BIOPROCESS APPLIED TO THE TREATMENT OF RESIDUAL WATERS OF COFFEE WET PROCESSING FOR THE INCORPORATION OF SELECTED YEAST STRAINS *

MARCIA REGINA BEUX **
CARLOS ITSUO YAMAMOTO ***
CRISTINA MARA GUOLO WINTER ****
WILTON FONTES RAMOS *****
FABIANA NICOL BARBIERI *****
JOSÉ LAURENTINO FERREIRA ******
CARLOS RICARDO SOCCOL *******

The aim of the present work was to isolate yeast strains able to degrade the degumming water originated from the wet processing of coffee, characterizing the formed metabolites. The drink prepared from the treated grains by wet processing is considered of better quality when compared with the grains treated by dry process. However, the great polluent potential of the residual water from this processing represents serious concern for the environment. The degumming water essentially presents organic contamination, with Oxygen Biochemical Demand (OBD) and Oxygen Chemical Demand (OCD) rates superior to 20.000 mgO_2/L. *Candida tropicalis, C. lypolitica*, *C. guilliermondii*, *C. parapsilopsis*, *Kloeckera apiculata* e *Rhodotorula rubra,* isolated from coffee cheeries were inoculated in flasks containing the degumming water and then submitted to constant agitation (120 rpm) for 48 hours at 28ºC. The isolated species revealed capacity to degrade the substrate, however *C.parapsilopsis* showed reduction percentages of OBD and OCD rates of 50.14% e 29.81%, respectivelly, showing the viability of the use of those microorganisms as biological depollutants.

KEY-WORDS: COFFEE; DEGUMMING WATER; YEAST.

* Parte do projeto de tese de Doutorado em Tecnologia de Alimentos da primeira autora.

** Doutoranda, Programa de Pós-Graduação em Tecnologia de Alimentos, Universidade Federal do Paraná (UFPR), Coordenadora da Área Biológica do Centro de Pesquisa e Processamento de Alimentos (CEPPA), Professora da Disciplina de Controle de Qualidade do Curso de Farmácia e Bioquímica, Pontifícia Universidade Católica do Paraná (PUC), Curitiba, PR (e-mail mrbeux@engquim.ufpr.br).

*** Doutor em Engenharia Química, Professor do Departamento de Engenharia Química, UFPR e Chefe do CEPPA.

**** Farmacêutica Industrial, Especialista em Vigilância Sanitária em Alimentos, Gerente-Técnica do Laboratório de Físico-Química de Alimentos, CEPPA, Curitiba, PR.

***** Técnicos em Saneamento Básico e Ambiental do Laboratório de Físico-Química de Águas e Efluentes, CEPPA, Curitiba, PR.

****** Físico do Laboratório de Alimentos do Instituto de Tecnologia do Paraná (TECPAR), Especialista em Controle Estatístico de Qualidade, Curitiba, PR.

******* Orientador do Projeto de Tese de Doutorado, Pós-Doutorado em Biotecnologia, Institut Français de Recherche Scientific Pour le Devellopement en Cooperat, Orstom, França; Professor Coordenador, Curso de Engenharia de Bioprocessos e Biotecnologia, UFPR (e-mail: soccol@ ufpr.br).

1 INTRODUCTION

Brazil is the largest producer of coffee, being responsible for 28% of the world production. The State of Paraná contributes with 5.8% of the total produced in the country (DEMARCHI, 2001).

Although it exists about twenty-five varieties of coffee, only two are commercially cultivated: the Arabic and the Robust. *Coffea arabica* produces coffee of better quality when compared to the elaborated from fruits of *Coffea canephora*, considered inferior due to the most acidic flavor (PETTIGREW, 1999).

In Brazil, with regard to the utilized harvest method, the fruit of the coffee tree must be clean and separated. It may be processed integrally by dry processing or without husk and mucilage by wet processing, originating the de-hulled and de-pulped grains. (MATOS, et al., 2003).

The wet processing of the fruit produces a softer drink and after the harvest the removal of the epicarp is held by mechanical de-huller or a de-pulper. In the sequence, the operation called degumming takes place, in which the mesocarp (the mucilage adhered to the endocarp) is removed. The degumming operation consists in the immersion of the de-hulled fruits in water for a 24 hours period, after which they will be washed in current water, generating great amounts of residual waters (DIAS & BARROS.,1993).

