

# LIGNOCELLULOSIC MATERIALS: A BINATIONAL PROJECT IN SINGLE CELL PROTEIN PRODUCTION

## *Materiales Lignocelulósicos: Proyecto binacional en producción de aminoácidos*

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### RESUMEN

*En este trabajo se analizan las proporciones de algunos aminoácidos de importancia nutricional en fuentes de proteínas convencionales, con las obtenidas a partir de materiales lignocelulósicos. También se comparan las reacciones de aminoácidos individuales con los aminoácidos esenciales totales (A/E) y la relación entre los aminoácidos esenciales totales y los totales recuperados como nitrógeno (E/T). Estos resultados junto a los valores químicos de los aminoácidos esenciales nos permitieron comparar las cualidades de estas proteínas obtenidas de las diferentes fuentes de carbono. Este tipo de análisis permitió seleccionar las proteínas más adecuadas para una función definida, ya sea como para una ración animal o como posible alimento humano.*

### INTRODUCTION

Lignocellulosic materials produced in the world and their potential conversion in to special products is still an open question. For our Latinamerican countries it is even a good chance of using efficiently our natural resources, especially the forest products of low economical value which are obtained from industrial use of *Pinus radiata* (Chile) (e.g.

### SUMMARY

*Rates of some amino acids having a nutritional significance in conventional protein sources together with those resulting from lignocellulosic materials are analysed here. Also a comparison of the relationship of individual amino acids to the essential total amino acids (A/E) together with the relation between the latter and the total ones recovered as nitrogen (E/T) is made. These results and chemical scores of essential amino acids allowed us to compare the qualities of these proteins obtained from the different sources of carbon. This kind of analysis made it possible to select the most suitable proteins for a particular purpose, either as animal or possible human food.*

barks, tannins, phlobaphenes, carbohydrate residues from organosolv pulping, etc.), from lignocellulosic waste materials (Brazil) (rice hull, orange bagasse), and from olive milling wastewater (alpechin, Spain). Many studies in the single cell proteins (SCP) and enzyme production have been examined, but further research with new lignocellulolytic microorganisms is still necessary (Duran, 1989, 1990). The ascomycete *Chrysonilia sitophila* Mont von Arx (TFB-

27441 strain) was isolated from a xilophagous insect (Campos *et al.* 1986; Ferraz *et al.* 1989). This strain, which exhibits high ligninolytic activity was efficient to degrade several organic materials (Duran *et al.*, 1987; 1988a; 1988b; O'Reilly *et al.*, 1991; Reyes *et al.*, 1992).

Since few lignocellulolytic microorganisms have been studied for direct SCP production from lignocellulosic materials (Tanaka *et al.* 1987), the *C. sitophila* (TBF-27441) as a potential microorganism for production of SCP with cellulolytic, proteolytic and ligninolytic enzymes were investigated using representative inexpensive carbon source materials from Chile, Brazil and Spain.

## MATERIALS AND METHODS.

**Samples:** Samples of bark were taken separately from 30 years old *Pinus radiata* as published (Baeza *et al.*, 1986). Phlobaphene and tannins were extracted as described previously (Baeza *et al.*, 1985). The insoluble material after solvent extraction (bark residue) was extracted with 1% NaOH to remove phenolic acids. This represents the lignocellulosic residue. Samples of rice hull from Goiás (Brazil) rice plantation were used. Olive milling wastewater the by product of olive oil technology was obtained from Spain (used as 10% as carbon source). Brown, pelleted orange bagasse (S.P. State), milled to 1 mm particle size was used as the sole carbohydrate source suspended in Czapeck solution.

Sugar residues were obtained from organosolv pulping of *Pinus radiata* chips with formic acid and posterior lignin precipitation and exhaustive formic-water solution evaporation (Baeza *et al.*, 1992). All the solid carbon sources were used as 1.5% concentration. The irradiation on rice hull and in olive milled wastewater were carried out with HQL-OSRAM mercury vapor lamp at a flux of 68 W/m<sup>2</sup> at 300 nm (using glass plates as the filter) (Duran *et al.*, 1986).

**Microorganism.** *C. sitophila* (TBF-27441 strain) was selected and cultured in a Czapek or Fries medium (Duran *et al.*, 1988a; Ferraz *et al.*, 1989). All the experiments were made by this method in triplicate. Amino acids from fungal mycelium were analyzed either by a Beckman Model 119 CL Amino acid Analyzer or a Aminochrom II, Labotron System. All the tables cited were averaged from duplicate hydrolysis reproducible to within 3-5%.

