Bioresource Technology 166 (2014) 142–150

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Utilization of coffee by-products obtained from semi-washed process for production of value-added compounds

Verónica Alejandra Bonilla-Hermosa, Whasley Ferreira Duarte *, Rosane Freitas Schwan

Department of Biology, Federal University of Lavras (UFLA), CP 3037 – Campus Universitário, CEP 37.200-000 Lavras, MG, Brazil

HIGHLIGHTS

- Production of bioethanol and volatile compounds using coffee residues as substrate.
- *H. uvarum* UFLA CAF76 was able to grow and ferment coffee residues.
- High ethanol yield factor (0.48 g/g) and efficiency (94.11%) were obtained.
- *H. uvarum* UFLA CAF76 produced important aroma compounds for industry.

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 9 May 2014
Accepted 11 May 2014
Available online 20 May 2014

Keywords:
Coffee pulp
Coffee wastewater
Bioethanol
Volatile compounds
Hanseniaspora uvarum

ABSTRACT

The semi-dry processing of coffee generates significant amounts of coffee pulp and wastewater. This study evaluated the production of bioethanol and volatile compounds of eight yeast strains cultivated in a mixture of these residues. *Hanseniaspora uvarum* UFLA CAF76 showed the best fermentation performance; hence it was selected to evaluate different culture medium compositions and inoculum size. The best results were obtained with 12% w/v of coffee pulp, 1 g/L of yeast extract and 0.3 g/L of inoculum. Using these conditions, fermentation in 1 L of medium was carried out, achieving higher ethanol yield, productivity and efficiency with values of 0.48 g/g, 0.55 g/L h and 94.11% respectively. Twenty-one volatile compounds corresponding to higher alcohols, acetates, terpenes, aldehydes and volatile acids were identified by GC-FID. Such results indicate that coffee residues show an excellent potential as substrates for production of value-added compounds. *H. uvarum* demonstrated high fermentative capacity using these residues.

1. Introduction

Coffee is the most frequently consumed functional beverage (Mussatto et al., 2011b). According to the International Coffee Organization (ICO, 2014), global coffee production during 2013 is estimated to be over 145 million 60 kg bags. Therefore, large amounts of residues are generated during coffee processing. Among these residues, coffee pulp is the main residue obtained during wet and semi-dry processing and represents 29% dry-weight of the whole berry (Murthy and Madhava Naidu, 2012). In semi-dry processing, fruit pulp covering the seeds is removed by a pulper, then, the mucilage coating the beans is washed before sun drying (Navia et al., 2011; Esquivel and Jiménez, 2012). Coffee pulp is essentially rich in sugars, proteins, minerals and it also contains appreciable amounts of tannins, polyphenols and caffeine, considered toxic in nature (Pandey et al., 2000). The water used for depulping and demucilage of cherries is known as coffee wastewater and has high concentrations of organic pollutants (Haddis and Devi, 2008). Residues from the coffee industry represent a serious environmental problem for coffee producing countries, due to unsafe disposal of them causing water and land pollution (Murthy and Madhava Naidu, 2012). There are few studies addressing their use in profitable applications. The main coffee residue, which potential has been most frequently studied is the spent coffee grounds. Most of the published studies have been focused on the use of spent coffee grounds (Choi et al., 2012; Mussatto et al., 2012) to produce bioethanol. This residue obtained after coffee extraction is composed mainly by carbohydrates such as mannose (46.8%), galactose (30.4%), glucose (19%), arabinose (3.8%) and proteins (13.6%). In addition to proteins and carbohydrates, different minerals (mg/kg) like potassium (3549.0), phosphorus (1475.1), magnesium (1293.3), calcium (777.4), iron (118.7), manganese (40.1), copper (32.3), and zinc (15.1), are present (Mussatto et al.,

* Corresponding author. Tel.: +55 35 3829 1358; fax: +55 35 3829 1100.
E-mail address: whasleyduarte@dbi.ufla.br (W.F. Duarte).

http://dx.doi.org/10.1016/j.biortech.2014.05.031
0960-8524/

2011a). Since coffee pulp and coffee wastewater contain considerable amounts of fermentable sugars and other nutrients (Mussatto et al., 2011b), these constitute interesting substrates for microorganism growth, and consequently, for producing value-added products such as bioethanol and aromatic volatile compounds.

