RESEARCH CONCERNING THE USE OF BEECHWOOD PREHYDROLYSATES TO GROW YEASTS

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This paper reports results concerning characterization and improvement of biological quality of beechwood prehydrolysate in order to adapt the *Candida Scottii* yeast to this nutritive medium. Some difficult aspects caused by the presence of lignin compounds in the prehydrolysate are mentioned. Studies carried out also made it possible to evaluate yeasts as regards the chemical composition and their nutritive value, tested in case of a mixed fodder used for chicken breeding.

Keywords: Dissolving pulp, Beechwood, Prehydrolysate, Fodder yeast, Candida Scottii, Proteins.

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INTRODUCTION

In dissolving pulp making (for threads, yarns, esters, ethers, foils), before being subject to kraft cooking (cooking with a solution of sodium hydroxide and sodium sulphure), wood chips undergo a water (or steam) hydrolytic treatment named hydrolysis or self-hydrolysis, which mainly aims to release hemicelluloses (Leschinsky et al. 2008; Sixta 2006; Sixta et al. 1992; Rozmarin et al. 1991; Honos 1984; Stanciu 1974). During this stage, hemicellulose separation takes place in a proportion of 80-85%. Prehydrolysis is carried out according to what is shown in Fig. 1 at a maximum temperature of 170-175ºC and at a bone dry wood ratio of 1:3.5. After prehydrolysis, the recovery of prehydrolysate mainly containing products resulted from hemicellulose transformation takes place. The raw material used, as well as the conditions of the prehydrolysis reaction, both drive pulp qualitative characteristics and prehydrolysate composition.

Prehydrolysate from the prehydrolysis process is a brown-reddish solution with a typical smell of furfural and acetic acid. Due to its complex composition (sugars, furfural, organic acids etc.) prehydrolysate is a convenient raw material for chemical and biochemical processing. Economically, it is considered that prehydrolysis recovery leads to the unfortunate loss of considerable amounts of polyosic substances and furfural. Apart the economic aspects, recovery of prehydrolysate is also driven by environmental aspects, as it has a high pollution capability (on average BOD_5 = 47g O_2/l and COD = 112 g O_2/l). Due to a composition rich in sugars, the prehydrolysate is a specific medium, suitable for fodder yeast culture and at the same time, is an effective solution concerning pollution abatement and in order to address the current lack of proteins.

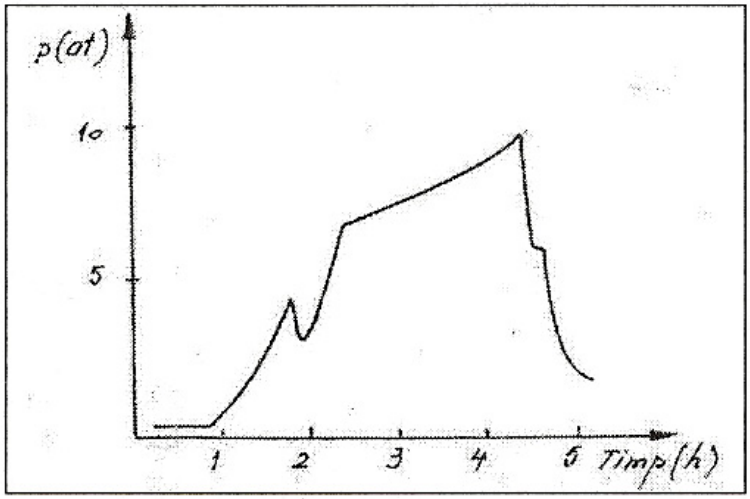

Fig. 1. Diagram of beechwood prehydrolysis

EXPERIMENTAL

Prehydrolysate from beech dissolving pulp making (water prehydrolysis with a bone dry wood ratio of 1: 3.5) and at a prehydrolysis temperature of 170ºC for 110 minutes) has been tested as a sublayer for yeast multiplication. The total reducing substances were determined by the Schöerl-Regenbogen method, monosugars have been identified by the thin layer chromatography (De Stefanis et al. 1968), and furfural was charged by means of the calorimetric method. Concentration of lignohumic substances has been determined by means of activated carbon adsorption (Stanciu 2003), and their nature has been confirmed by IR spectroscopy and elemental analysis.

