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Isolation and characterization of some dominant yeast strains for production of bioethanol from Arabica coffee (*Coffea arabica* L.) wet processing wastes

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ABSTRACT

The current study was initiated to isolate and characterize yeasts from wet Arabica coffee processing wastes for bioethanol production. Yeast isolates were retrieved from wet Arabica coffee processing effluent1, effluent2, effluent3, pulp1 and pulp2. The yeast isolates were screened and characterized for ethanol production following standard methods. All the isolates were first tested for carbohydrate fermentation in appropriate medium. Selected ethanol producing isolates were tested for various parameters. Yeast isolates from pulp (ACP12) and effluent (ACE12) showed significantly high counts at 20% glucose concentration with the highest population number of $2.16 \pm 1.00 \times 10^8$ and $1.21 \pm 1.00 \times 10^8$ CFU/ml, respectively. Isolate ACP12 showed higher population number ($9.7 \pm 1.00 \times 10^7$ CFU/ml) than the standard culture *(Saccharomyces cerevisiae*) with colonies count of $8.7 \pm 1.00 \times 10^7$ CFU/ml at 30 °C. Moreover, yeast isolate (ACP12) showed higher colonies count ($9.8 \pm 1.00 \times 10^7$ CFU/ml) compared to the standard strain ($8.7 \pm 1.00 \times 10^7$ CFU/ml) at pH 5.0. Based on morphological, physiological and biochemical characteristics, the two isolates (ACE12 and ACP12) were tentatively identified as genus *Saccharomyces*. Total sugar concentration on **(**90%) was obtained from pulp1. Isolate ACP12 showed the maximum ethanol production (6.2 g/l) from pulp1 compared to the standard isolate (5.49 g/l) and the other test yeasts. From this study, it can be concluded that isolate

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ACP12 has an inherent potential of ethanol production from coffee pulps compared to the rest yeast isolates and needs further supplementary activities to qualify it for industrial application.

***Keywords*:** Coffee wastewater, fermentation, yeast isolates

1. INTRODUCTION

In the 20th century, the world economy has been dominated by technologies that depend on energy obtained from fossil fuels (Sun and Cheng, 2002). However, the use of fossil fuels is responsible for 73 % of the CO_2 emission that increases the gas in the atmosphere and poses global warming (Demirbas *et al*., 2004; Wildenborg and Lokhorst, 2005) and facing the crisis of global warming and environmental degradation which mainly has been associated with excessive use of fossil fuels (Berndes *et al*., 2003). Among the various sources been explored, biofuels offer one of the best alternative options as they have much lower life cycle Green House Gas (GHG) emissions compared to fossil fuels (Kumar, 2012). These necessitate the search for alternative renewable energy sources with energy efficiency and reduce CO_2 emission. Among the bioconversion processes, bio-ethanol production is an appropriate technology for alternative energy and for the management of agro-industrial residues (Demirbas, 2006). Ethanol can be produced by fermentation of sugars using saccharophilic yeast strains such as *Saccharomyces cerevisiae*. It is an important industrial chemical with emerging potential as a bio-fuel. These days, many countries reduce the cost and amount of petrol consumption, by using a mixture of ethanol and petroleum (Sree *et al*., 2000). This can help to replace fossil fuels and thus can reduce CO_2 emission by 90% (Demirbas, 2006). Bioethanol can be produced using cheap sources of agricultural wastes such as coffee effluents. Substances in coffee wastewater are known to contain toxic chem*icals* and threaten the environment with pollution (Deepa *et al*., 2002). Thus, the use of effluent and pulps from wet coffee processing offers raw materials of a second generation liquid fuel wherever coffee is being processed, and reducing environmental pollution. This in turn, would diversify sources of energy production and can promote sustainable development by directly benefiting the inhabitants around coffee producing areas. In Ethiopia, about 5.6 million liters of ethanol is annually produced, but there is an urgent need to maximize this yield in the years to come (Ethiopian sugar develop agency using different cheap and locally available agricultural wastes (i.e., coffee wastes). Therefore, the current study was initiated to isolate, characterize some potential yeast strains for the production of ethanol from wet Arabica coffee processing effluents and pulps in order to utilize these agro-wastes for the production of bioethanol and reduction of reduce environmental pollution.

