ELSEVIER

Food Chemistry 53 (1995) 157–163

0308-8146/95/$9.50

Protein concentrates from yeast cultured in wood hydrolysates

J. C. Parajó, V. Santos, H. Domínguez, M. Vázquez

Department of Chemical Engineering, University of Vigo (Campus Orense), Las Lagunas, 32004 Orense, Spain

&

C. Alvarez

COREN Industries, Polígono Industrial de San Ciprián de Viñas, Orense, Spain

(Received 13 July 1994; accepted 24 August 1994)

Candida utilis NRRL Y-900 cells were cultured in glucose solutions generated by enzymatic hydrolysis of pretreated *Eucalyptus globulus* wood. Yeast biomass was subjected to chemical treatments with NH_4OH solutions in order to obtain protein concentrates with high nutritive value having low contents of nucleic acids and high digestibility. Under selected conditions, 95% of the initial nucleic acids were removed from yeast cells. The protein recovery after the chemical treatments accounted for 88% of the amount contained in untreated biomass. The protein concentrates showed high *in-vitro* digestibility, and their amino acid profiles were not significantly altered in relation to those of untreated cells.

INTRODUCTION

The development of alternative technologies for production of foods with high protein content is an interesting research subject owing to an insufficient supply from the traditional protein sources such as meat, fish or eggs. Since protein accounts for a quantitatively important part of microbial cells (including bacteria, yeast, fungi or algae), these microorganisms (also named single-cell protein or SCP) can be considered as natural protein concentrates. For example, more than 50% of the dry weight of yeast biomass corresponds to true protein.

Lignocellulosics are cheap and largely available raw materials for microbial biomass SCP production by means of saccharification–fermentation processes. The polysaccharide fraction of substrates can be hydrolysed to sugars in reaction media catalysed by acids or enzymes, and the hydrolysates (after supplementation with nutrients) are useful as fermentation media for culturing microorganisms.

The enzymatic hydrolysis of native lignocellulosics proceeds with slow kinetics and gives poor yields. Both drawbacks can be overcome by performing chemical treatments of the raw materials that cause both delignification and structural modifications of the cellulosic fraction (Fan *et al.*, 1982; Blanch & Wilke, 1983). Improvements in the economics of these processes are achieved if the chemical stages lead to marketable by-products from the hemicellulose and lignin fractions of the lignocellulosic substrate (for example, furfural, pentose sugars or phenolic compounds useful as dispersants or as chemical intermediates for polymer manufacture) (Wright, 1988).

The chemical–biotechnological processing of lignocellulosics to obtain SCP as a single product is not feasible today from an economical viewpoint, but the interest in developing integrated processes leading to a variety of marketable end-products (SCP being one of them) has been noted (Goldberg, 1988). On the other hand, the possibility of processing the microbial biomass for production of protein concentrates with high added-value attracts research in this field.

The nucleic acid (NA) content of yeast SCP is the major factor restricting its utilisation as food (Roth, 1980). As is well known, the ingestion by humans of more than 2 g NA/day can result in increased levels of uric acid in serum and urine, leading to health disorders such as uric gout or kidney stones (Waslien *et al.*, 1968; Edozien *et al.*, 1970). In order to satisfy the protein requirements of human diets by means of SCP intake, the upper limit allowed for NA concentration is 2% (w/w) (oven-dry basis) (FAO/WHO/UNICEF, 1970), in comparison with 5–20% of NA by weight reported for yeast biomass grown under a variety of culture conditions (Kilhberg, 1972).

The reduction of the NA content of yeast cells can

be performed by chemical or biotechnological methods. Enzymatic procedures for NA removal are based on the utilisation of endogenous or exogenous nucleases (Schlenck & Dainko, 1965; Maul *et al.*, 1970; Castro *et al.*, 1971; Sánchez Montero *et al.*, 1989; Martínez *et al.*, 1990). Several chemical treatments of SCP (based on the utilisation of alkalis, acids or salts) have been reported (Hedenskog & Ebbighaus, 1972; Kihlberg, 1972; Newell *et al.*, 1975; Otero & Cabello, 1980; Damodaran, 1986; Alvarez & Enríquez, 1988; Gibert, 1986). Among them, NH_4OH-based technologies show several advantages, such as the high selectivity in NA degradation and the accessibility of the chemical agent to the cytoplasmic protein without a previous step of cell wall disruption (Alvarez & Enríquez, 1988).