The presence of some toxic components (formic acid, methanol, acetone) has been recorded by gas chromatography (V2Achromatographic column, CHROMOSORB carrier w80-100, steady phase): diglycerol, supporting gas flow: 100 ml/min, chromatographic column length: 2 m, thermostat and detector temperature: 110ºC. In order to determine the type of yeast that best adapt on the prehydrolysate, multiplication of various stems in pure culture or polyculture has been studied. For each experiment, the biomass yield of consuming total reducing substances, phosphorus, nitrogen, and protein content has been determined.

In order to evaluate the biological value of proteins the aminoacid content has been determined by means of the AAA-81 analyzer. The yeast fat content was determined by extraction with a mixture made of ethilic alcohol (96°C) and benzene (1:3), for 8 hours. The main macro and microelements were charged by atomic absorption spectroscopy (Tănase et al. 1994; Paralescu et al. 1975; Tănase 2000; Tănase et al. 1999, 2002).

In order to denature the yeast samples, a mixture of H_2SO_4 + H_2O_2 (1:2.2) and HNO_3 + $HClO_4$(1:6) was used, with calcination at a temperature of 500°C and ash dissolution in concentrated hydrochloric acid (in case of lead measuring). Denaturation with HNO_3 and $HClO_4$ is recommended to measure the cadmium content. Destruction

with H_2SO_4 and H_2O_2 was carried out in approximately 13 hours, while for destruction with HNO_3 and $HClO_4$ is necessary to use 5 hours.

RESULTS AND DISCUSSION

In Table 1 a comparison of composition of beechwood prehydrolysate and softwood (spruce) prehydrolysate is shown (Filipov at al. 1977; Alexandrov et al. 1977; Orlov et al. 1977). Following the analysis of beechwood prehydrolysate, it was noticed that apart from the carbohydrates, it also contains a series of colloidal products (resulting from lignin degradation) that during hydrolysis participate in condensation reactions. On the other hand, reactions with sugar degradation products (mainly furfural) lead to the formation of some deposits (phenolfurfuralic resins and ligninfurfuralic resins) that cause difficulties in the prehydrolysate recovery (Stanciu et al. 1989, 2008). Prehydrolysate from the beech dissolving pulp manufacture has a specific composition having a high content of pentoses (76%), the weight being held by the free furfural xylose and colloidal substances (9.2 g/L) compared to the softwood prehydrolysate. Deposits made cause considerable difficulties in the prehydrolysate recovery (pipe and tank plugging) and the weight is in close correlation with the furfural contained in prehydrolysate (polycondensation reactions between phenolic compounds resulted after degradation of lignin, furfural tannins and its compounds).

Table 1. Composition of Hardwood and Softwood Prehydrolysates

Wood species	Dry subst. %	pH	TRS %	Sugar %	Furfural g/l	Prehydrolysis conditions	Author and reference
Beech	5.28	3.5	3.5	3.0	3.0	H= 1:3.5 at 170ºC for 120 minutes	Analysed by author
Spruce	4.15	3.7	1.90	1.32	1.2	T= 170 ºC	Orlov et al. (1977)
Hackmatak	6.9	3.7	2.70	2.05	1.4		

The lignin nature of these deposits was confirmed by IR spectroscopy (Fig. 2) emphasizing the bands from 1300 cm^{-1}, band characteristic for hardwood lignin from the OCH_3 group, bands from 1550 cm^{-1} and 1610 cm^{-1} characteristic of the ring (Table 2) and by elemental analysis (high carbon content of 59.7%) (Table 2). Deposits had a high heating value (6040 kcal/kg), similar to that of anthracite (6097 kcal/kg). Following the analysis of deposit solubility an almost full leaching of deposits was noticed by using some diluted solutions of sodium hydroxide (4-6%), even at ambient temperature and a minimum time of 15 minutes. Therefore, prehydrolysate cannot be used as such because it is necessary to improve its biological quality by diminishing formation of deposits, removal of furfural up to a maximum content of 0.35 g/L, and pH correlation. Thus, the sudden decrease of biomass yield was noticed when the nutritive medium contained 0.05% furfural.

