Xanthine-Containing Beverages as Fermentation Adjuncts of Baker's Yeast

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Abstract

The possible use of xanthine-containing beverages – tea (Camellia sinensis), robusta coffee (Coffee canephora) and decaffeinated coffee – as fermentation adjuncts of baker's yeast was investigated. Phytochemicals found in the beverage-containing musts were tannins, flavonoids, alkaloids and saponins. A standard solution of each beverage was acidic. Introduction of 1.0g of each beverage into the must caused an increase in velocity of fermentation and ethanol yield, although the yield was not statistically significantly higher ($p>0.05$) than that of the non-beverage containing ferment. The ethanol yield did not also vary ($p>0.05$) between the beverages. The increase in velocity of fermentation on addition of a beverage appeared to suggest an enhancement of the fermentation activity of baker's yeast. The velocity of fermentation was highest in the coffee-treated yeast, followed by the decaffeinated coffee-treated yeast and then the tea-treated yeast. The result showed that the beverages could enhance the fermentation activity and ethanol yield of the yeast.

Keywords: Baker's yeast, Beverages, Enhancement, Fermentation adjuncts

Introduction

Tea, coffee and decaffeinated coffee are popular xanthine-containing beverages in Nigeria. They are usually consumed socially and habitually as infusions or decoctions. Their parent plants are evergreen shrubs and trees (Senteza Kajubi *et al.*, 1974; Tourle, 2003). The methylated derivatives of xanthine found in these beverages include theobromine, theophylline and caffeine, which have mild stimulatory (except theobromine) effects on the central nervous system (CNS) (Reynolds, 1996), among other effects. Vitamins are also found in the beverages. Trignonelline in coffee is converted to nicotinic acid (also niacin) and other products on roasting. Roasted coffee contains 10-40mg of nicotinic acid/100g sample, depending on the degree of roasting and may therefore provide a significant source of this vitamin (Macrae, 1993). Diab (2004) found that the levels (in mg/100g) of vitamins B_1 and B_5 in coffee instant regular powder were 0.01 and 28.17, respectively; in decaffeinated coffee instant powder 0.00 and 28.07, respectively; and tea instant unsweetened powder 0.05 and 12.56, respectively. The vitamins are precursors of some transiently bound water-soluble coenzymes that take part in oxidation-reduction reaction. Niacin (B_5) is the source of the nicotinamide moiety in nicotinamide nucleotides (NAD^+) while thiamine pyrophosphate (TPP) is derived from vitamin B_1 (Nelson and Cox, 2000). These two coenzymes are very crucial to alcoholic fermentation.

Man had used enzymic reactions long before written history and these reactions were exploited in the fermentation processing of "unstable" or "undesirable" raw materials to exciting goods (or beverages) like wine and beer. Various beverages are produced using the yeast *Saccharomyes cerevisiae,* which is a multi-enzyme unicellular fungus. Ethanol is the principal end product of yeast in the alcohol beverage industry. Enzyme catalytic properties also depend on the presence of their cofactors, whether inorganic ions or organic molecules (Uboh, 2004). Enzymes speed up reactions and in fermentation processes this translates to saving time, reducing cost and enhancing profitability. To an industrialist, time is money. This study seeks to evaluate the possible use of some of our locally consumed xanthine-containing beverages as time saving fermentation adjuncts of baker's yeast.

Materials and Methods

Preparation of musts: Granulated pure cane sugar (sucrose), tea bags (black tea), tins of roasted soluble coffee (robusta coffee beans) and decaffeinated coffee (blend of coffee beans) and a tin of baker's yeast (*Saccharomyces cerevisiae)* were purchased from a major supermarket in Owerri metropolis, Imo State, Nigeria. The raw materials from which the beverages were made were the manufacturers' descriptions. Increasing amounts (g) of sucrose (50,100,150,200,250, respectively) were put into each of four sets of five one-liter capacity mixing bowls. Into three sets (the test sets) were put 1.0g each of the tea, coffee and decaffeinated coffee, respectively. The fourth set, the control set, did not contain any beverage. Ammonium sulphate (2.5g) was added to the content of each mixing bowl, and 500ml of boiling distilled water was poured in, with stirring.

Fermentation: Each hot must, was poured into a 2.5 liter fermentation jar and left on the bench to cool (30°C). The musts were each inoculated with 2.0ml suspension of activated baker's yeast suspension {prepared by sprinkling 5.0g of yeast onto 50.0ml of lukewarm (33°C) distilled water,

covered with muslin cloth and left to stand for 20minutes, then stirred}. A rubber balloon, which served as air-lock, was fitted over the mouth of each fermentation jar containing the must and secured tight using a rubber band. The bottles were kept in a cupboard (33°C) and allowed to ferment for four weeks. Adding 0.1mg of $Na_2S_2O_5$/ml of ferment, as recommended by Berry (1996) stopped fermentation.

Analysis of musts and ferments: Qualitative assay for phytochemicals in the beverage-containing musts and quantitative assay for ethanol content (% v/v) in ferments were respectively determined by the procedures described by Harborne (1973) and Amadi *et al*, (2004).

Double-reciprocal plot: The ethanol contents of the ferments were later expressed in molarity (M) and used as velocity to make a double-reciprocal plot.

Degree of enhancement (ε_a): The degree of enhancement of the yeast during fermentation was calculated using the formula recommended by Moss (2005): $\varepsilon_a = V\text{-}V_o / V_o$, where $V = V_{max}$ of enhanced yeast and $V_o = V_{max}$ of yeast in the absence of enhancer.

Statistical analysis: The means and standard deviation were analyzed by the use of the analysis of variance (ANOVA) at a p-value of 0.05 as described by Essien (2003).

