total nitrogen relation - 34.5%, pH (2% water solution after sterilization) - 7.1.

INTRODUCTION: Liver digest (papainic) is widely used in microbiology essentially in media for growing Trichomonas vaginalis and other protozoa, pathogenic and saprophitic fungi, bacteria and pleuropneumonia-like organism. It often could be included in media formulation by replacing other nutrient sources as peptones, yeast extracts, infusions (OXOID, 1982). In a previous work (Rodriguez et al., 1989) we refered two methods developed in the USSR for obtaining ox liver digest by carrying out the hydrolysis with pancreatic enzymes at different pH and temperature values in presence of several preservants. The quality of the product mainly depends, as for other protein hydrolysates, on the kind, state and activity of the employed enzyme, the substrate state and other technological parameters (Nekliudov et al., 1985). The aim of this work was the design of an appropiate method of the obtainment of ox liver digest using a papain preparate produced in our country as hydrolytic agent an a suspension of ox liver as protein substrate.

MATERIALS AND METHODS: As hydrolytic agent it was employed a papainic preparate (60 000 U.) produced in our country and stored by refrigeration and ox liver (refrigerated) from slaughterhouse as protein substrate. The objective of the first stage of the work was the obtainment of the liver digest at laboratory scale with the preliminary biological characterization. Second stage comprised the verification of the effectiveness of the method at industrial scale and characterization of the composition and growth promoting properties. The controlled parameters were varied according to an experimental design model 2x3 as follows: relation enzyme:substrate (X1) - 2:1 and 3:1 g/kg, pH (X2) - 6.5 and 7.5, and substrate:water solution relation (X3) - 1:2.5 and 1:3 kg/kg.
With the resulted values of the dependent variables (total nitrogen (Nt), amino nitrogen (Na), amino/total nitrogen relation (Na/Nt), dry weight (Dw), digests' yield) was adapted a polynome and its coefficients were calculated with a matricce system and the significance by a t-Student test ($p<0.05$); the model

addecuation was determined by a Fisher test. For the statistical analysis of the results it was applied a variance analysis and a Duncan test ($p<0.05$) in cases that media differed. In tables are given the media values and standart errors. Polynomes were also calculated for the amino nitrogen content at different times (t - from 1 to 4 h).
At the end of the hydrolytic stage yield was calculated, and for the best experience (No. 5) it was developed a mathematical model (regression equation) to develop a functional relationship between the Na content and t (López, 1988). Statistical analysis of the design model and mathematical regression model were calculated in an IBM-PC fully compatible microcomputer with the "MICROSTAT" statistical package in MC-DOS system.
Chemical analysis were developed by the following methods: amino nitrogen - by potenciometric titration with formaldehyde (Chechetkin et al., 1984); total nitrogen (Kjeldhal method) with the "KJELTEC SYSTEM" from TECATOR (Tecator, 1987); humidity/dry weight and chlorides as described by Matrozova (Matrozova, 1977); pH - by a potenciometric method with a digital pH meter PHM-83 from RADIOMETER.
The biological evaluation of the developed nutrient base was carried out in the first and second stages of the work by testing the biological reactivity (Acethyl-Methyl-Carbinol, Indole and sulphidric gas production and carbohydrate fermentation) as described by Sonnerwirth (Sonnerwirth, 1983); and by testing different culture media such as MacConkey Agar, C.L.E.D. and Kligler Iron Agar in wich peptone was replaced by the liver digest for growing strains from the "American Type Culture Collection" (ATCC): Escherichia coli 25922, Streptococcus faecalis 19433, Enterobacter aerogenes 13048, Staphilococcus aureus 25923, Proteus vulgaris 13315, Klebsiella pneumoniae 13883, Shigella flexneri 12022 and Salmonella typhibismuth 1454 from the "GISK Tarasievich Type Culture Collection" (TTCC). Control media from OXOID ltd. were tested also for comparing the results. The preparation of media and further work with the strains was developed as recommended in the OXOID manual (OXOID, 1982).

RESULTS AND DISCUSIONS: As shows in table 1 and in regression equatios resulted at different times of the hydrolytic proces, the amino nitrogen content increased through the time for all experiences and at 4 h is highest for exp. No. 5 and had an addecuated level for exp. No. 6, 7, 8 and 1.
The higher the substrate:solution relation (1:3) the greater the amino nitrogen content in the hydrolyzate. Howseover the pH and enzyme:substrate relation values had a less signifficant influence on the Na content than substrate:solution relation it was observed that the lesser the level of these two first parameters the higher the Na level.

