

as acyl acceptors (Folk, 1971; Nio et al., 1986; Ikura et al., 1981). The potential for modifying proteins in a rational manner warrants more extensive studies and the development of practical sources of transglutaminase.

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Comparison of Yeast Biomass Production in Five Wood Aqueous Extracts

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Hot water aqueous extracts of the woods of five trees—mimosa (*Albizia julibrissin*), mulberry (*Morus rubra*), mesquite (*Prosopis glandulosa*), pecan (*Carya illinoensis*), and live oak (*Quercus virginiana*)—were compared for composition and ability to support yeast growth. The mesquite extract yielded the highest biomass, whereas the oak extract yielded the lowest. In the aerobic growth study, *Rhodotorula rubra* biomass production correlated with the amount of reducing sugar consumed but not with the amount of D-glucose or total carbohydrate present.

In a semiarid ecosystem, trees are often utilized in ways that differ from those common in wetter habitats. Their foliage and fruit may be important sources of nutrients for domestic livestock (Simpson and Solbrig, 1977; Bhatia and Ratan, 1983) or could serve as feedstock for industrial ethanol production (Felker et al., 1980). Likewise, while wood from trees is primarily used for fuel or as a building material, the wood of mesquite trees has been used as a substrate for the production of both bacterial (Thayer,

1976; Thayer and Murray, 1977) and yeast (Wilson and Thayer, 1978, 1982; Stanlake, 1986) biomass. The bacterial or yeast biomass could then be included as a nitrogen source in an animal ration. This report extends this line of study by comparing yeast biomass production in aqueous mesquite extract to that in aqueous extracts of four other hardwoods.

MATERIALS AND METHODS

Wood samples of mimosa (*Albizia julibrissin*), mulberry (*Morus rubra*), mesquite (*Prosopis glandulosa*), pecan (*Carya illinoensis*), and live oak (*Quercus virginiana*) were collected as green stems measuring 1-4 cm in diameter

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Table I. Composition of Wood Extracts

wood type	pH	total carbohydrate, g/L	reducing sugar, g/L	D-glucose, g/L	protein, mg/L
mimosa	4.6	3.00	0.31	0.10	6.2
mulberry	4.8	3.00	1.21	0.50	10.5
mesquite	4.7	3.00	1.25	0.50	12.5
pecan	4.7	4.00	1.02	0.60	23.0
oak	4.6	8.20	1.24	1.05	52.5

from living trees in Abilene, TX. The wood was cut into sections 2–6 cm in length and air-dried for 21 months. The sections were split lengthwise, yielding fragments ranging in weight from 0.4 to 6 g, and 50 g of each wood type was extracted for 1 h with 200 mL of boiling water in either a flask or retort. Duplicate extracts were then filtered through a double layer of cheesecloth and combined. These aqueous extracts were stored frozen to inhibit microbial growth.

Total carbohydrate content for each wood extract was determined by the phenol–sulfuric acid method of Dubois et al. (1956). Reducing sugar content was quantified by the *o*-toluidine method (Fox, 1980), and D-glucose content was assayed by the hexokinase/glucose 6-phosphate method (Carroll et al., 1970) in a kit form produced by Sigma Chemical Co., St. Louis, MO. D-Glucose was used as the standard in calibrating the preceding tests. Protein content was determined by the dye-binding technique of Bradford (1976) using 0.5 mL of sample and 4.5 mL of Coomassie Blue G-250 reagent (Stanlake, 1986), with bovine serum albumin (Dade Diagnostic, Inc., Aquado, Puerto Rico) serving as the standard.

The yeast strains *Saccharomyces bayanus* var. champagne, *Saccharomyces cerevisiae* var. distillers, and *S. cerevisiae* var. sautern, used in the fermentation studies, were obtained from the Fairleigh Dickinson Research Center, Abilene, TX. The *Rhodotorula rubra* strain used in the aerobic growth studies was obtained from Presque Isle Cultures, Presque Isle, PA. All yeast cultures were maintained on Sabouraud dextrose agar (Difco Laboratories) slants.

Fermentation studies were carried out in 10 mm by 100 mm screw-cap tubes containing gas collection tubes and 2 mL of the appropriate extract. The cultures were inoculated with 0.1 mL of a standardized yeast suspension in water and overlaid with 0.1 mL of sterile mineral oil. Incubation was for 7 days at 30 °C. Cell counts were done in quadruplicate in an improved Neubauer counting chamber, and gas production was assessed by visual inspection of the gas collection tube. In the cell count, clumps of cells and cells with buds were counted as one unit. Aerobic growth studies were carried out in flasks incubated in an environmental shaker at 30 °C and 200 rpm. Direct cell counts were determined as before, and cell mass was determined as dry weight after 72 h of incubation.

RESULTS AND DISCUSSION

The carbohydrate and protein content for the wood extracts are given in Table I. The pHs of the five extracts were acidic. The oak extract contained approximately twice the amounts of total carbohydrate, glucose, and protein as the other wood extracts, but in proportion to its total carbohydrate content, it was low (13%) in reducing sugar as was mimosa. D-Glucose accounted for 32–85% of the reducing sugar content of the five wood extracts, being highest in oak.