Table 2. The Main Absorption Bands of Deposits Resulted from Beechwood

2	2850	Valence vibration in C-H
3	1710	Valence vibration in C=O
4	1680	Valence vibration in C=O
5	1610	Valence vibration in C-C in ring
6	1550	Valence vibration in C-C in ring
7	1470	Deformation vibration of CH_2 group
8	1430	Deformation vibration of CH and CH_2 groups
9	1330	Deformation vibration of OH group
10	1220	Valence vibration of C=O group
11	1175	Valence vibration of C-O-C group
12	1150	Valence vibration of C-O-C group
13	1120	Valence vibration of C-O-C group
14	1040	Valence vibration of C-O-C group
15	940	Valence vibration in C=O group
16	885	Vibration typical to glucosidic bound pyranosic ring

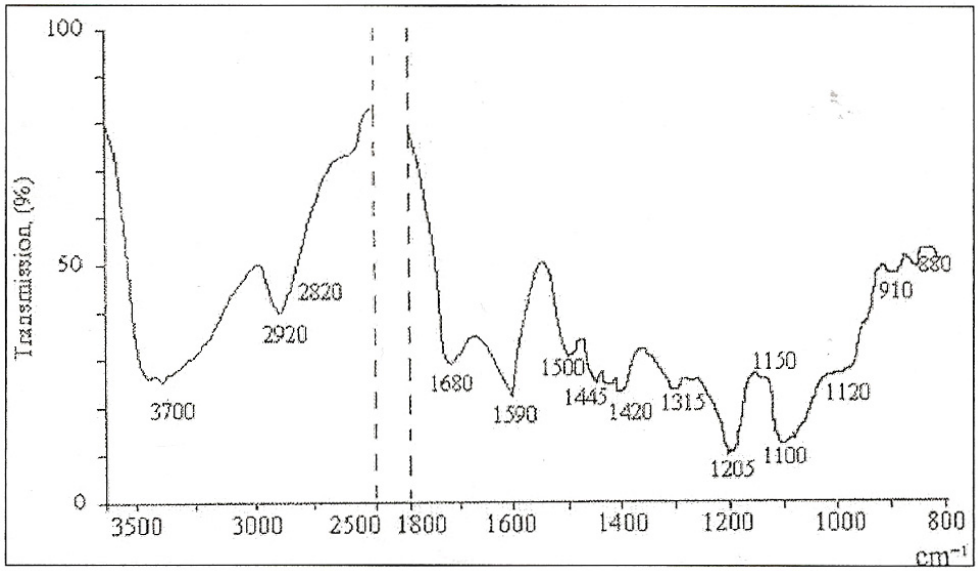

Fig. 2. IR spectrometer of deposits resulted from beechwood prehydrolysis

Furfural is undesirable in the yeast producing medium because this kind of medium mainly contains pentoses. Diminishing deposit formation can be carried out by adding lignosulphonates (0.04-0.05% sodium lignosulphonate) in the water that results from prehydrolysis or in the prehydrolysate. The use of lignosulphonates is based on their surface-active properties that explain their use as dispersing aids and protective colloids. In studies carried out the use of sodium lignosulphonate in the prehydrolysis stage has emphasized the following advantages compared to introduction in the prehydrolysate:

- Deposits occur only at temperatures below 90°C, while they occur even at 98°C when prehydrolysate is obtained without lignosulphonate charge,

- Deposits built-up by progressive cooling of prehydrolysate (and in prehydrolysate resulted after furfural removal), are very brittle and can release easily from the walls of the storage tanks,

- The use of sodium lignosulphonate is more efficient at high temperature (170ºC) when the amounts of deposits go down by 30-40%.