The generated fermented degumming water is rich in organic matter and due to its high pollutant capacity its disposal became undesirable, without treatment in water courses (ROLZ et al.,1982).

Selected strains of microorganisms may chemically decompose the polluent constituents present in the residues being considered as the more ecological treatment employed until the present moment (FURTADO, 1997).

Certain strains may degrade phenols, hydrocarbons and even chlorinated aromatic compounds. While some microorganisms are particularly efficient in decontaminate polluted soils, others are more utilized in industrial wastewater treatment. For example, *Pseudomonas halodesnitrificans*, in absence of oxygen transforms by reduction,

nitrates in molecular nitrogen, inert gaseous element (DESPOLUIÇÃO..., 2000).

As stated by MACÊDO (2000) biodepollution may be defined as the incorporation of microorganisms to the wastewater treatment station, being considered a progress in biotechnology. When applied to liquid or solid organic residues, changes the physical-chemical properties, enhancing the degradation rates and making it suitable for disposal in water courses. The degradation rates may be evaluated by the Oxygen Biochemical Demand (OBD) and by Oxygen Chemical Demand (OCD). The OBD is a well utilized parameter and gives information about the concentration of oxygen necessary to biologically oxidize the organic material present in water (KAWAI, 1991; SPERLING, 1996). The OCD, is also being employed as it offers faster operations. The oxygen present in organic matter that can be oxidized, is measured by utilizing a oxidizing agent extremely acid (BRAILE & CAVALCANTI,1979).

The aim of the present work was to isolate yeast strains capable of degrading the degumming water originated from the wet processing of coffee and to characterize the formed metabolites.

2 MATERIAL AND METHODS

This research was realized at Centro de Pesquisa e Processamento de Alimentos (CEPPA) and at Laboratório de Processos Biotecnológicos of Universidade Federal do Paraná, during the period of July 2001 to July 2003.

The coffee cherries samples of 2000/2001 crops and the degumming water were gently given by Instituto Agronômico do Paraná (IAPAR).

2.1 ISOLATION AND SELECTION OF YEAST STRAINS

The coffee cherries were selected and the envelope (husk, pulp and mucilage) manually separated from the grain. From each sample, 25 g of the envelope was weighed which was macerated in gral with the aid of a pistil. It was added to the macerate 100 mL of distilled water, which was filtered in volumetric flask and the volume was completed to

150 mL. This solution was distributed in three Erlenmeyer flasks of 250 mL (50 mL/flask). The Erlenmeyer flasks were incubated in shaker for 3 days at 28º C and 120 rpm (PAGNONCELLI, 2002).

2.1.1 Culture media preparation

For yeasts isolation, six different culture media were employed: yeast medium agar (YM), starch agar (AA), saccharose agar (ASC), potato dextrose agar (PDA) dichloran rose bengal chloramphenicol agar (DRBC) and coffee extract agar (AEC), as specified in Table1.

TABLE 1 - CULTURE MEDIA COMPOSITIONS (YM, AA, ASC, PDA, DRBC AND AEC) UTILIZADED FOR YEASTS STRAINS ISOLATION

	Culture media					
Composition	YM agar	AA	ASC	PDA	DRBC	AEC
- Bacteriological agar	20 g	20 g	20 g	20 g	20 g	20 g
- Distilled water	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL
- Macerated coffee cherries	-	-	-	-	-	500 g
- Chloramphenicol	-	-	-	-	0.1 g	-
- Dextrose	10 g	10 g	10 g	20 g	10 g	-
- Dicloran	-	-	-	-	0.002 g	-
- Dihidrogen sodium phosphate	-	-	-	-	1 g	-
- Yeast extract	3 g	3 g	3 g	-	-	-
- Malt extract	3 g	3 g	3 g	-	-	-
- Potato infusion	-	-	-	200 g	-	-
- Bacteriological peptone	5 g	5 g	5 g	-	5 g	-
- Rose bengal	-	-	-	-	0.025 g	-
- Magnesium sulfate	-	-	-	-	0.5 g	-

Note: adjust pH to 4.5.
YM = yeast medium, AA = starch agar, ASC = saccharose agar, PDA = potato dextrose agar, DRBC = dichloran rose bengal chloramphenicol agar, AEC = coffee extract agar.