The chemical processing of SCP can result in alteration of its amino acid profile (with generation of compounds toxic or without biological value) or reduced in digestibility. Both aspects have to be taken into account for an evaluation of the overall process.

In the present investigation, results are reported on the generation and chemical processing of *Candida utilis* NRRL Y-900 cells grown on enzymatic hydrolysates of pretreated *Eucalyptus globulus* wood. Wood was delignified with HCl-catalysed acetic acid solutions and the solid residue was subjected to a swelling treatment with NaOH solutions to enhance its susceptibility to the enzymatic hydrolysis. Glucose solutions were generated from the cellulosic fraction obtained in the above treatments by means of an enzymatic treatment with a mixture of cellulases and β-glucosidase. These hydrolysates, after supplementation with nutrients, were used as media for *Candida utilis* growth. The microbial biomass was processed to obtain protein concentrates with low NA content. Additional data were obtained on the selectivity of NA removal and on both amino acid profile and digestibility of the protein concentrates.

MATERIALS AND METHODS

Composition and chemical processing of wood

The samples of *Eucalyptus globulus* wood used in this work were taken from the same lot utilised in previous studies (Vázquez *et al.*, 1992). The wood content in moisture, lignin, cellulose and hemicelluloses was determined using standard methods (Browning, 1967). Wood delignification was performed under the operational conditions listed in Table 1, which were selected on the basis of a previous work reported by authors (Vázquez *et al.*, 1992). Delignified samples were filtered off and subjected to swelling treatments with NaOH solutions in order to cause structural modifications that improve their behaviour as hydrolysis substrates. The operational conditions used for this stage are also listed in Table 1. The composition of the solid residues obtained in both chemical treatments was determined by the same methods previously used for untreated wood.

Table 1. Operational conditions used for the chemical and biotechnological processing of *Eucalyptus* wood

(a) Delignification stage
- Normal boiling temperature
- Liquid/solid ratio = 10 g/g
- Acetic acid concentration = 95% (w/w)
- Catalyst (HCl) concentration = 0·15% (w/w)
- Reaction time = 5 h

(b) Swelling stage
- Temperature = 110°C
- Liquid/solid ratio = 9 g/g
- Sodium hydroxide concentration = 5%
- Reaction time = 1 h

(c) Enzymatic hydrolysis
- Temperature = 48·5°C
- pH = 4·85 (citric acid–sodium citrate buffer 0·05 M)
- Liquid/solid ratio: 20 g/g
- Cellulase/substrate ratio: 8 IU/g
- β-glucoidase/substrate ratio: 80 IU/g
- Maximum reaction time: 48 h

Generation of sugar solutions from processed solid residues

The solid residues obtained in the swelling stage, with increased cellulose content, were used as hydrolysis substrates in media containing cellulases (from *Trichoderma reesei*) and β-glucosidase (from *Aspergillus niger*). The enzymatic concentrates used were kindly provided by Novo Industries (Denmark). The operational conditions used for hydrolysis are listed in Table 1. At given reaction times, samples from the hydrolysis media were withdrawn and analysed for sugars using reported methods (Vázquez *et al.*, 1992).

Microorganism and culture conditions

Lyophilised broths of *Candida utilis* NRRL Y-900, obtained from the Northern Regional Research Center (USDA, Peoria, Illinois, USA) were proliferated and maintained in agar slants containing glucose and yeast extract. The enzymatic hydrolysates were supplemented with 1% peptone and 1% yeast extract, sterilized and used for cell proliferation under the following experimental conditions: orbital agitation (250 rpm); temperature, 30°C; 500 ml Erlenmeyer flasks with 100 ml culture medium. Samples from the fermentation media were withdrawn at selected fermentation times and centrifuged. The glucose concentration of supernatants was determined by the same methods as used in wood analysis. The cells were resuspended and used to determine the biomass concentration of fermentation media by spectrophotometric measurements.

Analytical characterisation and chemical processing of yeast biomass

Candida utilis cells were collected at the end of the log phase and assayed for true protein and nucleic acid content by the biuret and Schmidt–Tannahauser–Schneider methods, respectively (Herbert *et al.*, 1971).