2. MATERIALS AND METHODS

Description of the Study area

Samples collection

Wet Arabica coffee wastewater (three effluents) and two pulp samples were collected from Agaro and Gomma districts

Isolation of yeasts from Arabica coffee wastes

Yeasts were isolated from the samples on pre-solidified plates of yeast extract peptone glucose (YEPD) agar and isolation done by serial dilution. All the inoculated plates were incubated at 25-28 °C for 2 to 3 days**.** The yeast isolates were purified and preserved on YEPDA slants at 4 °C for further study

Characterization of yeast isolates

The two yeast isolates ACP12) and (ACE12) were selected for further characterization

Carbohydrate fermentation

Yeast fermentation was undertaken on minimal broth medium containing (g/l) of 4.5 yeast extract, 7.5 peptone, with nine (9) carbohydrate sources of 1ml added to the medium separately, and Bromo cresol blue was incorporated with Durham tubes as indicators of fermentation and gas production (Warren and Shadomy, 1991). Two yeast isolates (ACP12 and ACE12) were selected for further characterization since they showed rapid fermentation on the tested carbohydrates and screened for ethanol production (Warren and Shadomy, 1991).

Tolerance of isolates different physicochemical and nutritional factors

Tolerance to glucose concentration

The yeast isolates (1 ml) of 24 hrs old yeast culture) were each inoculated into 100ml Yeast mannitol (YM) minimal medium containing with different glucose concentrations (10%, 20%, 30%, 40%, 50%, and 60%) (Subashini *et al.*, 2011)

Tolerance to ethanol

The ability of isolates to tolerate various concentrations of alcohol was tested according to Subashini *et al.*, (2011). A 1 ml of 24 hrs old culture grown in YEPD (yeast extract 10 g/l, peptone 20 g/l and D-glucose 20 g/l) broth was inoculated to 100 ml yeast mannitol broth (g/ml) containing different concentrations of ethanol 4%, 8%, 12%, 16%, 20%, 24% (v/v) Growth (Colony Forming Unit CFU/ml) was estimated after 7 days of incubation at 30 °C.

Tolerance to temperature

One ml of 24 hrs old yeast culture grown in YEPD(yeast extract 10 g/l, peptone 20 g/l and D-glucose 20 g/l) broth was inoculated aseptically into 100 ml yeast mannitol and incubated at 10, 20, 25, 30, 40, 50, and 60 °C. Growth (Colony Forming Unit CFU/ml) was estimated after 7 days of incubastion. (Subashini *et al.*, 2011).

Tolerance to pH

One ml of 24 hrs old yeast culture was inoculated into 100 ml flasks containing yeast mannitol broth (g/ml) with pH adjusted to 2.5, 3.5, 4.5, 5.5, and 6.5 using 1N HCl and NaOH. (Subashini *et al.*, 2011). Growth (Colony Forming Unit CFU/ml) was estimated after 7 days of incubastion.at 30 °C.

Morphological characterization

Cultural and microscopic characterization of the yeast isolates was made following the methods of Barnett *et al.,* (2000).

Total sugar determination in the coffee waste samples

The sugar content in the coffee effluents was calculated by Fehling method using the following formula (Urbaneja *et al*., 1996).

$$\textbf{Sugar Contents}\ (\%) = \frac{\boldsymbol{300ml \cdot f \cdot 100}}{\boldsymbol{V}}$$

where: f = - Fehling factor (0.051); V = volume used in the titration (titrate value) (ml).
Fermentation of coffee wastes

The flasks containing the coffee effluent (750 ml) were diluted with 250 ml of distilled water (v/v). The flasks were covered, autoclaved for 15 minutes at 121 °C and allowed to cool at room temperature.