The culture media were sterilized and distributed aseptically in Petry dishes (20 mL/ plate).

2.1.2 Inoculation

After three days of fermentation the Erlenmeyer flasks were removed from shaker and transferred to a sterile chamber. The inoculation was done in the agar surface from serial dilutions. In the surface of each plate 0,1 mL of each dilution was inoculated, being homogenized with the aid of a sterile Drigalski loop. The plates were inoculated with three replicates (PAGNONCELLI, 2002).

2.1.3 Incubation

The plates were incubated at 28º C for 5 days.

2.1.4 Screening and colonies selection

The colonies were selected by their morphological characteristics presented in each culture media. The criteria utilized were: aspect (creamy and wrinkled), color (very white, white, white yellowish, yellow, pink, media color), size (>2 mm or <2 mm) and edge (regular or irregular). The selected colonies were submitted to GRAM staining (PAGNONCELLI, 2002).

2.1.5 Maintenance of selected strains

The strains were inoculated in YM agar slants, which were incubated at 28º C for 48 hours and maintained at 4º C being re-inoculated every 3 months.

2.1.6 Identification of the selected yeast strains

The isolated strains were identified by the system API 20C AUX (BIOMÉRIEUX, 1993). This system allows the evaluation of 21 characteristics (20 biochemical and 1 morphological). This are

separated in 7 groups of 3 and to each positive reaction is attributed a numerical value, composing a code of 7 digits. This code corresponds to species identification. The characteristics evaluated are the fermentation of the following components: glucose, glycerol, 2-ceto-d-glucanate, arabinose, xylose, adonitol, xylitol, galactose, inositol, sorbitol, methyl-d-glucoside, n-acetyl-glucosamin, celobiose, lactose, maltose, saccharose, trealose, melezitose, rafinose and the presence of hifes.

2.2 INOCULATION OF THE YEASTS ISOLATED FROM COFFEE CHERRIES IN THE DEGUMMING WATER

2.2.1 Characterization of degumming water

The degumming water composition was defined from the analysis of pH, humidity, ashes, lipids, proteins, carbohydrates, caloric value and total sugars realized following analytical methods of IAL (1985).

2.2.2 Inoculum preparation

The isolated yeast strains were inoculated in tubes containing 10 mL of YM broth and the tubes were incubated at 28º C for 48 hours.

2.2.3 Degumming water preparation as fermentation substrate

The degumming water was distributed in Erlenmeyer flasks with 250 mL capacity (50 mL/Erlenmeyer flask) and the flasks were sterilized at 121ºC/20 minutes.

2.2.4 Inoculation and Fermentation

For each Erlenmeyer flask containing the sterile degumming water, 5 mL of every tube containing the 48 hour inoculum in YM broth was transferred. The Erlenmeyer flasks were incubated in shaker for 3 days at 28ºC and 120 rpm.

2.3 EVALUATION OF THE DEGUMMING WATER DEGRADATION

The degumming water degradation rates were evaluated by Oxygen Chemical and Biochemical Demands, before and after 48 hours of inoculation, by the method described in **Standard Methods for Examination of Water and Wastewater** (APHA, 1998).

2.4 CHARACTERIZATION OF THE FORMED COMPOUNDS AFTER FERMENTATION BY THE YEAST STRAIN THAT SHOWED THE GREATEST DEGRADATION CAPACITY

From the results obtained in the analysis of OBD and OCD, the yeast strain that showed the best degradation capacity of the degumming water was selected. The formed metabolites were characterized by gas chromatography (Chromatograph Varian, model CG3800 coupled to mass detector Varian, model Saturn 2000).

Aliquots of 50 mL were removed from degumming water samples without yeast and after 24h, 48h e 72h of incubation. Every aliquot was extracted twice with 100 mL diclhoromethane and hexane solution (1:1). The extracts were combined and passed through a filter containing anhydrous sodium sulfate. The solvents were put in a rotative evaporator (Quimis) and the resultant residue re-suspended and added of acetone to 5 mL. The solutions obtained were analyzed by gas chromatography coupled to a mass spectrometer (GC/MS). The conditions of the GC/MS system operation are demonstrated in Table 2.

The compounds identification was realized by comparation with the spectrum stores in libraries of the chromatographic system utilizing as standards NIST and Saturn.