The amino acid profile of biomass was determined by the Pico-Tag method (Waters Assoc.). The *in-vitro* digestibility of yeast cells was measured by the HCl–pepsin method (De Groot & Slump, 1967). Yeast biomass samples were subjected to treatments with ammonium hydroxide under the following operational conditions: NH_4OH concentration 3·6%; temperature, 75°C; reaction time = 30 min; cell concentration in reaction media = 6·6 g oven-dry biomass/100 g solution. The protein and NA content of the concentrates obtained from cell processing, as well as their amino acid profile and digestibility, were assayed by the same methods previously used for untreated cells.

RESULTS AND DISCUSSION

Chemical processing of wood

Eucalyptus wood treatments with HCl-catalysed acetic acid solutions allowed an efficient processing of the raw material. Under the experimental conditions used in this study, most of the hemicellulose and lignin fractions were degraded, whereas the cellulosic fraction remained almost intact in the solid phase. Since the reaction products from hemicelluloses (pentose sugars and furfural) have commercial value and the lignin degradation products show little chemical alteration (showing potential for use as chemical intermediates), the economic features of the overall proposed process are improved in relation with technologies leading to a single product.

The delignification step did not cause structural modifications in the cellulosic fraction, because of the essentially non-polar character of the concentrated solution of acetic acid used for this purpose. In order to reduce the crystallinity of substrate and to promote other related effects (such as increases in available surface area and pore size or decrease in polymerisation index), a subsequent swelling treatment with NaOH solutions was performed (see Vázquez *et al.*, 1992). Figure 1 summarises some material balances concerning the chemical processing of wood. On the basis of the results presented, the marked delignification achieved with this process, as well as the high selectivity in relation to cellulose hydrolysis are notable. Little chemical alteration was caused by the alkaline step, but this stage notably improved the susceptibility of the substrate to the enzymatic hydrolysis owing to the structural modifications induced in the solid phase.

Generation of sugar solutions from treated wood samples

Samples subjected to delignification and swelling treatments, having enhanced cellulose content with respect to that of the raw material, were used as substrates for the enzymatic hydrolysis of the polysaccharide fraction. Figure 2 shows the behaviour of processed wood samples as hydrolysis substrates under the experimental conditions selected (see Table 1). The results confirmed