## RESULTS AND DISCUSSION

The evaluation of single cell proteins produced by *C. sitophila* with different carbon sources was performed.

Amino acids content of common carbon sources, as saccharose and others like wood components and ligno-cellulosic materials were determined. Most of the amino acids in all mycelial protein obtained from the substrate showed a distribution similar or higher to that of the FAO standard requirement, excepting in the methionine content using saccharose (not showed).

Methionine was deficient in rice hull, but after pre-

irradiation a high content was found (10.43%). Both sources exhibited a tyrosine and phenylalanine deficiency. A significant methionine content was observed when orange bagasse was used (6.70 %).

In Table 1 it is compared the mycelial protein, that exhibited the better qualities as compared to the FAO standard requirements, following the ratio of some essential amino acids of nutritional significance with know proteins (FAO, 1970).

Biological quality in a protein can be assessed not only by the absolute amount of each essential amino acid but also by their internal balance. Isoleucine, for example, should keep a molar ratio not lower than 1:3 (0.33) with leucine. Threonine is another essential amino acid found in short supply in vegetable proteins and encountered in high quality proteins in almost equimolar rates with serine. Table 1 shows that low quality proteins, usually of vegetable origin, are rich in arginine and histidine, in relation to lysine. These data confirm to improvement of the internal amino acid balance throughout fermentation, with the exception of isoleucine/leucine which, nevertheless remains at acceptable values in sugar residue, rice hull and irradiated alpechin. The high lysine/arginine rate of mycelial protein when orange bagasse and alpechin/hv as inductor were used, was the result of the low level of arginine found in *C. sitophila*. Likewise, the low content of tyrosine in the fungus was responsible for the extremely low tyrosine/phenylalanine rates observed in orange bagasse, alpechin/hv and tannin cultures. Low phenylalanine content in novel proteins would be desirable for phenylalanine intolerant individuals.

Table 2 shows the nutritional significant amino acid for two known proteins as casein and egg proteins. A comparison of the rates of individual amino acids, total essential amino acids (A/E rate) and total essential amino acid to total amino acids recovered as nitrogen (E/T rate), for the various substrates, is shown also in Table 2. The E/T rates of all substrates compared rather well with those for the casein reference, while some substrate exhibited much higher values than the value of 2.02 for the FAO provisional reference protein (FAO-WHO, 1965). All the substrates had A/E rates equal to or greater than those of egg protein for isoleucine, excepting by sugar residue and alpechin/hv. Leucine was equal as the standard proteins. Lysine was deficient only in bark, rice hull and tannin. In term of total aromatic compounds almost all the substrate induced a deficiency in these amino acids, except for alpechin/hv as carbon source. In general all the mycelia were sulfur compounds deficient except for with rice hull/hv and in orange bagasse. Threonine and tryptophan were higher than the standard carbon sources. Valine was deficient in rice hull as carbon source.

Chemical scores were calculated using National Academy of Science/National Research Council Essential Amino Acids Scoring Pattern (NAS, 1980) (Table 3). The total sulphur-containing amino acids were the most limiting amino acid in the proteins obtained when saccharose, rice hull, alpechin/hv, tannin and phlobaphene were used as substrates. Higher values than those of the standard proteins in the case of sugar residue and rice hull/hv were obtained. Histidine the

**Table 1. Rates of some aminoacids of nutritional significance in conventional and non-conventional protein sources (FAO. 1970).**