Fermentation was carried out in 1000 ml capacity Erlenmeyer flask with optimum inoculum 3 g/l of yeast isolates following standard method (Turhan *et al*., 2010). The flasks were incubated at 30 °C and fermented for 72 hrs.

The powdered pulp (20 g) was hydrolyzed with 1000 ml of distilled water contained in a flask for 4 hrs. The flasks were covered, autoclaved and allowed to cool at room temperature. Fermentation was carried out in 1000 ml capacity Erlenmeyer flask with 3 g/l of yeast isolates and standard *S. cereviciae* with incubation temperature of 30 °C for 72 hrs (Franca *et al.*, 2008).

Determination of ethanol amount in the fermentation broth

After centrifugation at 10,000 rpm for 5 minutes, the supernatant was filtered. Ethanol concentration was measured using Ebulliometer

Cell biomass determination

After 72 hrs, the fermentation broth with coffee effluents and pulps in each flask was filtered and centrifuged at 10,000 rpm for 5 minutes. Each yeast biomass (pellet) was measured using Methler balance (Scaltec). A standard yeast *S. cerevisiae* was obtained from Mycology Laboratory, Addis Ababa University and used as a positive control for some experiments.

Data analysis

Data were analyzed statistically on the basis tolerance to different factors and/or concentration of substrates and ethanol yield using SPSS window version 22.0, SPSS Inc, Chicago, IL, USA. Analysis of variance (ANOVA) was used to indicate significant mean differences at 95% confidence interval.

3. RESULTS

Isolation of fermentative yeasts

A total of fifteen (15) yeast isolates were retrieved from five samples of Arabic coffee effluent 1, effluent 2, effluent 3, and Arabica coffee pulp 1 and pulp 2 (Table1). The isolates from effluent 1 were designated as ACE1 (ACE11, ACE12 and ACE13). The yeast isolates from effluent 2 were designated as ACE2 (ACE21, ACE22 and ACE23). Similar designation (ACE3) was given to the isolates from effluent 3 (i.e., ACE31, ACE32 and ACE33). The yeast isolates from pulp1and pulp2 were designated by ACP1 (ACP11, ACP12and ACP13) and ACP2 (ACP21, ACP22 and ACP23), respectively. Most of the yeast colonies exhibited smooth surfaces with circular margins (Table 1).

The colour of the pure colonies of effluents and pulps showed creamy white but some colonies of pulps slightly red and pinkish. The cells were found to be of various shapes such as round; oval, spherical and ellipsoidal (Table 1).

Screening for fermentative yeast isolates

The yeast isolates were capable of utilizing nine (9) different carbon sources (Table 2). All the isolates utilized glucose, galactose, fructose and maltose. The most versatile fermenters were ACE12 and ACP12 and taken for further morphological and physiological characterization (Table 2).

Physiological characterization of the yeast isolates

Sugar tolerance

The growth of ACP12 and ACE12 was gradually increased with concentrations of sugar. The results indicated that the two isolates had maximum population at 20% glucose concentration (Table 3).

The yeasts isolated from pulps (ACP12) and effluents (ACE12) recorded maximum population count at 20% glucose concentration with the mean counts of $2.16\pm1.00\times10^8$ and $1.21\pm1.00\times10^8$ CFU/ml, respectively (Table 3). However, as the sugar concentration increased from 20% to 60%, the growth of both isolates and standard yeast *S.cerevisiae* was decreased gradually. At glucose concentrations of 30-60%, isolate ACP12 showed higher counts compared to the rest strains. There was significant difference ($p<0.05$) within the yeast isolates in terms of glucose concentrations tolerance.

Ethanol tolerance

Difference ($p<0.05$) in ethanol tolerance was observed among the yeast isolates and the standard *S. cerevisiae* (Table 4).