Apart from furfural, other harmful substances for yeast multiplication have been identified, such as: formic acid (0.04%), acetone (0.07%) and methanol (0.15%). In order to reduce the furfural concentration in the prehydrolysate used for yeast multiplication, its minimum formation in the prehydrolysis process should be tracked. Then its maximum removal should be secured. Furfural removal is carried out in the furfural, removing columns equipped with sieve plates. Volatile inhibitors and mainly furfural can be removed by steam entrainment. After removing the furfural, the prehydrolysate has a sugar content of 27 g/L and an organic acid content of 10 g/L (expressed in acetic acid) being able to be used as a sublayer for yeast multiplication. The prehydrolysate has a strongly acid pH (3.5) unsuitable to yeast development, a fact that requires its neutralization with basic substances such as lime milk or ammonia water. Neutralization with lime milk at a pH =7.8-8.3 leads to reduction of the total reducing substances by 15%. Prehydrolysate neutralization immediately after leaving the furfural-removing columns leads to a ceasing of polycondensation reactions whose speed was already reduced by using sodium lignosulphonate at prehydrolysis, by modifying the behaviour of polycondensation substances that do not make compact deposits by settling a more or less loose sludge according to how neutralization was carried out. In order to perform a suitable clarification and make a deposit easy to remove from the clarifier, lime milk neutralization should be performed up to a minimum pH of 4.7.

The disadvantage of using lime milk (issue of deposits) was removed by means of prehydrolysate neutralization from pH = 3.5 at pH = 5, with ammonia water (concentration 25%) (485 kg/t of yeast). In this case it is mainly noticed that the furfural content goes down by over 50%. Simultaneous aeration at prehydrolysate neutralization is also recommended because it drives the removal of inhibitors from the prehydrolysate (45-55 m^3air/m^3 prehydrolysate for 1.5-2 hours) to a greater extent. The prehydrolysate cannot be used as such for yeast multiplication due to the nutritive substances, vitamins or microelements. Therefore, the following was introduced in the medium: phosphate diammonium as a phosphorus source (6 kg P_2O_5/100 kg TRS), and H vitamin as a vitamin source and microelements as well as molasses (0.52% in regard to prehydrolysate).

As regards the yeast species used, it has been proved that the *Candida Scottii* yeast shows the best results. This yeast can be used both as pure culture or mixed culture in conjunction with other *Candida* yeast species or *Trichosporum cutaneum* (Stanciu et al., 1992, Stanciu et al., 1996, Stanciu, 2004). Morphologically, the *Candida Scotti* yeasts look like oval colonies arranged as a chain made of 5-12 cells with sprouts from everywhere (Figure 3). Colonies developed on solid agarized media are white-cream, with a narrow wrinkled surface, with fine granulation. The colony profile is crowned. (Fig. 3).

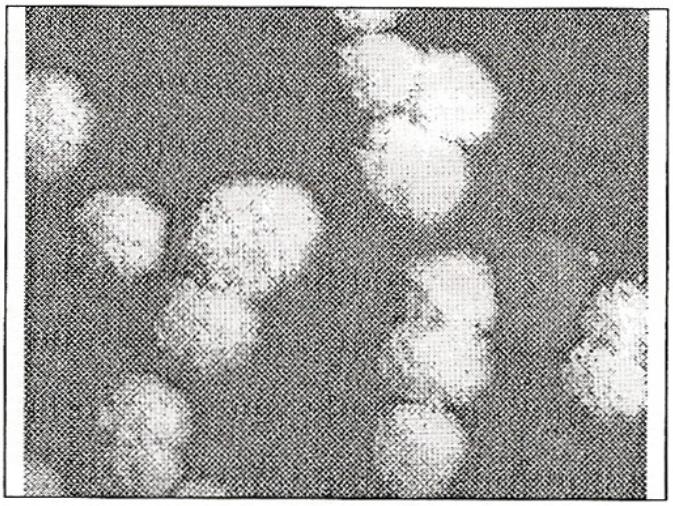

Fig. 3. Picture of *Candida scottii* colonies

Candida scottii yeasts assimilate well both hexoses and pentoses as well as organic and uronic acids. Table 3 shows the behaviours of the *Candida scottii* yeasts multiplied on beechwood prehydrolysate compared to various types of yeasts and polycultures.