The emission of greenhouse gases due to the consumption of fossil fuels and the nonrenewable nature of them, encourage the search for new sources of clean energy, which can be produced in a sustainable manner. Burning ethanol instead of gasoline reduces carbon emissions by more than 80% while eliminating the release of acid-rain-causing sulfur dioxide (Mussatto et al., 2011b). Furthermore, there is a worldwide growing interest to discover new and cheap carbohydrate sources for production of second generation bioethanol. The agricultural residues are attractive materials since they are abundant, an inexpensive feedstock, avoid direct and indirect competition with human food and animal feed and provide better energy supply security. It is considered that agroindustrial residues will become the main feedstock for ethanol production in the near future. Recent studies indicate the excellent potential of coffee residues for bioethanol production (Kwon et al., 2013; Choi et al., 2012; Mussatto et al., 2012; Shenoy et al., 2011).

On the other hand, a novel approach for value-added products from agroindustrial residues is the production of volatile aroma compounds for industry application using yeast and fungi (Murthy and Madhava Naidu, 2012; Medeiros et al., 2001; Pandey et al., 2000). The chemical synthesis of aroma compounds and their direct extraction from plants present economic and environmental disadvantages. Moreover, nowadays there has been a remarkable preference by consumers for natural food additives (Medeiros et al., 2001). Hence, biotechnological production of these compounds is a suitable alternative, since it occurs at mild reaction conditions, presents high enantioselectivity, does not generate toxic wastes and the products obtained may be labeled as "natural" (Bicas et al., 2010). The utilization of agroindustrial wastes as feedstock could bring economic feasibility for the commercial process, reducing costs in the culture medium (Bicas et al., 2010).

Many types of yeast have the capacity to produce specific single flavor molecules by either *de novo* synthesis or converting an added substrate/precursor molecule (Vandamme, 2003). During alcohol fermentations, yeasts can produce alcohols (2-phenylethanol), terpenes (citronellol, linalol, geraniol), esters (2-phenylethyl acetate) and other compounds with fruity, floral and green odors. Specifically, apiculate yeasts can impact the sensory quality of wine owning to the higher production of aroma compounds (De Benedictis et al., 2011; Moreira et al., 2011).

The application of coffee residues in such bioprocesses would reduce waste treatment costs, reduce environmental pollution, provide alternative substrates and produce economically important compounds. The aim of this study was first to evaluate the production of ethanol and volatile compounds by eight yeast strain utilizing coffee residues (coffee pulp and coffee wastewater) as substrate for fermentation, and then to investigate the effects of culture medium composition and the inoculum size on fermentation performance of pre-selected yeast, as well as to determine the volatile compounds produced under these optimum conditions.

2. Methods

2.1. Raw material

Coffee pulp and coffee wastewater from the depulping and demucilage process of coffee beans (*Coffea arabica* L., variety Catuaí 99 vermelho) were supplied by a coffee-producing unit located in the Southern area of the state of Minas Gerais (Brazil), during the 2012 harvest. As soon as obtained, the materials were frozen at −20 °C. The coffee pulp was dried at 60 °C for 48 h until reaching constant weight, later the coffee pulp was ground and stored in hermetical glass flasks.

2.2. Physicochemical analysis

The physicochemical characteristics of the coffee pulp and wastewater were determined according to standard procedures. The parameters analyzed were: water content, ash, lipids and protein (AOAC, 1970), reducing sugars, non-reducing sugars and total sugar (Nelson, 1944), crude fiber (Van der Kamer and Van Ginkel, 1952), cellulose, hemicelluloses, and lignin (Van Soest and Wine, 1968), soluble pectin, total pectin and solubility (Bitter and Muir, 1962), total phenolic compounds by the Folin–Ciocalteu method, pH, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (APHA, 1992).