Yeast isolated from Arabica coffee pulp (ACP12) showed the highest count ($9.6\pm1.00\times10^7$ CFU/ml) followed by isolate from Arabica coffee effluent (ACE12) with maximum mean count of $7.8\pm1.53\times10^7$ CFU/ml. ACP12 exhibited the maximum tolerance up to 16% ethanol with a mean count of $7.8\pm1.00\times10^7$ CFU/ml similar to that of the standard strain *S. cerevisiae* ($6.8\pm1.00\times10^7$ CFU/ml). As the concentration of ethanol increased from 4% to 24%, cell number gradually drastically decreased.

Temperature tolerance

The yeast isolate from Arabica coffee pulp (ACP12) showed higher mean count ($9.7\pm1.00\times10^7$ CFU/ml) at 30 °C followed by the standard culture *S. cerevisiae* with maximum population of $8.7\pm1.00\times10^7$ CFU/ml (Table 4). However, the yeast isolated from Arabica Coffee effluent (ACE12) showed $6.8\pm1.54\times10^7$ CFU/ml) at the same temperature. The growth of selected yeast isolates increased from 15 °C to 30 °C. Beyond 30 °C, the growth of the test yeasts and the standard strain was declined but isolate ACP12 performed better at 40 and 50 °C compared the rest isolates (Table 5). There were significant differences ($p<0.05$) among the yeast isolates with regard to their temperature tolerance.

pH tolerance

The isolates from Arabica coffee pulp (ACP12) and effluent (ACE12) gave the maximum mean counts of $9.8\pm1.00\times10^7$CFU/ml), $7.8\pm1.00\times10^7$CFU/ml) and the standard strain $8.7\pm1.00\times10^7$ CFU/ml) at pH 5.0, respectively (Table 6). In all cases, there was a decline in yeast growth above pH 5.5

Determination of sugar content

The maximum reducing sugar concentration of 90% (Fig. 2) was produced from distilled water hydrolysate of coffee pulp1 followed by pulp 2 (85%), effluent1 (51%), effluent2 (43.71%) and effluent3 (40.26%), respectively.

Fermentation and bioethanol concentration

The alcohol yields from different substrates by the yeast isolates (Table 7) ranged from 4-6.2% (g/l). The isolates showed different pattern of ethanol production of 4.5% (g/l) for standard *S. cerevisiae*, 6.20% (g/l) for isolate ACP12 and 5.01% (g/l) for isolate ACE12 from pulp1.

Biomass yield at the end of fermentation

The maximum cell density was recorded for ACP12 and ACE12 compared to standard *S. cerevisiae* in batch fermentation with initial sugar concentration of 20% standard sucrose, pulp 1 and pulp 2 as well as coffee effluents (Table 8). ACP12 showed the higher cell densities in all the substrates with the highest biomass from pulp1.

4. DISCUSSION

Some yeasts with fermentative potential were retrieved from samples of Arabica coffee wet processing wastes. Microscopic and cultural characteristics of the yeast isolates shared similarities with the descriptions given by Lodder (1971) and Boekhout and Kurtzman (1996). Accordingly, the coffee wastes (pulps and effluents) isolates (ACP12 and ACE12) were tentatively assigned to a genus *Saccharomyces* type unicellular ascomycete. Furthermore, the features of isolates were similar with the previous findings (Berhanu Abegaz Gashe *et al.*, 1982; Samuel Sahle and Birhanu Abegaz Gashe, 1991; Tamene Milkessa, 2009)

All the test isolates were capable of utilizing 6-9 sugars indicating their potential to utilize different sugars to produce more ethanol. Among the test isolates, ACP12 and ACE12

were capable to ferment eight (8) sugars out of the nine (9) sugars compared to the standard *S. cerevisiae* that was able to utilize les sugars. Consequently, they were selected for ethanol production.