Table 1.- Amino-nitrogen content through the hydrolysis process.

| Exp. No. | Na, % x h | | | | Std. err. |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| 1 | 0.20 b | 0.23 b | 0.25 b | 0.26 b | 0.005 |
| 2 | 0.15 c | 0.17 d | 0.18 c | 0.19 c | 0.005 |
| 3 | 0.10 d | 0.11 e | 0.12 d | 0.15 d | 0.006 |
| 4 | 0.10 d | 0.11 e | 0.13 d | 0.15 d | 0.006 |
| 5 | 0.22 a | 0.26 a | 0.28 a | 0.31 a | 0.005 |
| 6 | 0.20 b | 0.22 c | 0.25 b | 0.28 b | 0.001 |
| 7 | 0.20 b | 0.22 c | 0.25 b | 0.28 b | 0.007 |
| 8 | 0.19 c | 0.24 b | 0.25 b | 0.27 b | 0.003 |
| Std. err. | 0.001 | 0.001 | 0.001 | 0.016 | |

Regression equations:

$$Na(1^{th}\ h)\ Y=0.170-0.010X_1-0.022X_2+0.033X_3+0.008X_1X_2+0.003X_1X_3+0.015X_2X_3-0.005X_1X_2X_3$$

$$Na(2^{nd}\ h)\ Y=0.194-0.009X_1-0.026X_2+0.041X_3+0.016X_1X_2+0.004X_1X_3+0.021X_2X_3$$

$$Na(3^{th}\ h)\ Y=0.214-0.011X_1-0.026X_2+0.040X_3+0.014X_1X_2+0.04X_1X_3+0.019X_2X_3$$

$$Na(4^{th}\ h)\ Y=0.236-0.014X_1-0.024X_2+0.049X_3+0.011X_1X_2+0.004X_1X_3+0.014X_2X_3-0.006X_1X_2X_3$$

The evaluation of the Na, Nt, Dw content of the final product and process' yield are shown in table 2, and their characteristic equations are related for the better understanding and convinient comparisson of these results, they were recalculated for 95% dry weight (characteristic Dw of these powdered products). Experinces No. 1, 6, 5 and 7 showed the highest Nt, Na content and Na/Nt relation. Exp. No. 5 to 7 showed the better total yield.

Table 2.- Composition and yield of the final product obtained at laboratory scale

| Exp. No. | Composition, % | | | | Yield, % |
|---|---|---|---|---|---|
| | Nt * | Na * | Na/Nt * | Dw | |
| 1 | 13.25 a | 4.22 c | 31.83 d | 6.43 c | 35.90 |
| 2 | 11.92 d | 3.32 f | 27.63 f | 6.07 d | 37.61 |
| 3 | 10.46 g | 2.46 h | 23.43 h | 5.03 f | 37.83 |
| 4 | 10.96 f | 2.75 g | 25.42 g | 5.87 e | 38.58 |
| 5 | 12.92 b | 4.82 a | 37.28 a | 7.03 b | 47.07 |
| 6 | 13.02 b | 4.24 b | 32.54 c | 7.30 a | 45.64 |
| 7 | 12.22 c | 4.11 d | 33.63 b | 6.77 c | 49.14 |
| 8 | 11.55 e | 3.41 e | 29.54 e | 6.47 c | 41.81 |
| Std. err. | 0.006 | 0.004 | 0.020 | 0.020 | |

* - values recalculated to 95% Dw.

Polynomes' equations

Nt: $Y=12.04-0.18X_1-0.74X_2+0.39X_3+0.13X_1X_2+0.03X_1X_3+0.2X_2X_3-0.33X_1X_2X_3$

Na: $Y=3.67-0.24X_1-0.48X_2+0.48X_3+0.13X_1X_2+0.10X_1X_3+0.10X_2X_3-0.16X_1X_2X_3$

Na/Nt: $Y=30.16-1.38X_1-2.16X_2+3.18X_3+0.83X_1X_2-0.83X_1X_3+0.50X_2X_3-0.69X_1X_2X_3$

Dw: $Y=6.37+0.06X_1-0.34X_2+0.52X_3+0.08X_1X_2-0.06X_1X_3+0.06X_2X_3-0.22X_1X_2X_3$

Yield: $Y=41.70-0.79X_1+0.14X_2+4.22X_3-0.86X_1X_2-1.40X_1X_3-0.58X_2X_3-0.62X_1X_2X_3$

The functional relationship between the Na content and t for experience No. 5 resulted:

$$Na = 0.195 + 0.029 * t$$

The evaluation of the biological reactivity of the liver digest obtained according to the experience No. 5 at laboratory scale (Table 3) showed satisfactory and characteristic results with all the tested strains.