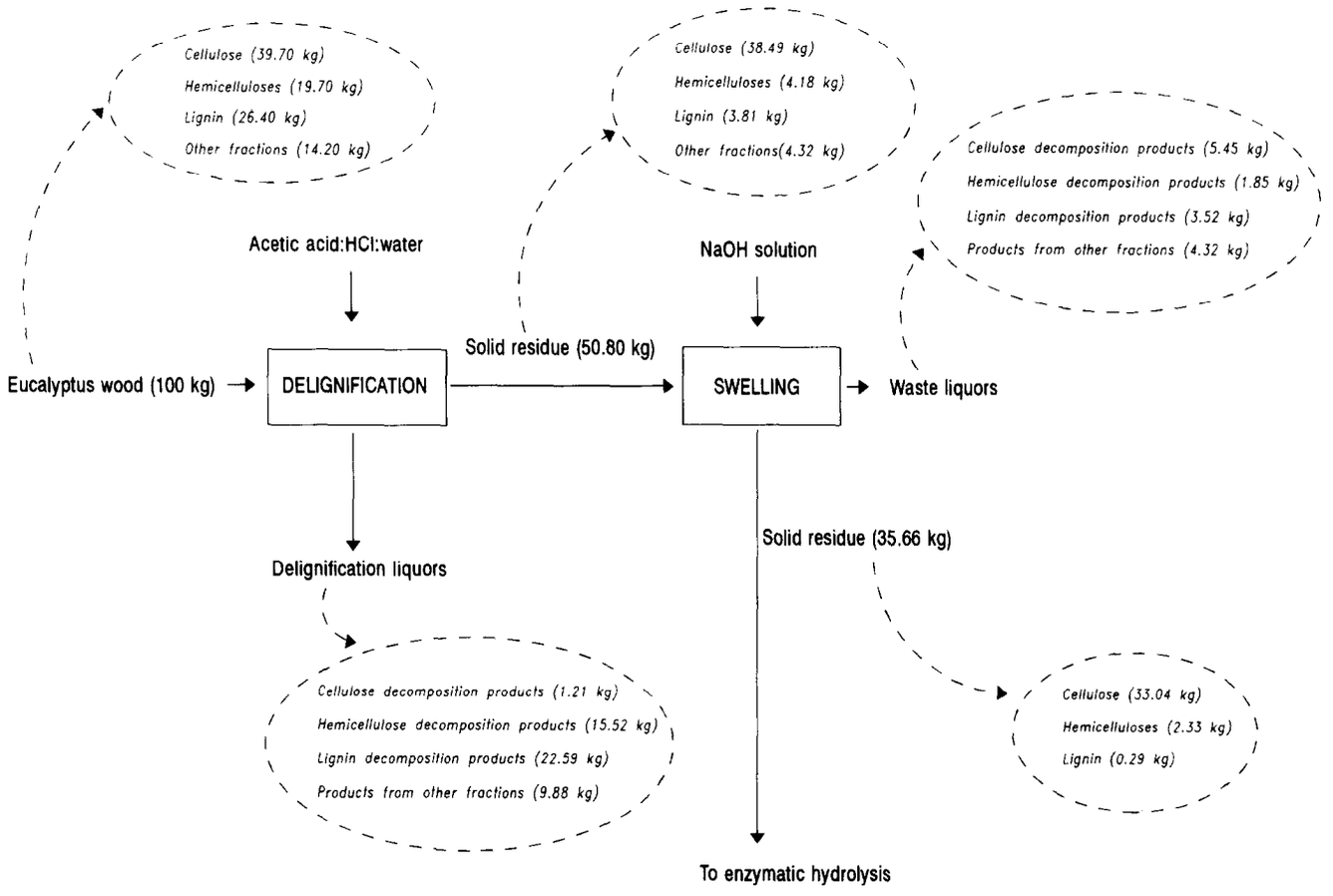

Fig. 1. Some material balances in the chemical processing of wood (data expressed on oven-dry basis).

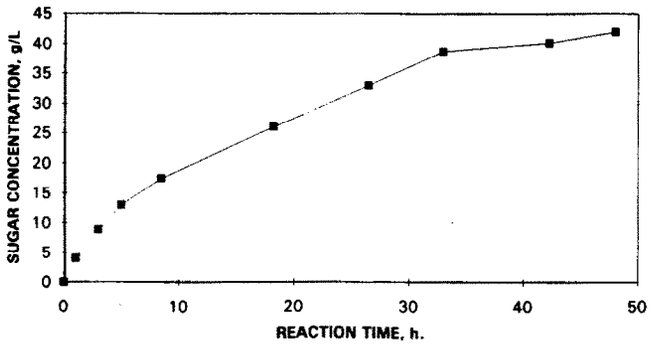

Fig. 2. Kinetic behaviour of treated wood samples as substrates for enzymatic hydrolysis.

that the solid residues obtained from chemical processing of wood were highly susceptible to enzymatic hydrolysis. The maximum concentration reached after 48 h reaction time was 42 g/litre, with a high polysaccharide conversion (82%). The conversions achieved have to be evaluated taking into account the limited cellulase/substrate ratio used in this work (8 IU/g).

Production and analytical characterization of SCP

Wood hydrolysates obtained as described in the above section were supplemented with nutrients, sterilized and used as a carbon source for culturing *Candida utilis* NRRL Y-900 cells. This strain has been selected because of its fast growth and high biomass yield: in fermentation runs lasting 48 h, biomass yields in the range 0·479–0·496 g biomass/g glucose consumed were obtained with this microorganism (Vázquez *et al.*, 1993). These data are near the theoretical yield (0·5 g biomass/g glucose consumed) (Solomon & Layokun, 1988).

Figure 3 shows the dependence with time of *Candida utilis* cell concentration and glucose consumption in batch experiments performed under the selected operational conditions. The results refer to experiments carried out in media formulated from biomass hydrolysates and in media containing commercial glucose, which were performed in order to provide information on differences of hydrolysates for bioconversion. No significant differences were observed between the bioconversion of hydrolysates and that of standard glucose solutions. Under the experimental conditions used, the glucose contained in the media was almost quantitatively consumed after 10–12 h, with a typical kinetic pattern.

Yeast cells collected at the end of the log phase were subjected to analytical determinations of true protein and NA. The results obtained are shown in Table 2. The true protein content of yeast biomass was near the values reported for experiments performed with the same microorganism in media containing 20 g glucose/litre and with a limited initial cell concentration (both aspects leading to a slow growth kinetics) (Vázquez *et al.*, 1993). On the other hand, the NA content of yeast cells increased from 6% reported for the above slow fermentations up to 10·8% obtained in this work.