2.3. Yeasts strains

Eight strains isolated from coffee fermentation, cocoa fermentation, sugar cane spirit (cachaça) fermentation and soil were evaluated in the present study. They were preselected according to their capacity to produce ethanol and aroma compounds in published studies. The isolates involved were: *Saccharomyces cerevisiae* UFLA CA11, *Pichia anomala* UFLA CAF70, *Kluyveromyces marxianus* UFLA CH1-1, *Candida tropicalis* UFLA CES-Y573, *Pichia guilliermondi* UFLA CAF725, *Haseniaspora uvarum* UFLA CAF76, *Torulaspora delbruekii* UFLA CAF58 and *Pichia stipitis* NCYC 1541 (National Collection of Yeast Cultures, Norwich, UK). All strains, except *P. stipitis*, were from the microbial collection of the Microbial Physiology Laboratory/Department of Biology from the Federal University of Lavras (UFLA) in Brazil.

2.4. Inoculum preparation

Yeast strains maintained at −80 °C were re-activated and multiplied using YPD medium (10 g/L yeast extract, 20 g/L peptone and 20 g/L glucose) as described below. Using a platinum loop, yeast strains were inoculated into tubes containing 1 mL YPD and were incubated at 28 °C. After 24 h, the content of these tubes were transferred to new tubes containing 9 mL YPD and incubated for 24 h at 28 °C. The resulting yeast cultures (10 mL) were transferred to Erlenmeyer flasks containing 90 mL YPD, which were subsequently incubated for 24 h at 28 °C; for all steps the agitation was 135 rpm. Yeast cells were later recovered from the medium by centrifugation (5000 rpm, 10 min, 20 °C) and washed twice with sterile peptone water (Duarte et al., 2013). Finally, the biomass obtained was inoculated in the fermentation medium containing coffee pulp and coffee wastewater.

2.5. Screening of the yeast strains

Eight yeasts strains were screened based on their fermentation performance (sugar consumption, ethanol production and fermentation kinetics). Fermentation experiments were performed using 250 mL Erlenmeyer flasks containing a mixture of 10 g of coffee pulp and 100 mL of coffee wastewater, this medium was sterilized at 121 °C for 15 min. The flasks were inoculated with an initial concentration of 0.3 g/L of cells (dry weight) of each strain. The incubation was performed in a rotary shaker at 27 °C and 105 rpm. The fermentation time was determined based on the maximum sugar consumption, analyzed by HPLC. During the experiments, samples were taken for sugars (sucrose, glucose and fructose), alcohols (ethanol and glycerol) and volatile

compounds determinations. All the assays were performed in duplicate. After this preliminary test, the strain with the highest ethanol yield, lower residual sugar level and highest kinetic parameters was used in a new fermentation assay.

2.6. Evaluation of the effect of the culture medium composition and the inoculum size on fermentation performance

At the second stage, three variables were tested aiming to improve the fermentation performance with the selected yeast strain from the previous stage (item 2.5). It was carried out in a $2 \times 3 \times 2$ factorial experiment. The variables tested were: coffee pulp concentration (6% and 12% w/v), yeast extract concentration (0, 1 and 2 g/L) and inoculum size (0.3 and 1 g/L dry weight), which combinations resulted in 12 assays.

The pre-selected yeast strain was inoculated in the culture medium containing 100 mL of coffee wastewater and different concentrations of coffee pulp and yeast extract as described above. The fermentation conditions were the same as previously described (item 2.5). Samples were taken for determination of sugars and alcohols. All the assays were performed in duplicate. The assay with significant and positive effect on the fermentation performance was chosen to conduct the fermentation in 1 L of medium containing the coffee residues (described below).

2.7. Fermentation with optimized conditions

The assay obtained in the last stage was reproduced in 1 L of culture medium and incubated at the same fermentation conditions. This experiment was carried out in triplicate. At initial and final time of fermentation, samples were taken for determination of sugars, ethanol and volatile compounds. The content of total phenolic compounds was determined (Folin–Ciocalteu method), in the residual solid, after fermentation, for its comparison with the concentration of phenolic compounds in the coffee pulp.

2.8. Evaluation of the fermentation performance

To evaluate the fermentation performance some parameters were calculated. The ethanol yield factor ($Y_{p/s}$, g/g) was determined as the ratio between the maximum ethanol concentration (g/L) and total sugars consumed (g/L). Ethanol volumetric productivity (Q_p, g/L h) was defined as the ratio between the maximum ethanol concentration (g/L) and the respective fermentation time (h). The conversion efficiency of sugars to ethanol (E_f, %) was calculated as the ratio between the obtained $Y_{p/s}$(g/g) and the theoretical value (0.51 g/g) of this parameter (Mussatto et al., 2012). The percentage of sugars consumed (*Conv*, %) was defined as a ratio between the total sugars consumed (g/L) and the initial sugar concentration (g/L).