In the fermentation studies (Table II) all three yeast strains grew in all five wood extracts. Cell counts were lowest in the oak extract with but one exception, *S. bay-*

Table II. Fermentation of Wood Extracts [Gas (Cells/mL)]

wood type	yeast type		
	<i>S. bayanus</i>	<i>S. cerevisiae</i> var. distillers	<i>S. cerevisiae</i> var. sautern
mimosa	-5.9×10^6	-1.6×10^7	-2.0×10^7
mulberry	-6.9×10^6	-1.7×10^7	-2.2×10^7
mesquite	$+1.7 \times 10^7$	$+3.5 \times 10^7$	$+2.5 \times 10^7$
pecan	-1.0×10^7	-2.2×10^7	-2.5×10^7
oak	-6.8×10^6	-1.5×10^7	-1.8×10^7

Table III. Biomasses and Yields for the Aerobic Growth of *R. rubra* in Wood Extracts

wood type	biomass, g/L	red sugar consumed		yield		
		g/L	%	red sugar consumed	carbohyd present	red sugar present
mimosa	0.45	0.18	47.4	2.50	0.15	1.18
mulberry	0.59	0.99	72.3	0.59	0.20	0.43
mesquite	0.83	1.15	79.3	0.72	0.10	0.57
pecan	0.78	1.05	85.4	0.74	0.26	0.63
oak	0	0.10	7.3	0	0	0

anus var. champagne, grown in mimosa extract. In all cases cell counts were highest in the mesquite extract. Fermentation resulted in gas production only in the mesquite extract, and even then gas production was feeble, indicating that ethanol production would be low at best.

In the aerobic growth studies (Table III) the oak extract failed to support the growth of *R. rubra*. The highest biomass value recorded for *R. rubra* (0.83 g/L) was in the mesquite extract and was 26% greater than the average biomass of the four growth-supporting extracts. Pecan was a close second followed more distantly by mulberry and mimosa. The yield of biomass per mass of mesquite wood extracted, however, was a low 0.33%. A rank comparison of the five wood extracts, based on *R. rubra* biomass vs reducing sugar consumed, showed 100% correlation. Biomass production, however, does not correlate with yields based on either reducing sugar consumed, reducing sugar available, or total carbohydrate available. The most aberrant results were for the mimosa extract wherein the biomass produced was greater than the total of reducing sugar available. This could best be explained by the catabolization of one of the nonreducing carbohydrate components of the extract. The failure of the oak extract to support the aerobic growth of *R. rubra* and the corresponding low cell counts for the fermentative growth of the other yeasts are most likely due to the presence of an inhibitor, possibly tannin, rather than the absence of an essential nutrient.

While the carbohydrate and protein contents of the different extracts varied, all contained adequate nutrients for the growth of yeasts. Yet in both the aerobic and fermentation studies the mesquite extract yielded the highest yeast biomass. Therefore, its choice as a substrate for yeast biomass production can be based on its comparative value as a substrate as well as its abundance in many semiarid ecosystems.

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Fruit Development Effect on Fatty Acid Composition of *Persea americana* Fruit Mesocarp

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Various samples of lipids of avocado (*Persea americana*) mesocarp belonging to four varieties (Lula, Bacon, Fuerte, Zutano) and taken at various stages of fruit development were analyzed for fatty acids by gas chromatography (GC). These cultivars were grown under the same agroclimatic Mediterranean-like conditions and during the same growing season. Evolution of fatty acids seemed to be similar among the avocado varieties studied. Intercorrelation among fatty acids and their relation to variety and fruit development were investigated by analyzing the data (154 samples) with pattern recognition techniques such as principal component analysis (PCA) and factorial discriminant analysis (FDA). These statistical techniques showed a good partition of samples among the different cultivars studied, and the first axis determined the maturity index during fruit development for all cultivars studied. By FDA, supplementary data (32), representing new avocado samples of these cultivars, were successfully classified among the cultivar groups and/or among the different stages of fruit development.

The avocado (*Persea americana* Mill., family Lauraceae) is an oleaginous fruit (Mazliak, 1970). The terminology of maturity and fruit quality is generally associated with the oil content in mesocarp (Lewis, 1978). The oil level in avocado mesocarp constitutes the basis of maturity regulations for marketed avocados, and therefore, considerable interest in determination of avocado oil content has appeared (Lewis et al., 1978; Lozano et al., 1982). Among the various horticultural varieties, four of them are now available in Corsica Island (France): Zutano variety, a Mexican race originating from the cool subtropical highland of Mexico area; Bacon and Fuerte varieties, hybrids between the Mexican and the Guatemalan races; Lula

variety, hybrid between the West Indian and Guatemalan races. In the case of avocado fruit, unlike some other fruits, maturation is not associated with external changes in color. On the other hand, consumer and physiological maturity do not coincide, and therefore, consumer maturity is particularly difficult to determine. Since only sensory evaluation could determine consumer maturity, a chemical and/or a physical index of fruit development (Lozano et al., 1987), related to the results of sensory evaluation, is needed. Recently, the change in the class of lipids associated with the development of avocado fruit has been reported in a recent synthesis of research works done on this theme (Ahmed and Barmore, 1980).

In this study, we present the results obtained upon the changes in fatty acid composition during fruit development of the lipids contained in the mesocarp of the four varieties described above. Since the chemical composition is dependent on many factors such as geographic and climatic conditions, comparative results have been obtained from the same area (Mediterranean climate) and the same growing season. The 154 samples investigated in this study have been obtained from randomized selected fruits and multivariate statistical analyses, which have been successfully applied in lipids research (Gaydou et al., 1984, 1985), and have been used to distinguish among the different varieties and stages of fruit development. Supple-

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