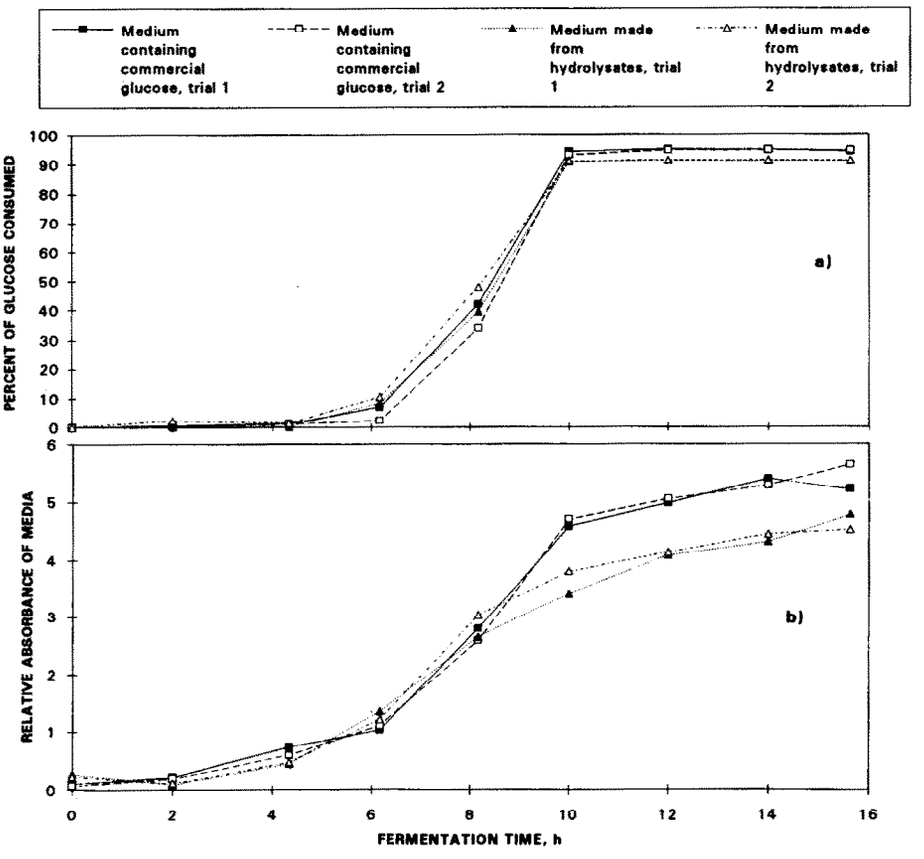

Fig. 3. Kinetics of *Candida utilis* growth on media generated from wood hydrolysates. (a) Sugar consumption as a function of fermentation time. (b) Dependence of cell concentration (measured as absorbances) on the fermentation time.

Table 2. Composition of yeast cells grown on hydrolysates and changes in composition associated to the NH_4OH treatments

(a) Data relative to yeast cells cultured on hydrolysates
- Nucleic acid content: 10·8% (w/w) (oven-dry basis)
- True protein content: 53% (w/w) (oven-dry basis)

(b) Treatment yield and composition of concentrates
- Yield of treatments: 71 g recovered/100 g untreated cells (oven-dry basis)
- Nucleic acids content: 0·8% (w/w) of treated samples (oven-dry basis)
- True protein content: 66% (w/w) of treated samples (oven-dry basis)

This fact agrees with the expected behaviour, since increased growth rates result in higher NA contents of cells (Kilhberg, 1972).

Taking into account that the NA content is the main factor limiting the nutritive quality of SCP, fast fermentations lead to a less suitable product than slow ones. Since the maximum profitability required for industrial processes is in contradiction with the utilisation of slow reaction stages, the yeast cells were subjected to chemical treatments in order to reduce the NA content of processed samples below the recommended limit of 2 g/100 g treated biomass (FAO/WHO/UNICEF, 1970).

Production and analytical characterisation of protein concentrates from SCP

The reduction of NA content of yeast cells can be accomplished by enzymatic or chemical methods. Enzymatic methods for NA removal can be based on the utilisation of exogenous or endogenous nucleases. High specificity and selectivity of reaction are common to enzymatic technologies, but important drawbacks have been reported, including the cost of catalyst and inhibition effects (for methods using exogenous nucleases) or difficulties in the control of the reaction (for methods involving endogenous nucleases). Chemical treatments of yeast cells allow a fast and extensive reduction in the NA content of SCP, but the reaction is not specific, and changes in amino acid composition limiting the nutritional value of concentrates can occur.