The test isolates showed tolerance to high (20%) glucose concentrations compared to standard yeast (*S. cerevisiae*) (Table 2). Similarly, Osho (2005) reported that wine yeast (*S. cerevisiae)* strains could tolerate a maximum of 20% sugar concentration. Recently, a maximum of 20% sugar tolerance was reported by Ali and Khan (2014) ethanol production from 20% of glucose concentration within 72 hrs of incubation, but the yield abruptly decreased when the concentration of sugar increased. Bekatorou *et al*., (2006) showed that high substrate concentration would lead to catabolic repression by glucose and sucrose and may lead to several problems such as incomplete fermentation, development of off flavors and undesirable by products as well as decreased biomass and yeast vitality.

From this study, the isolate from coffee pulp (ACP12) recorded more tolerance (up to 16%; v/v) ethanol than isolate from coffee effluent (ACE12) and the standard strain *S. cerevisiae* (Table 4). The data also showed as the concentration of ethanol increased from 4% (v/v) to 24% (v/v), the growth of isolates slightly, but not significantly decreased. This is similar to the report of Subashini *et al*. (2011) that showed *S. cerevisiae* was tolerant to ethanol high (15%) concentration with cell count of 6.2×10^7CFU/ml, but slightly lower than the level of alcohol tolerance of 16%-16.5% (v/v) by some yeast strains (Casey and Ingledew 1986; Teramoto, *et al*.(2005),

With regard to temperature, maximum yeast cell biomass of ACP12 and ACE12 yeast isolates counts 7.1×10^7 CFU/ml and 5.6×10^7 CFU/ml were recorded at 30 °C irrespective of the strain type respectively (Table 5). Similar study was done by Subashini *et al*., (2011), described that , yeast isolated from molasses showed higher population (9.4×10^6 CFU/ml) at 30 °C, followed by the yeast isolate from batter (7.7×10^6 CFU/ml) and growth was decreased when the temperature increased the trends similar with the findings of Subashini *et al*., (2011)

The isolates and showed maximum population (9.8×10^7 CFU/ml of ACP12 and 7.8×10^7 CFU/ml of ACE12) at pH 5 and 9.5×10^7 CFU/ml ACP12 and 7.6×10^7 CFU/ml at pH 5.5, respectively. Similarly, Linden *et al*. (1992), showed the optimal pH for ethanol production and maximum population of yeast cells (*S. cerevisiae)* were around 5 and 5.5.

The maximum reducing sugar concentration of 90% was produced from distilled water hydrolysate of coffee pulp1 compared to the other coffee wastes.

The isolates showed different pattern of ethanol production from coffee wet processing wastes (Table7). Maximum amount of ethanol produced by ACP12 isolate (6.2 g/l from pulp 1 substrate compared to the two isolates from the other samples (Table7).

Similarly, Ayele Kefale (2011) reported, the maximum bioethanol concentration of 7.4 g/l from Arabica coffee pulp, with longer hydrolysis time. However, ethanol production was much lower than batch fermentation of acid hydrolysate of coffee husk (13.6 g/l) (Franca *et al*., 2008) and from wheat stillage hydrolysate 11 g/l (Davis *et al*., 2005) using *S. cerevisiae.*

The biomass accumulation of ACP12 and the standard yeast was high (1.6-2.4 g/l) compared to ACE12 (1.0-1.2 g/l) showing biomass accumulation was directly proportional to the ethanol yield. Optimal conversion of carbohydrates to ethanol requires maximum number of cells that are tolerant to high concentration of both substrate and product to efficiently produce good amount of ethanol yield (Walker *et al.*, 2006).

5. CONCLUSION

The two screened and further tested yeast isolates showed closest morphological similarities to genus *Saccharomyces.* All the isolates were capable of utilizing 6-10 carbohydrates. Yeast isolate from pulp (ACP12) showed remarkable tolerances to different levels of sugar, ethanol concentration, temperature and pH with production of high amount of ethanol yield from coffee wastes. This study demonstrates that Arabica coffee wet processing wastes have a potential to be used as feedstock for bioethanol production in Ethiopia which in turn contributes much to the proper management of environmental pollution.