3 RESULTS AND DISCUSSION

3.1 IDENTIFICATION OF THE COLONIES ISOLATED FROM COFFEE CHERRIES BY BIOCHEMICAL TESTS API 20CAUX

The results obtained by the identification system API 20C aux showed

that the isolated colonies from coffee cherries represented six distinct yeast species: *Candida parapsilopsis*, *C.guilliermondii*, *C.lypolitica*, *C.tropicalis*, *Rhodorotula rubra* e *Kloeckera apiculata*.

TABLE 2 - CONDITIONS OF ANALYSIS IN THE GC/MS SYSTEM

PARAMETERS	CONDITIONS
Injector temperature	200”C
Injection mode	Split (1:60)
Column initial temperature	80”C
Heating ramp	8”C/min
Final temperature	300”C (2 min)
Trail gas flux (He)	1 mL/min
Column	CP Sil 8CB low bleed 25 m x 0,25 mm
Interface temperature	170”C
Trap temperature	180”C
Manifold temperature	60”C
Ionization mode	Electron impact (IE) a 70eV

VAN PEE & CASTELEIN (1971) isolated 8 yeast species, present even at the surface as in the mucilage of the cherries of *Coffea canephora* (robust) cultivated in Congo Republic. From those, three (*Candida guilliermondii, Candida parapsilopsis* e *Candida tropicalis*) coincided with the strains isolated in this experiment demonstrating that the distant cultivation regions presented similar microbiota.

3.2 DEGUMMING WATER CHARACTERIZATION

The results of the characterization of degumming water are demonstrated in Table 3.

TABLE 3 - DEGUMMING WATER CHARACTERIZATION BY THE ANALYSIS OF pH, PERCENTUAL COMPOSITION AND TOTAL SUGARS

ANALYZED PARAMETER	RESULTS OBTAINED
-pH	4.12
-Humidity (g/100 mL):	97.24
-Ashes (g/100 mL):	0.29
-Lipids (g/100 mL):	0.10
-Proteins (g/100 mL):	0.34
-Carbohydrates (g/100 mL):	1.86
-Caloric value (Kcal/100 mL):	10.38
-Total sugars (g/100 mL)	0.86 (glucose)

The degumming water shows low pH value (below 4.5), which favors the preferential development of yeasts with utilization of the carbohydrates and total sugars as carbon source in the fermentation substrate.

3.3 EVALUATION OF DEGUMMING WATER DEGRADATION

The reduction percentiles of the OBD and OCD values of the degumming water obtained after 48 hours of inoculation compared to the initial values are demonstrated in Table 4.

TABLE 4 - REDUCTION PERCENTILES OF OBD AND OCD OF DEGUMMING WATER

Microorganism	% reduction OBD/48 hours	% reduction OCD/48 hours
Candida parapsilopsis	50.14	29.81
Candida guilliermondii	35.23	9.99
Candida lypolitica	22.63	9.02
Rhodorotula rubra	21.79	3.63
Kloeckera apiculata	15.75	-
Candida tropicalis	5.44	-

Initial OCD: 35.294.11 mg O_2/L. Initial OBD: 21.930.12 mg O_2/L.

As stated by BAILLY et al. (1992) the degumming water, evaluated in wet processing plants situated in Mexico, presented essentially organic contamination (with high values of OBD and OCD). Values of OBD 19.000 mg O2/L and of OCD 34.000 mg O2/L were found, showing that they are quite close to the ones obtained in the degumming water analyzed.

Although highly pollutant, the residual water shows good degradability (OCD/OBD between 1.5 to 2) which favors the treatment by biological means.

By comparing the degradation percentiles obtained it is observed that *Candida parapsilopsis* showed degradation percentiles very superior to the others, designating its depollutant potential.

ROLZ et al. (1982) isolated a strain of *Penicillium crustosum* capable of degrading chlorogenic acid (carbon source) and caffeine (nitrogen source) present in the residues of coffee processing (pulp, de-pulping and degumming water), making viable the use of this microorganisms as biodepollutants.

3.4 CHARACTERIZATION OF THE COMPOUNDS SYNHETIZED BY THE STRAIN *Candida parapsilopsis* IN THE DEGUMMING WATER

The comparison between the chromatograms of the various degumming water fractions (without yeast, 24h, 48h e 72h) revealed the appearance of 3 new compounds in relation to the initial fraction. In the 48 hours fraction it was observed the appearance of compound 1, which is accentuated in the 72 hours fraction, in which it was also observed the synthesis of compounds 2 and 3 (Figure 1).