Most of enzymatic and chemical methods require a previous step of cell wall disruption, to make the cytoplasmic protein accessible to the hydrolysing agent. Among the chemical agents used for NA removal, NH_4OH shows several advantages, including (i) the cell wall disruption step can be avoided, because the cytoplasmic membranes are permeable to the chemical agent and to the reaction products, (ii) extensive NA removal can be achieved, and (iii) the reaction can be performed with good selectivity. NH_4OH-based treatments have been applied to various yeast cells, including *Saccharomyces cerevisiae*, *Kluyveromyces fragilis* and *Candida utilis*. Alvarez and Enríquez (1988) reported reductions in NA contents of *S. cerevisiae* and *K. fragilis* from 12–7% to 1·2–1·4%. Under selected conditions, Parajó *et al.* (1994) obtained protein concentrates with 0·5% NA from *Candida utilis* cells grown on 20 g/litre standard glucose solutions during 48 h (conditions leading to a NA content of 9·3%). This work studied the potentiality of NH_4OH treatments for reducing the NA content of *C. utilis* cells grown with fast kinetics (and so, having high NA concentration) in fermentation media formulated from wood hydrolysates.

The operational conditions used for cell treatments with ammonium hydroxide solutions were selected according to reported data (Parajó *et al.*, 1994). Table 2 lists the results obtained for the extraction yield (measuring the percent of substrate recovered after treatments) and analytical results on the true protein and NA contents of concentrates. It can be noted that the NA concentration of the processed product is below the limit of 2% (w/w), and that the concentrates obtained have a content in true protein higher than that of untreated yeast cells. Taking into account the extraction yield, it can be calculated that 95% of the initial nucleic acids were removed during treatment, and that 88% of the initial protein was recovered in the treated substrates. Both results compare favourably with related data reported for chemical processes of NA removal in yeast cells (Hendenskog & Ebbighaus, 1972; Kihlberg, 1972; Newell *et al.*, 1975; Otero & Cabello, 1980; Damodaran, 1986; Gibert, 1986; Alvarez & Enríquez, 1988).

Amino acid profile and digestibility of the protein concentrates obtained

The nutritive value of protein substrates depends on their contents in NA and true protein, but also on other factors (such as amino acid profile and digestibility) which merit consideration.

The protein fraction of untreated SCP is characterised by a well-balanced amino acid profile, with deficiency in sulphur-containing amino acids. Table 3 shows reported results for the amino acid pattern of yeast protein (Kilhberg, 1972; Anderson *et al.*, 1988; Gálvez *et al.*, 1989; Singh & Neelekantan, 1989; Lyutskanov *et al.*, 1990). Experimental data on the amino acid profile of untreated and treated biomass of *Candida utilis* cultured in wood are also included in the same table. In the present case, the content in sulphur-containing amino acids (cystine, methionine and tryptophan) was not determined owing to the limitations of the analytical procedure. It can be noted that the experimental results obtained in this study for untreated *Candida utilis* yeast cells are in good agreement with data reported for other yeasts. Owing to the comparatively high contents of yeast biomass in lysine and threonine, its utilisation for supplementation of cereal diets (poor in the above amino acids) has been proposed (Kilhberg, 1972).

Previous studies (De Groot & Slump, 1967) reported modifications in the chemical composition of proteins caused by alkaline treatments of substrates. New amino acids (such as lysinoalanine, alanthionine and ornithinoalanine) can be generated in alkaline media under

Table 3. Amino acid profiles of yeast and protein concentrates (results expressed as g amino acid/100 g protein)

Amino acid	Reported range for yeast protein[a]	This work	
		Untreated cells	Protein concentrates
Aspartic acid	8·2–11·4	8·4	9·6
Threonine	4·1–6·5	6·2	7·0
Serine	3·7–7·0	5·7	5·9
Glutamic acid	13·4–18·0	16·7	13·0
Proline	2·3–5·2	4·1	5·4
Glycine	3·8–5·8	5·1	4·5
Alanine	6·4–10·8	7·4	6·7
Cystine	0·7–1·9	—	—
Valine	5·3–7·8	6·5	5·3
Methionine	1·1–2·0	—	—
Isoleucine	4·3–6·7	5·6	5·9
Leucine	7·0–11·0	8·3	8·8
Tyrosin	2·5–4·0	4·0	4·3
Phenylalanine	3·6–5·1	4·5	5·0
Lysine	5·9–11·1	8·1	8·5
Histidine	1·5–4·0	2·5	2·6
Arginine	5·6–7·4	6·5	7·6
Tryptophan	0·7–1	—	—