Results and Discussion

Phytochemicals can be described as substances in plants that are responsible for their taste, color, and smell, among other things. They can also be described as a group of non-nutrients produced by plants that may have beneficial effect on the body. Some of the phytochemicals in the beverage-containing musts, as shown in Table 1, included alkaloid [like caffeine theobromine and theophylline, which are responsible for the boost experienced by their consumers (Reynolds, 1996)], polyphenols (tannins and flavonoids) that have free radical scavenging (antioxidant) activities (Tourle, 2003) and saponins, which are popularly known to have haemolytic properties. When saponins are taken by mouth they are comparatively harmless (Evans, 2002).

Table 1: Some phytochemicals in the musts

	Phytochemicals			
Sample	**Tannins**	**Flavonoids**	**Alkaloids**	**Saponins**
Control	-	-	-	-
Tea	+	+	+	+
Decaffeinated Coffee	+	+	+	+
Coffee	+	+	+	+

- = absent, + = present

Saponins also exhibit antioxidant properties (Gordon, 1993). The presence of these phytochemicals portrayed the beverages as being potentially healthful drinks. The common side effects of xanthine – containing beverages are sleeplessness, anxiety, tremor, palpitation and withdrawal headache (Reynolds, 1996).

The addition of the respective beverages to the musts increased, though not statistically significant ($p > 0.05$), the velocity of fermentation of the yeast; indicating enhancement (Table 2, Table 3). This degree of enhancement (Table 3) increased in the order: coffee > decaffeinated coffee > tea.

Table 2: Amount of beverages in musts, pH of 0.8% beverage solution and grand mean ethanol content in ferment[q]

Sample	Beverage in must (g)	pH of a 0.8% beverage solution[k]	Grand mean ethanol (% v/v) in ferment[m]
Control	0.00		1.58±1.67[c]
Tea	1.00	6.44±0.02[a]	2.94±2.32[c]
Decaffeinated Coffee	1.00	5.28±0.00[b]	4.65±3.88[c]
Coffee	1.00	5.27±0.01[b]	4.71±4.09[c]

[q] values are means ± standard deviation (S.D) [k] = five determinations, [m] = five ferments, Values on the same column bearing the same superscript letter are not significantly different at a p-value of 0.05.

Table 3: Influence of beverage on V_{max}, k_m and ε_a values of yeast[b]

Sample	V_{max} (μM/min)	k_m (mM)	ε_a
Control	8.71×10^{-12}		
Tea	1.25×10^{-11}	2.31×10^{-3}	0.30
Decaffeinated Coffee	2.04×10^{-11}		0.57
Coffee	2.22×10^{-11}		0.61

[b] calculated from Fi

The enhancement of the yeast's fermentation activities did not however alter its measure of affinity for its substrate. We observed (experimentally) that the rates of frothing and inflation of the balloons were highest in the musts that contained coffee, higher in musts containing decaffeinated coffee and high in musts containing tea, relative to those that did not contain any beverage.

The activity of the decaffeinated coffee-treated yeast was higher than that of the tea-treated yeast, but was lower than that of the coffee-treated yeast (Table 3). This may suggest that the levels of the alkaloids supposedly in the beverages did not play any role(s) in the fermentation (at least at the concentration of the beverages used). Tea contains more caffeine than coffee on weight to weight basis (Sweetman, 2002; Wardlaw and Kessel, 2002).

A standard solution of the respective beverage was found to be acidic (Table 2); with those of the coffee and decaffeinated coffee being, respectively, more acidic ($p < 0.05$) than that of the tea. These beverages may have retained the acids that are normally found in their raw materials that

impart acidity. The pH optimum of industrial fungi tends to be a plateau (of between pH 8.0 to 2.0) rather than a single value (Brown, 1988). Maximum ethanol production by *Saccharomyces cerevisiae* occurs between pH 5.0 to 5.5 (Narendranath and Power, 2005). These suggested that the beverages might have shifted the pH of their respective musts toward the supposed pH optimum for yeast activity i.e. where ethanol production is maximum, thus, improving the yeast's ethanol yield (supposedly its turnover number, k_{cat}) as shown in Table 2. Ibegbulem *et al.* (2005) showed that increasing concentrations of coffee improved yeast's ethanol yield over a given time. The other factor that may have contributed markedly to the increased rates of fermentation could have been the levels of the vitamins {mostly niacin (B_5) and thiamin (B_1)} in the beverages, which we did not estimate. Presumably, the increase in [NAD^+] pulled the aldolase reaction more in the cleavage direction, thereby producing more glyceraldehyde 3 – phosphate to meet the increased demand for metabolites down the pathway {as more reduced nicotinamide nucleotide (NADH) would be available for alcohol dehydrogenase}. The presumption that the enhancement exercise started at the glyceraldehyde 3 –phosphate dehydrogenase stage (due to increased [NAD^+]) may explain why the yeast's affinity for its substrate was unchanged (since sucrose was the yeast's substrate and not the intermediate of alcoholic fermentation). Thiamine may not have had any marked influence since Diab (2004) showed that its contents of the beverages were low.

We also did not feel that the rate limiting enzymes in glycolysis – mainly phosphofructokinase-1 (PFK –1) and pyruvate kinase – could have hindered the rate of fermentation as the level of their allosteric inhibitor, adenosine triphosphate (ATP), is always very low in alcoholic fermentation.

From the foregoing, it would appear that the beverages enhanced the yeast's fermentation activity by a possible synergy of their effects on the pH of the fermentation media and their supposed niacin content. The beverages could therefore serve as fermentation adjuncts that will help yeast produce more ethanol within a given time frame.

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