The identification of the formed compounds was realized through the comparison of their spectra with the ones stored in the libraries NIST and Saturn. The mass spectrum in relative percentage composition and the identidy suggested are presented in Table 5.

FIGURE 1 - CHROMATOGRAMS OF THE SAMPLE WITHOUT YEAST AND 72 HOURS SAMPLE

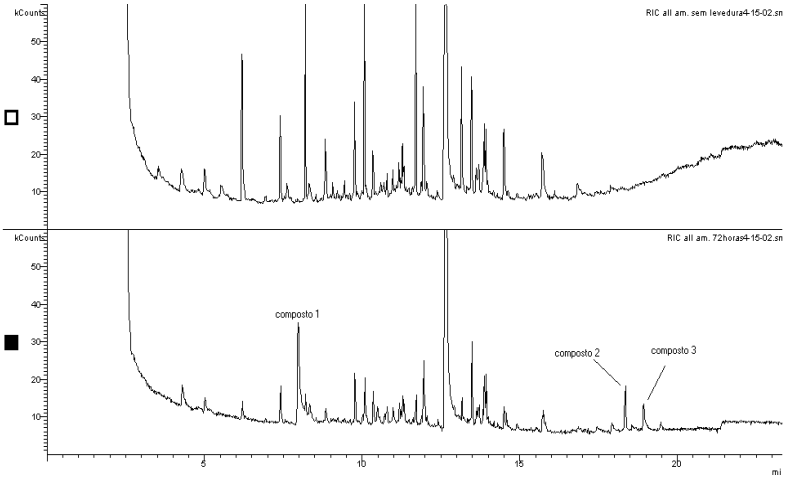

TABLE 5 - MASS SPECTRUM AND SUGGESTED IDENTIFICATION

Compound	Mass spectrum	Suggested Identification	
	Relative quantity (%)	NIST	Saturn
Compound 1	138(32); 108(10); 107(100); 79(11); 78(11); 77(11).	Benzeneethanol, 4-hidroxy- CAS N" 501-94-0	Benzeneethanol, 4-hidroxy- CAS N" 501-94-0
Compound 2	386(17); 372(26); 371(100); 315(18); 119(7); 91(11).	2,4-Bis(dimethyilbenzyl)-6-t-butylphenol CAS N" -x-	2,4-Bis(dimethyilbenzyl)-6-t-butylphenol CAS N" -x-
Compound 3	331(7); 330(28); 316(28); 315(100); 237(13); 119(5); 91(11).	Phenol, 2,4- bis (1-methyl-1-phenylethyl)- CAS N" 2772-45-4	Phenol, 2,4- bis (1-methyl-1-phenylethyl)- CAS N" 2772-45-4

Both the libraries returned the same results for the compounds identity. The first one reveals the formation of a alcoholic compound characteristic of the primary metabolism of the yeasts. Compounds 2 and 3 are considered secondary metabolites of fermentation showing the utilization of alternative metabolic pathways by the yeast, this is because of the presence of small amounts of fermentable sugars in the substrate.

4 CONCLUSION

From the six yeast species (*Candida tropicalis*, *C. lypolitica*, *C. guilliermondii*, *C. parapsilopsis*, *Kloeckera apiculata* e *Rhodotorula rubra),* isolated from the coffee cherries, *Candida parapsilopsis* was the strain that revealed the best reduction percentile of OBD and OCD (respectively 50.14% e 29.81%), degrading the degumming water and showing the viability of using this microorganisms as biological depollutants.