[a]Yeasts considered: *Saccharomyces cerevisiae, Saccharomyces fragilis, Klyveromyces marxianus, Kluyveromyces fragilis* and *Candida pseudotropicalis*. Data obtained from the following references: Kilhberg (1972); Anderson *et al.* (1988); Singh & Neelekantan (1989); Gálvez *et al.* (1989); Lyutskanov *et al.* (1990).

certain operational conditions from the corresponding amino acids. The content in lysinoalanine has been considered as a criterion to evaluate the alkali damage of substrates (De Groot & Slump, 1967). Table 3 shows that a close interrelationship exists between the data obtained for untreated cells and those corresponding to the protein concentrates resulting from the NH_4OH treatments. The chromatograms obtained for the protein concentrates did not show peaks corresponding to the new amino acids or unidentified compounds.

According to published results (Roth, 1980), untreated yeast biomass shows a high percent of biological utilisation. However, processed protein concentrates can show a different behaviour: decreased digestibilities have been reported for some alkali-treated substrates. In order to obtain information on this possibility, protein concentrates obtained by NH_4OH treatments of cells were subjected to *in-vitro* digestibility tests. The results obtained (88% digestibility) confirmed the high nutritive value of the processed product obtained.

CONCLUSIONS

The scheme proposed for *Eucalyptus* wood processing allowed an efficient utilisation of the raw material. The solid residues obtained by chemical processing showed a favourable behaviour as substrates for enzymatic hydrolysis. Glucose concentrations of 40 g/litre, corresponding to a high polysaccharide conversion, were obtained in hydrolysis trials. The fermentation media made from hydrolysates were suitable carbon sources for *Candida utilis* growth. Almost quantitative conversion of the glucose contained in fermentation media was achieved in 12 h, with a typical kinetic pattern. The yeast cells contained 10·8% NA, far above the maximum recommended concentration in food (2%). The NH_4OH treatments performed for improving the nutritional properties of biomass led to a substantial reduction in the NA content of concentrates (with 95% of NA removal). Reduced protein losses were caused by these treatments (12% of the initial amount). Additional favourable characteristics of the protein concentrates obtained are both their amino acid profiles (similar to the corresponding untreated yeast cells) and their high *in-vitro* digestibility. These results confirm the importance of the processing scheme studied.

REFERENCES

Alvarez, R. & Enríquez, A. (1988). Nucleic acid reduction in yeast. *Appl. Microbiol. Biotechnol.*, **29**, 208–10.

Anderson, P. J., McNeil, K. E. & Watson, K. (1988). Thermotolerant single cell protein production by *Kluyveromyces marxianus* var. *marxianus*. *J. Ind. Microb.*, **3**, 9–14.

Blanch, H. W. & Wilke, C. R. (1983). Sugars and chemicals from cellulose. *Rev. Chem. Engng*, **1**, 71–118.

Browning, B. L. (1967). In *Methods of Wood Chemistry*. John Wiley & Sons, New York, USA.

Castro, A. C., Sinskey, A. J. & Tannenbaum, S. R. (1971). Reduction of nucleic acid content in *Candida* yeast cells by bovine pancreatic ribonuclease A treatment. *Appl. Microbiol.*, **22**, 422–7.

Damodaran, S. (1986). Removal of nucleic acids from yeast nucleoprotein complexes by sulfitolysis. *J. Agric. Food Chem.*, **34**, 26–30.

De Groot, A. P. & Slump, P. (1967). Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. *J. Nutr.*, **98**, 45–56.

Edozien, J. C., Udo, U. U., Young, V. R. & Scrimshaw, N. S. (1970). Effects of high levels of yeast feeding on uric acid metabolism of young men. *Nature*, **228**, 180.

Fan, L. T., Lee, Y. H. & Gharpuray, M. M. (1982). The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis. *Adv. Biochem. Engng*, **23**, 157–87.

FAO/WHO/UNICEF/ (1970). *Single Cell Protein*. Protein Advisory Group, Technical Report no.4.