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Table 1. Colony characteristics of yeast isolates

	Characteristics			
Strain	Surface	Margin	Colour	Shape of cells
ACE11	Smooth	Irregular	Creamy white	Round/Oval
ACE12	Smooth	Circular	Creamy white	Round/Oval
ACE13	Rough	Irregular	Creamy white	Ellipsoidal
ACE21	Smooth	Circular	Creamy white	Spherical/Oval
ACE22	Smooth	Circular	Creamy white	Ellipsoidal
ACE23	Smooth	Irregular	Creamy white	Spherical/Oval
ACE31	Smooth	Irregular	Creamy white	Round/Oval
ACE32	Smooth	Circular	Creamy white	Round
ACE33	Smooth	Circular	white	Oval
ACP11	Smooth	Irregular	white	Round/Oval
ACP12	Smooth	Circular	Creamy white	Round/Oval
ACP13	Rough	Circular	Slightly red /Pinkish	Round/Oval
ACP21	Smooth	Circular	Creamy white	Round/Oval
ACP22	Rough	Circular	Creamy white	Ellipsoidal
ACP23	Smooth	Circular	Pinkish	Round/Oval
Saccharomyces cerevisiae	Smooth	Circular	Cream, white	spheroidal, ellipsoidal

Table 2. Carbohydrates fermentation by yeast isolates

Isolates	Fermentation									
	Glucose	Galactose	Fructose	Sucrose	Maltose	Lactose	Raffinose	Trehalose	xylose	Total carbohydrate fermented
ACE11	+++	++	++	+	+	+	+	+	-	8
ACE12	+++	++	+	++	+	+	++	+	+	9
ACE13	+	+	+	+	+	-	-	+	+	7
ACE21	++	++	+	-	+	+	+	+	-	7
ACE22	++	+	+	+	+	++	+	+	-	8
ACE23	+	+	+	+	+	++	_	+	-	7
ACE31	++	+	++	++	++	+	+	+	+	9
ACE32	++	++	+	-	+	+	_	+	+	7
ACE33	+++	++	++	++	++	+	-	+	-	7
ACP11	++	+	++	+	+	+	+	+	-	8
ACP12	+++	+	+	+	+	++	+	+	+	9
ACP13	+	+	+	+	+	-	+	-	-	7
ACP21	++	+	+	-	+	-	+	+	+	7
ACP22	+	+	++	-	+	+	+	-	-	6
ACP23	++	+	+	+	+	_	_	+	-	6
S. cerevisiae	++	+	+	++	+	_	+	+	_	7

+ = Fermentative, ++ = moderately fermentative, +++ = Highly fermentative (Durham tube empty),
- = No carbohydrate utilization

Table 3. Growth of yeast isolates at different glucose concentrations

Glucose (%)	Mean count (CFU×10^8/ml) of yeast isolates		
	ACP12	ACE12	*S.cerevisiae*
10	1.00±0.77^{g}	0.50 ±0.48 ^{j}	1.00± 0.56 ^{i}
20	2.16±1.00^{a}	1.21±1.00^{c}	1.53 ±1.00^{b}
30	1.12±1.00^{d}	1.00± 0.91 ^{f}	1.06±0.94 ^{e}
40	1.00± 0.65 ^{h}	1.00± 0.43 ^{k}	1.00± 0.48 ^{j}
50	1.52± 0.49 ^{j}	1.00± 0.23 ^{l}	1.00± 0. 36 ^{l}
60	1.00± 0.37 ^{l}	1.00± 0.13 ^{m}	1.00± 0.21 ^{m}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p < 0.05$)

Table 4. Tolerance of the yeast isolates to different ethanol concentrations

Ethanol (%)	Mean count (CFU×10^7/mL) of yeast isolates		
	ACP12	ACE12	*S.cerevisiae*
4	9.6±1.00^{a}	7.7±1.53^{de}	8.9±1.00^{b}
8	8.7±1.00b^{c}	6.4±1.00^{gh}	7.7±1.00^{de}
12	8.1±1.00^{cd}	5.7±1.53^{h}	7.2±1.00^{ef}
16	7.8±1.00^{de}	4.5±2.00^{i}	6.8±1.00^{fg}
20	2.4±2.00^{j}	2.0±1.9 ^{j}	4.0± 2.40 ^{j}
24	2.01±0.20^{k}	1.0± 0.90 ^{k}	1.0±1.00^{k}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p < 0.05$)