RESUMO

DESENVOLVIMENTO DE BIOPROCESSO APLICADO AO TRATAMENTO DE ÁGUAS RESIDUAIS DO BENEFICIAMENTO ÚMIDO DO CAFÉ PELA INCORPORAÇÃO DE LINHAGEM SELECIONADA DE LEVEDURAS

O objetivo deste trabalho foi isolar cepas de leveduras capazes de degradar a água de degomagem do beneficiamento úmido do café, caracterizando os metabólitos formados. A bebida preparada a partir de grãos tratados por via úmida é considerada de melhor qualidade quando comparada à elaborada com grãos tratados por via seca. No entanto, o alto potencial poluente da água residual do beneficiamento representa sério problema para o ambiente. A água de degomagem apresenta essencialmente contaminação orgânica, com taxas de demanda bioquímica de oxigênio (DBO) e demanda química de oxigênio (DQO) superiores a 20.000 mgO_2/L. *Candida tropicalis*, *C. lypolitica*, *C. guilliermondii*, *C. parapsilopsis*, *Kloeckera apiculata* e *Rhodotorula rubra,* isoladas de cerejas de café, foram inoculadas em frascos contendo água de degomagem e esses, submetidos à agitação constante(120 rpm) por 48 horas, a 28^oC. *Candida parapsilopsis* apresentou percentual de redução da taxa de DBO de 50,14% e de DQO de 29,81%, evidenciando a viabilidade do uso desses microrganismos como biodespoluentes.

PALAVRAS-CHAVE: CAFÉ; ÁGUA DE DEGOMAGEM; LEVEDURAS

REFERENCES

1 APHA. American Public Health Association. **Standard methods for examination of water and wastewater.** 20th ed. Washington, DC,1998.

2 BAILLY, H.; SALLÉE, B.; GARCIA-GARCIA, S. Projecto de tratamiento de águas residuales de benefícios húmedos.1.Diagnóstico de la contaminación. **Café Cacao Thé**, v.36, n.2, p.129-136, avril-juin. 1992

3 BIOMÉRIEUX. **Analytical profile index**. 2éme ed. Marcy-L´Étole, France, 1993 (Api 20C aux) (Ref.20290).

4 BRAILE, P.M.; CAVALCANTI, J.E.W.A. **Manual de tratamento de águas residuárias industriais**: São Paulo: CETESB, 1979. 764 p.

5 DEMARCHI, M. **Café**: aspectos econômicos. [Curitiba]: SEAB, Dep. de Economia Rural, dez. 2001.

6 DESPOLUIÇÃO: os microorganismos ganham terreno. **França-Flesh Meio Ambiente**, n. 9, p. 6-7, [2000].

7 DIAS, M.C.L.de L.; BARROS, A. do R. Avaliação de métodos para remoção da mucilagem de sementes de café (*Coffea arabica* L.). **Revista Brasileira de Sementes**, v.15, n.2, p.191-202, 1993.

8 FURTADO, M.R. Controle ambiental chega ao subsolo. **Química e Derivados**, v.32, n.354, p.8-19, out 1997.

9 IAL. **Normas analíticas do Instituto Adolfo Lutz**: métodos químicos e físicos para análise de alimentos. 3.ed. São Paulo, 1985.

10 KAWAI, H. **Avaliação do desempenho de estações de tratamento de esgotos**. São Paulo: CETESB, 1991. 38 p.

11 MACÊDO, J.AB. de Programa de bioaumentação (Bioaugmentation): uma tecnologia avançada para tratamento de efluentes. **Revista Nacional da Carne**, v.24, p-106-116, 2000.

12 MATOS, A.T. et al. **Remoção de DBO e DQO em águas residuárias de lavagem.** Disponível em: <www.coffeebreak.com.br/ocafezal.asp ?SE=8&ID=203>. Acesso em 31/03/2003.

13 PAGNONCELLI, M.G.B. **Estudo da biodiversidade de bactérias lácticas isoladas de frutos de café no Estado do Paraná**: isolamento, identificação e avaliação de seu potencial nos processos de ensilagem da casca de café. Curitiba, 2002. 102 f. Dissertação (Mestrado em Tecnologia Química), Curso de Pós-Graduação em Tecnologia de Alimentos, Universidade Federal do Paraná.

14 PETTIGREW, Jane. **Café**. São Paulo: Nobel, 1999. 96 p.

15 ROLZ, C. et al. Biotechnology in washed coffee processing. **Process Biochemistry**, v.17, n.2, p. 8-10, Mar./Apr.1982.

16 SPERLING, M. V. **Introdução à qualidade das águas e ao tratamento de esgotos**. Belo Horizonte: UFMG, 1996. 243 p.

17 VAN PEE, W.; CASTELEIN, J. The yeast flora of fermenting robusta coffee. **East African Agricultural and Forestry Journal.**, v.36, p.308-310, Jan. 1971.