Table 3.- Biological reactivity of the ox liver digest at laboratory scale

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

In the MacConkey Agar medium with the liver digest included in the formulation as nutrient base (in substitution of bacteriological peptone) was observed a characteristic growth of tested strains in comparisson with OXOID's medium (Table 4).

Table 4.- Colonies' characteristics of microorganisms in MacConkey Agar medium (Laboratory scale)

| Microorganism | Media | |
|---|---|---|
| | Experimental | OXOID |
| E. coli | red, non mucoid | red, non mucoid |
| E. aerogenes | pink, mucoid | pink, mucoid |
| S. faecalis | red, minute, round | red, minute, round |
| S. aureus | pink, opaque | pink, opaque |

At the second stage was defined a method for the liver digest obtainment: preparation of the water solution - addition of the minced ox liver and agitation - pH and temperature regulation - addition of the enzyme preparate - pH and temperature adjustment - acidification and heating - separation of the hydrolysate - filtration - concentration - filtration - spray drying - pH regulation.

The characteristic (mean values) of the final product resulted as follows: humidity - 7.62%, total nitrogen - 12.04%, amino-nitrogen - 4.43%; amino/total nitogen relation - 34.53%, pH (2% water solution after sterilization) - 7.12; powder's colour - light cream; apparience - fine powder, without grumes; colour of the 2% water solution - light yellow.

The results of the biological reactivity test of the dehydrated product (results from 3 industrial batches) evaluated with ATCC strains were the same as those obtained at laboratory scale (Table 3).

In C.L.E.D. medium elaborated at industrial scale (3 batches) with the experimental liver digest the tested ATCC strain showed a characteristic colony development (Table 5) and similar to their growth in the OXOID's medium.

Table 5.- Colonie's characteristics of microorganisms in C.L.E.D. medium (industrial scale)

| Microorganism | Media | |
|---|---|---|
| | Experimental | OXOID |
| E. coli | yellow, opaque colonies with a slighthy deeper coloured centers | yellow, opaque colonies with a slighthy deeper coloured centers |
| P. vulgaris | translucent blue colonies with rough periphery | translucent blue colonies with rough periphery |
| S. aureus | deep yellow colonies | deep yellow colonies |
| K. pneumoniae | translucent blue colonies | translucent blue colonies |
| E. aerogenes | yellow colonies, larger than E. coli | yellow colonies, larger than E. coli |

We observed for all the ATCC strains characteristic changes and reactions in Kligler Iron Agar experimental medium in comparisson with the OXOID's medium elaborated with bacteriological peptone (Table 6).

Table 6.- Evaluation of the liver digest growth promoting characteristics in Kligler Iron Agar medium

| Microorganism | Media | | | | | |
|---|---|---|---|---|---|---|
| | Experimental | | | OXOID | | |
| | Butt | Slope | $H_2S$ | Butt | Slope | $H_2S$ |
| E. coli | AG | A | - | AG | A | - |
| E. aerogenes | AG | A | - | AG | A | - |
| S. flexneri | A | ALK | - | A | ALK | - |
| S. typhimurium | AC | ALK | + | AC | ALK | + |
| P. vulgaris | AC | ALK | + | AC | ALK | + |

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

CONCLUTIONS: At laboratory scale were determinated the addecuated values of the main process parameters of the ox liver hydrolysis (pH - 6.5, relation enzyme:substrate - 2:1 g/Kg and substrate:water solution relation - 1:3 kg/kg). At industrial scale was developed and verified a method for the obtainment of ox liver digest. The results of the chemical and biological characterization of the elaborated product demostrated satisfactory results mainly the good growth promoting characteristics that allow its employment as nitrogen source in different culture media.

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