Table 3 Behaviours of the *Candida scottii* Yeast Multiplied on Beechwood Prehydrolysate Compared to Different Types of Yeasts and Polycultures

Parameters	Type of culture			
	CS	CA	CR	P
Biomass, %	64.40	35.85	37.84	39.39
TRS consumption, %	85.64	78.30	78.31	76.74
Nitrogen consumption, %	46.43	52.84	53.27	55.99
P_2O_5 consumption, %	59.68	48.39	48.86	69.90
Protein consumption, %	53.51	47.22	48.00	47.37

Legend: CS- Candida Scottii, CA – Candida arboreea, CR- Candida robusta, P- polyculture, (Candida arboreea + Candida robusta).

Comparing behaviours of the *Candida scottii* yeast with those of pure species such as *Candida arboreea*, *Candida robusta*, and a mixed culture (*Candida arboreea* + *Candida robusta*), the efficiency of the *Candida scottii* yeast is noticed. It provides a biomass yield of 64.4%, a TRS consumption of 85.64% and a high protein content of 53.51%. In a mixed culture, the *Candida scottii* yeast has a stimulating influence on the other species as it secretes the H-vitamin-biotin. The protein content is comparable with that recorded at yeasts grew on n-paraffins (50-52%) and higher than those grew on waste sulphite liquors and molasses (45%). The full scale multiplication process takes place at a TRS concentration of 8-12 g/L, 5 g acetic acid /L, at a pH = 4.8-9.8 at a temperature of 37-39ºC and a fermentation time of 3.5-4.0 hours. The chemical composition of yeast obtained on sublayers containing the prehydrolysate is illustrated in Table 4.

Table 4 Chemical Composition of Yeast Obtained with the Beechwood Prehydrolysate Compared to Other Yeasts Multiplied on Various Sublayers

Yeast cultivated on:	Moisture, %	Ash, %	Fats, %	Non-nitrated extractible substances
Beechwood prehydrolysate	9.27	7.37	1.35	30.69
Waste sulphite liquors	5.9-9.5	6-8	2.2-7.0	33-35

Aminoacid composition in the proteins of fodder products is a major indicator of their food value (Stanciu et al. 1992). The quality of protein from yeast cultivated on the beechwood prehydrolysate is emphasized by means of the values concerning the high content in the three basic aminoacids shown in Table 5: lisine, methionine, and indole amino-propionic. The resulting yeasts contain proteins with a high biological value considering the aminoacid content is ranged at the level of yeasts cultivated on waste liquors (lisine 36.8 g/kg, methionine 7.1 g/kg, arginine 29.3 g/kg).

Table 5 Aminoacid Content in Yeasts Cultivated on Various Sublayers

Aminoacid	Content in g/kg		
	Beechwood prehydrolysate	n-paraffin	Waste sulphite liquors
Lisine	36.8	38.7	40.5-44.0
Histidine	7.2	9.8	11.3-14.9
Arginine	29.3	22.6	22.5-32.0
Treonine	20.4	28.3	25.0-27.6
Glicine	18.4	23.5	-
Alanine	18.6	33.0	-
Valine	17.2	30.7	30.8-33.1
Leucine	39.6	37.9	36.0-37.2
Methionine	7.1	6.9	8.5-30.0
Cystine	5.5	4.9	-
Indole amino-propionic	4.2	6.1	3.0-5.3

For a full chemical characterization of yeast, the content in main macro and microelements has been assessed by atomic absorption spectroscopy (Table 6). Determination of heavy metals in yeasts (especially lead and cadmium) has a major importance on animal feeding (Tănase et al., 1994, Stanciu, 1996). Table 6 shows the working conditions for spectrometric measurements. Spraying has been carried out in the air - acetylene flame.