Gálvez, A., Ramírez, M. J. & García, M. (1989). Chemical composition of a mixture of Single Cell Protein obtained from *Kluyveromyces fragilis* and whey proteins. *Archivos Latinoamericanos de Nutrición*, **40**, 252–62.

Gilbert, E. (1986). Production of single-cell protein (SCP) with reduced nucleic acid content. In *Int. Congr. Biochem. Eng.*, H. Chimiel & W. P. Hammes, Bonn, Germany, pp. 464–8.

Goldberg, I. (1988). Future prospects of genetically engineered single-cell protein. *Tibtech*, **6**, 32–4.

Hedenskog, G & Ebbinghaus, L. (1972). Reduction of the nucleic acid content of Single-Cell Protein concentrates. *Biotechnol. Bioengng*, **24**, 447–57.

Herbert, D., Phipps, P. J. & Strange, R. E. (1971). Chemical analysis of microbial cells. *Meth. Microbiol.*, **5B**, 249–300.

Kilhberg, R. (1972). The microbe as a source of food. *Ann. Rev. Microbiol.*, **26**, 427–66.

Lyutskanov, N., Koleva, L., Stateva, L. Venkov, P. & Hadjiolov, A. (1990). Protein extracts for nutritional purposes from fragile strains of *Saccharomyces cerevisiae*: Reduction of the nucleic acid content and applicability of the protein

extracts. *J. Basic Microbiol.*, **30**, 523–8.

Martínez, M. C., Sanchez Montero, J. M., Sinisterra, J. V. & Ballesteros, A. (1990). New insolubilized derivatives of ribonuclease and endonuclease for elimination of nucleic acids in single-cell protein concentrates. *Biotechnol. Appl. Biochem.*, **12**, 643–52.

Maul, S. B., Sinskey, A. J. & Tannenbaum, S. R. (1970). A new process for reducing the nucleic acid content of yeast. *Nature*, **228**, 181.

Newell, K. A., Robbins, E. E. & Seeley, R. D. (1975). Process of making yeast protein isolate having reduced nucleic acids content. US Patent number 3, 867,255.

Otero, M. A. & Cabello, A. J. (1980). Single-cell protein low in nucleic acids by alkaline treatment. *Biotechnol. Lett.*, **2**, 379–84.

Parajó, J. C., Santos, V., Domínguez, H., Vázquez, M. & Alvarez, C. (1994). Protein concentrates with high nutritive value from wood. In: *Biotec94*. Sociedade Portuguesa de Biotechnologia, Algarve, Portugal.

Roth, F. X. (1980). Microorganisms: a source of protein for animal nutrition. *Animal Res. Dev.*, **12**, 7–19.

Sánchez-Montero, J. M., Sinisterra, J. V. & Ballesteros, A. (1989). DNase activity of micrococcal endonuclease insolubilized on corn cob. *Appl. Biochem. Biotechnol.*, **22**, 205–14.

Schlenk, I. & Dainko, J. L. (1965). Action of ribonuclease preparation on viable yeast cells and spheroplasts. *J. Bacteriol.*, **89**, 13–18.

Singh, K. & Neelekantan, S. (1989). Amino acid composition of yeast single cell protein grown on paneer whey. *J. Dairy Sci.*, **56**, 813–15.

Solomon, B. O. & Layokun, S. K. (1988). Studies on the potential of cashew apple for bioconversion to single-cell protein. *Proc. Biochem.*, **8**, 97–9.

Vázquez, D., Lage, M. A., Parajó, J. C. & Alonso, J. L. (1992). Empirical assessment on the cellulase digestibility of processed *Eucalyptus* wood. *Appl. Biochem. Biotechnol.*, **37**, 123–39.

Vázquez, D., Lage, M. A., Parajó, J. C. & Alonso, J. L. (1993). Obtención de proteína unicelular (single cell protein) por procedimientos basados en el fraccionamiento de biomasa celulósica. I. Procesamiento de la madera de eucalipto. *Afinidad*, **4**, 213–20.

Waslien, C. I., Calloway, D. H. & Margen, S. (1968). Uric acid production of men fed graded amounts of egg protein and yeast nucleic acid. *Am. J. Clin. Nutr.*, **21**, 892–7.

Wright, J. D. (1988). Ethanol from biomass by enzymatic hydrolysis. *Chem. Eng. Prog.*, **10**, 63–74.