Source	ILE/LEU	TYR/PHE	LYS/HIS	LYS/ARG	THR/SER
Milk	0.65	1.05	2.95	2.12	0.78
Egg(Whole)	0.75	0.74	2.66	2.66	0.59
Meat (bovine)	0.64	0.82	2.51	1.35	1.05
Rice	0.54	0.91	2.34	0.68	0.77
Maize	0.35	1.34	1.39	0.82	0.70
Sorghum	0.34	0.55	1.41	0.72	0.71
Wheat Flour	0.67	0.46	1.69	0.76	
Peanuts	0.67	0.71	1.45	0.33	0.40
Yeast	0.57	0.95	2.58	1.70	1.04
Orange Waste	0.83	0.49	1.15	0.60	0.94 (a)
FAO	0.88	1.00			
Soybean	0.75	0.75	2.34	0.93	0.93
Casein	0.54	1.10	3.95	2.03	0.73
SCP					
Induced By:					
Orange Waste	0.68	0.02	2.69	4.31	1.08
Saccharose	0.76	0.95	2.59	1.42	1.12
Sugar Residue	0.30	0.82	2.33	0.88	0.85
Bark	1.47	1.02	3.78	1.78	0.75
Rice Hull	0.37	1.00	--	1.86	2.90
Rice Hull/hv	-	1.00	--	--	0.84
Alpechin	0.58	0.64	3.30	1.49	1.25
Alpechin/hv	0.35	0.29	4.75	4.00	1.12
Lignocel.Res	0.73	0.87	2.45	1.10	1.08
Tannin	0.81	0.14	0.56	0.27	1.12
Phlobaphene	1.18	0.87	2.68	0.60	0.78

a) Reyes et al 1992.

**Table 2. Nutritional Significant Amino Acids (a,b).**

A/rate (c)	S.	B.	SR.	R.	Rhv	AL.	ALhv	L.	O.	T.	PHL	C.	E
Isoleucine	126	263	78	102	0.3	109	90	141	103	180	220	106	129
Leucine	167	186	261	273	304	187	254	189	150	223	187	194	172
Lysine	144	96	136	95	158	147	158	152	159	30	122	169	125
Total arom.	176	128	194	0.6	0.6	155	211	165	85	115	149	226	194
Phenylalani	90	63	107	0.3	0.3	94	163	88	83	101	80	108	114
Tyrosine	86	66	97	0.3	0.3	61	48	77	2	14	69	118	81
Total Sulfur containing Amino acids	36	53	78	0.6	301	38	17	19	148	53	25	52	107
Cysteine	2	0.2	39	0.3	0	19	0.2	2	0.2	2	11	6	46
Methionine	34	53	39	0.3	301	19	17	17	148	15	51	51	61
Threonine	148	103	107	495	197	105	115	128	140	152	111	88	99
Tryptophan	37	27	30	33	38	27	27	35	29	35	32	24	31
Valine	167	135	116	0.3	0.3	260	128	151	186	214	155	137	141
E/Rate (d)	2.4	3.2	2.7	2.4	2.2	3.1	3.0	2.5	2.8	2.36	2.6	2.9	3.2
												(e)	(f)

a) S=Saccharose; B: Bark; SR: Sugar residue; R: Rice hull; Rhv: Rice hull irradiated; AL: Alpechin;ALhv: Alpechin irradiated; L:Lignocellulosic residue; C: Casein; E: Eggs; O: Orange residue; T:Tannin;PHL:Phobaphene. (b) Tryptophan taken as minimum of 1.3 g per 16 g N; (c) Individual amino acid (mg) per g of total essential amino acids; (d) Total essential amino acids (g of amino acid) per g of total recovered nitrogen; (e) Calculated from data Ref.(Dong et al., 1987); (f) Ref. (Houston et al.,1969).

Amino Acid	Table 3. Chemical scores calculated limiting Amino Acids (a,b).											
	C	S.	B.	SR.	R.	Rhv	AL.	Alhv	L.	O.	T.	PHL
H.	118	127	77	140	0.6	0.6	123	94	150	155	120	115
I	116	117	333	80	95	0.2	124	102	128	109	164	222
L	129	93	136	162	152	150	128	174	113	96	123	144
Ly	154	109	98	115	73	107	137	149	123	139	22	102
Th	117	163	150	132	555	195	143	157	151	179	166	135
Try	100	118	118	118	118	118	118	118	118	118	118	118
Va	133	134	145	105	0.2	0.2	258	128	131	172	170	137
TSC	102	54	105	129	0.8	401	69	31	29	252	77	42
TAA	144	93	90	115	0.3	0.3	101	138	95	51	60	86

a) Abbreviation as Table 2; b) calculated using the scoring in NAS (1980) calculations based on total amino acids recovery. Chemical score (g aminoacid/16 g of test protein)(g amino acid/16 g of reference pattern) x 100.

second limiting factor with all the substrates.

Although chemical scores have inherent limitations and therefore are only an approximation of the nutritional quality (Dong *et al.*, 1987), these values are complements of those obtained with the other methods.