Table 5. Temperature tolerance by the yeast isolates

Temperature (ºC)	Mean count (CFU×10^7/ml) of yeast isolates		
	ACP12	ACE12	*S. cerevisiae*
15	2.2±1.00^{k}	1.2±1.0^{m}	1.7±1.00^{l}
20	2.6 ±1.00^{i}	1.5±1.00^{m}	2.3±1.00^{jk}
25	7.1±.58^{c}	5.6±1.53^{fg}	5.9±1.00^{f}
30	9.7±1.00^{a}	6.7±1.54^{d}	8.7±1.00^{b}
40	6.3±1.00^{e}	5.4±1.00^{g}	5.5±1.00^{g}
50	3.6±1.53^{h}	2.1±1.00^{k}	3.2±1.53^{i}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p < 0.05$)

Table 6. pH tolerance of the yeast isolates

pH	Mean count (CFU×10^7/mL) of yeast isolates		
	ACP12	ACE12	*S.cerevisiae*
2.5	1.4±1.00^{j}	1.2±1.00^{j}	1.2±1.00^{j}
3.5	2.6±1.00^{i}	1.5±1.00^{j}	2.3±1.00^{i}
4.5	4.8±0.58^{f}	3.5±1.00^{h}	4.3±1.00^{g}
5	9.8±1.00^{a}	7.8±1.00^{c}	8.7±1.00^{b}
5.5	9.5±1.00^{a}	7.6±2.65^{c}	8.7±1.53^{b}
6.5	5.8 ±0.58^{d}	4.5±1.00^{fg}	5.3±1.00^{e}

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p < 0.05$)

Table 7. Morphological characteristics of the two selected yeast isolates

Types of sample	Sugar content (%)	Yeast isolates	Alcohol contents (g/l)
Pulp 1	90	ACE12	5.01^{bc}
		ACP12	6.20^{a}
		S. cerevisiae	5.49^{b}
Pulp 2	85	ACE12	4.14^{ef}
		ACP12	5^{cd}
		S. cerevisiae	4.34^{de}
Effluent 1	51	ACE12	2.1^{i}
		ACP12	2.5^{hg}
		S. cerevisiae	2.3^{i}
Effluent 2	43.71	ACE12	1.86^{j}
		ACP12	2.01^{i}
		S. cerevisiae	1.98^{j}
Effluent 3	40.26	ACE12	0.96^{j}
		ACP12	1.23^{j}
		S. cerevisiae	1.01^{j}

Means ± SD from two replications and mean values followed by different letters in the same column indicate significant differences ($p < 0.05$).

Table 8. Comparison of ethanol production from Arabica Coffee effluents, pulps and standard sucrose

Types of Samples	Isolates and standard isolates		
	Net weight (g/l)		
	ACP12	ACE12	Standard *S.cerevisiae*
Pulp 1	2.4±1.52[a]	1.2±0.60 [k]	2.3 ±0.20[b]
Pulp 2	2.2±0.30[d]	1.1±0.80 [l]	2. 2±1.00[ef]
Effluent 1	2.2±0.40[e]	1.0±0.10[m]	2. 1±0.51[g]
Effluent 2	1.9±0.80 [h]	1.0±0.11[n]	1. 7 ±0.60[j]
Effluent 3	1.8±0.01[i]	0.5±0 [mn]	1. 6 ±0.50[j]

Means ± SD from two replications and mean values followed by different letter(s) in the same row indicate significant differences ($p < 0.05$)