Table 6 Instrumental Conditions to Determine Macro and Microelements in Fodder Yeasts

Element	Wave length (nm)	Slot width (nm)	Lamp intensity (mA)	Air- acetylene ratio
Mo	313.3	0.30	6	50/70 N_2O-C_2H_2
Cu	324.3	0.30	5	75/55
Co	240.7	0.10	5	70/70
Mn	279.5	0.20	5	75/55
Zn	213.9	0.20	6	70/50
Cd	228.8	0.30	7	75/55
Pb	283.3	0.40	5	75/55

Denaturation of fodder yeast samples has been carried out in an acid way, using a mixture of H_2SO_4 +H_2O_2 (1:2.2) HNO_3 + $HClO_4$ (1:6), calcination at 500ºC and ash solution in concentrated hydrohloric acid C in case of lead measurement. Destruction with H_2SO_4 +H_2O_2 is carried out in approximately 13 hours while the destruction with HNO_3 + $HClO_4$ is carried out in 5 hours. To this effect, the existing standards envisage the following maximum allowable requirements for heavy metals in food stuffs and fodder ingredients: maximum lead 5 mg/kg and cadmium 1 mg/kg.

Table 7 Content of Microelements in Yeasts Multiplied on Beechwood Prehydrolysate (in µg/g)

Element	Destruction with H_2SO_4 +H_2O_2 (1:2)			Destruction with HNO_3 + $HClO_4$ (1:6)		
	P_1	P_2	P_3	P_1	P_2	P_3
Mo	18.28	14.50	19.45	26.10	25.72	36.46
Zn	Traces	26.51	2.25	164.90	93.68	87.90
Co	4.24	5.85	7.28	5.32	4.20	4.51
Cu	11.13	16.50	18.00	18.29	19.49	19.80
Mn	301.80	291.36	303.51	297.10	303.11	295.90
Pb	10.90	10.75	16.21	11.80	10.20	16.80
Cd	1.60	1.85	2.72	1.14	1.48	2.88

The values of content in microelements measured for the delivery obtained are recommended to be used in feeding swine and poultry. Yeasts cultivated on the prehydrolysate assimilates Mn, Zn, Mo and Co very well. The nutritive value of yeast multiplied on beechwood prehydrolysate expressed by means of metabolizable energy (EM= 2780 Kcal) or net energy (EN-1677 Kcal) is similar to that concerning the other types of yeasts. These results were obtained by incorporating yeasts cultivated on prehydrolysate in a proportion of 4% in a structure of mixed fodder for chicken of 28.75 g/capita at a specific consumption of 2.45 kg/kg growth. This data of bioproductive effect is comparable with that registered at a reference lot that was fed with fodder containing 4% yeast cultivated on -paraffin.

CONCLUSIONS

Beechwood prehydrolysate has a specific composition and has a high content of total reducing substances, furfural, organic acids, and lignin substances. The lignin deposits resulting from prehydrolysis represent a major problem of chemical or biochemical recovery of prehydrolysate.

These deposits resulted from the polycondensation reactions between phenolic compounds, furfural, and its by-products in prehydrolysate. As regards the possibility of removing deposit formation or it is about a full removal or only a reduction (by removing furfural), hydrolysis reactions and lignin polycondensation cannot be eliminated. In the initial state, beechwood prehydrolysate has a low biological capacity and cannot be used as such at producing fodder yeasts.

The biological quality of prehydrolysate is improved following homogenization, aeration, dilution, furfural removing, neutralization operations that lead to reducing the content of inhibitors. Due to its properties (biomass yield, high protein content and inhibitor strength) introduction of *Candida scottii* yeast is recommended both as pure culture or mixed culture combined with other *Candida* yeast species.

Besides yeast production, the complex recovery technology concerning the prehydrolysate comprises furfural and methanol production and using acetic water in the prehydrolysis process can be incorporated into a biorefining concept.

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