In summary, considering the casein as pattern, the rate of important amino acids of significant nutritional values, E/T and A/E rates together with chemical scores indicated in our case that bark, orange bagasse and alpechin are the best carbon source for good quality single cell protein from *C. sitophila*.

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#### REFERENCES

Food Policy and Food Science Service, Nutrition Div.FAO.(1970). Amino Acid content of foods and biological data on proteins. FAO nutritional studies. Publication N 24, Rome, Italy.

Baeza, J.; Freer, J.; Rojas, N.; Palma, G.; Duran, N. (1986). Characterization of polyphenols extracted from wood. Bol. Soc. Chil. Quim 31:115-121

Baeza, J.; Freer, J.; Rojas, N.; Duran, N. (1985). A conductometric method for determination of phenolic groups in phobaphene and tannins from wood. Analyst. 110:1407-1409

Baeza, J.; Urizar, S.; Erismann, N de M.; Freer, J.; Schmidt, E.; Duran, N. (1992). Organosolv pulping V: Formic acid delignification of *Eucalyptus grandis* and *Eucalyptus globulus*. Bioresource Technol. 37:1-6

Campos, V.; Salas, E.; Duran, N.; Rodríguez, J.; Baeza, J. (1986). Isolation of cellulolytic *Chrysonilia sitophila* from *Tribolium ferrugineus*. Bol. Micol. 2:161-165

Dong, F.M.; Rasco, B.A.; Gazzaz, S.S. (1987). A protein quality assessment of wheat and corn distillers' dried grains with soluble. Cereal Chem. 64:327-332

Duran, N. (1989). Bioconversion to single cell protein: Recovery of lignocellulosic materials to produce human food as an integrated process. Part 1. Alimentos (Chile) 14: 39-50

Duran, N. (1990). Bioconversion to single cell protein: Recovery of lignocellulosic materials to produce human food as an integrated process. Part 2. Alimentos (Chile). 15: 51-61

Duran, N. & Gomez, E. (1986). Biomass Photochemistry-IX: Photochemical pre-treatment of cellulose and its effect on cellulase efficiency. J. Photochem. 35:109-115.

Duran, N.; Reyes, J.L.; Baeza, J.; Campos, V. (1988a). Biomass photochemistry XII: Chemical and photochemical pre-treatment of rice hull and its fungal degradation. Biotechnol. Bioeng. 32: 564-568

Duran, N.; Rodríguez, J.; Gomes, E.; Campos, V.; Baeza, J. (1988b). Biomass photochemistry: XI Photochemical pre-treatment of cellulose and its fungal degradation. Biotechnol. Bioeng. 31: 215-219

Duran, N.; Rodríguez, J.; Ferraz, A.; Campos, V. (1987). *Chrysonilia sitophila* (TFB-27441): A hyperlignilolytic strain. Biotechnol. Lett. 9:357-360

FAO-WHO, Protein requirements report by a joint FAO-WHO expert group. (1965). World Health Org. Tech. Rep. Ser. N301.

Ferraz, A. & Duran, N. (1989). Effect of various conditions of the *Chrysonilia sitophila* (TBF-27441) growth. Rev. Microbiol. 20:240-245

Houston, D.F.; Allis, M.E.; Kohler, G.O. (1969). Amino acid composition of rice and rice by-products. Cereal Chem. 46:527-537

National Academy of Science (NAS). (1980). Recommended dietary allowance, NAS, Washington, DC, pp. 39-54.

O' Reilly, S.; Erazo, S.; Campos, V.; Salas, E.; Baeza, J.; Ferraz, A.; Rodríguez, J. Duran, N. (1991). The effect of carbon sources on the single cell proteins and extracellular enzymes production by *Chrysonilia sitophila* (TBF-27441). Appl. Biochem. Biotechnol. 27:267-276

Reyes, J.L.; Amaya-Farfan, J.; Duran, N. (1992). Utilization of lignocellulosic materials: Orange waste as carbon source for single cell protein. Proc. Second Braz. Symp. on Chem. of Lignin and other Wood Components (N. Duran and E. Esposito, Eds.) FASESP Publ. (Campinas, S. P. Brazil) pp.410-415

Tanaka, M. & Matsuno, R. (1987). Conversion of lignocellulosic materials to single cell proteins (SCP): Recent development and problems. Enzyme Microbiol. Technol. 7: 197-206.