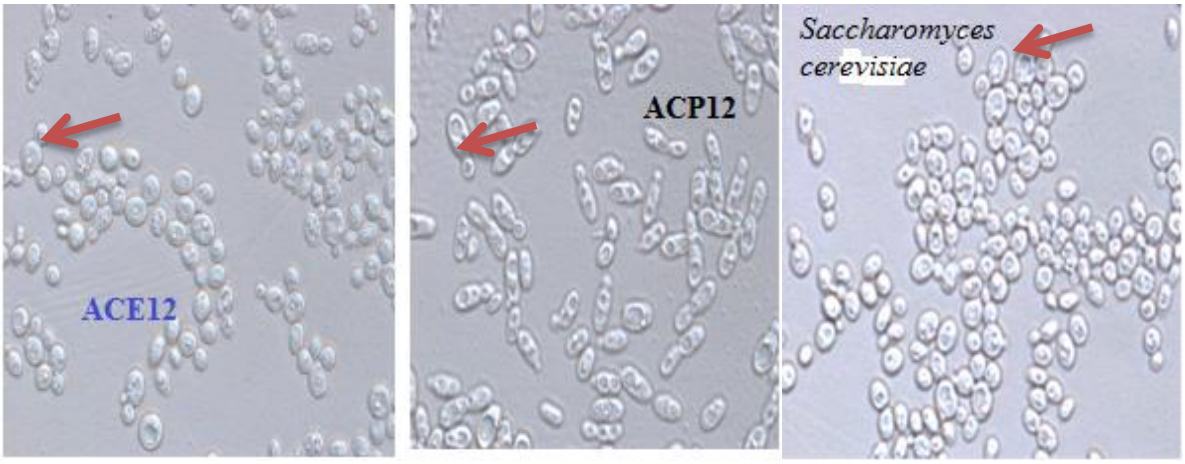

Fig. 1. Asexual reproduction (arrows) of yeast isolates and standard *S. cerevisiae*

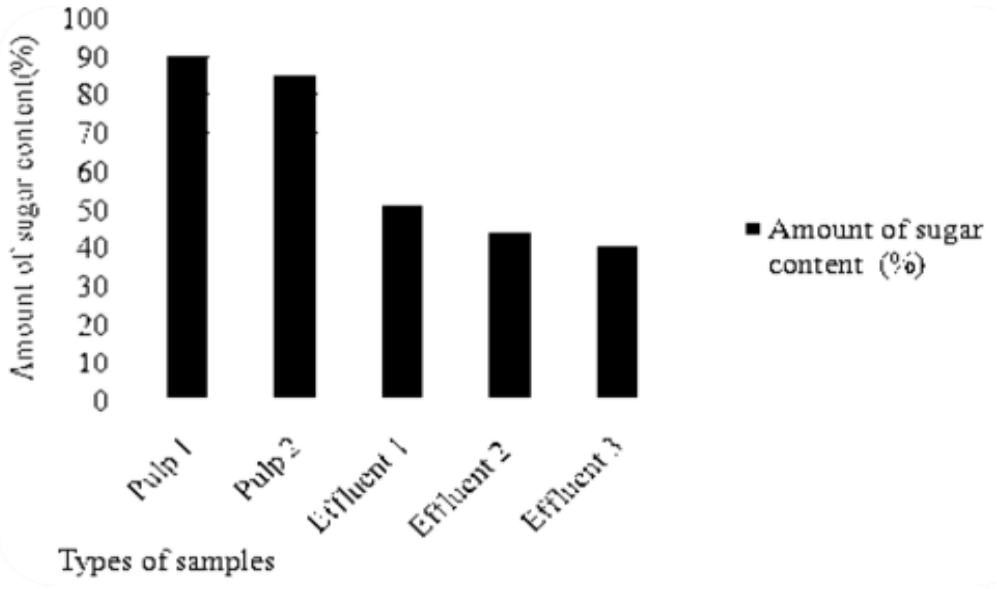

Fig. 2. Total sugar content of Arabica coffee wet processing wastes