2.9. HPLC analysis

The concentration of carbohydrates (sucrose, glucose and fructose), ethanol and glycerol was determined by High-Performance Liquid Chromatography (HPLC). Analyses were carried out using a Shimadzu chromatograph (Shimadzu Corp., Japan) that was equipped with a refractive index detector (RID-10A). For carbohydrates quantification was used a Shimadzu ion exclusion column (Shim-pack SCR-101C, 7.9 mm x 30 cm) that was operated at a temperature of 80 °C using ultrapure water as the eluent at a flow rate of 0.6 mL/min. Ethanol and glycerol was determined using a Shimadzu ion exclusion column (Shim-pack SCR-101H, 7.9 mm x 30 cm) that was operated at 30 °C using 100 mM perchloric acid as the eluent at a flow rate of 0.6 mL/min. The compounds were identified by comparison of their retention times with the retention times of certified standards. The quantification of compounds was performed using calibration curves constructed with different concentrations of standard compounds, injected at the same conditions of samples (Duarte et al., 2013, 2011). All samples were examined in duplicate.

2.10. Extraction of volatile compounds and GC-FID analysis

The extraction of volatile compounds was performed by headspace solid-phase microextraction (HS-SPME). A sample of 5 mL of fermented medium was added in a 15 mL vial with 4-nonanol (internal standard) at a final concentration of 249.5 μg/L. Extraction was performed by constant magnetic stirring of sample during 10 min of equilibration at 60 °C. After this period, a 50/30 μm DVB/CAR/PDMS fiber (Supelco, Belletonte, PA, USA) was exposed into the sample headspace for 15 min with a constant stirring and the same temperature. The time of volatiles desorption from the SPME fiber was fixed at 5 min.

The volatile compounds were analyzed using a gas chromatograph (GC) Shimadzu model 17A, equipped with an flame ionization detector (FID) and using a capillary column of silica DB Wax (30 m × 0.25 mm i.d. × 0.25 μm) (J&W Scientific, Folsom, Calif.,U.S.A.). The oven temperature was maintained at 50 °C for 5 min, raised to 190 °C by increments of 3 °C/min, and then maintained at 190 °C for 10 min. Injector and detector temperatures were kept at 230 and 240 °C, respectively. The carrier gas (N_2) was maintained at a flow rate of 1.2 mL/min. Injection was made in the split mode (1:10). The volatile compounds were identified by comparing their retention times with those of standard compounds injected under the same conditions. Quantification of the volatile compounds was expressed as 4-nonanol (internal standard) equivalents. The relative concentrations of the investigated compounds were calculated by relating the area of the internal standard to the area of the compound of interest (Duarte et al., 2011). Analyses were performed in duplicate.

2.11. Statistical analysis

The software SISVAR 5.1 (Lavras, MG, Brazil) was used for the Scott–Knott test. Principal Component Analyses were performed with XLstat 7.5.2 software (Addinsoft's, New York, NY, USA).

3. Results and discussion

3.1. Physicochemical analysis

Table 1 shows the composition of coffee pulp and coffee wastewater. The majority of values found for coffee pulp were similar to those reported by other authors (Esquivel and Jiménez, 2012; Navia et al., 2011; Ulloa et al., 2003; Elias, 1979), with exception of proteins, which presented a relative higher content (14.79%) when compared with other studies that found values from 7% to 12% protein (Navia et al., 2011; Ulloa et al., 2003). The coffee pulp presented significant sugar content (9.7%), which is expected given the origin of such residue, i.e., fruit pulp. On the other hand, coffee wastewater presented lower component values than coffee pulp but it had similar physicochemical characteristics since the wastewater catches components from pulp and mucilage during the depulping and the demucilaging process. The protein content in the wastewater was also measured in a significant concentration (4.26%). According to State Council for Environmental Policy (COPAM) of Minas Gerais/Brazil, the values of COD and BOD are higher than those permitted for effluent discharge in fluvial receptors (Table 1).

Table 1
Physicochemical characteristics of coffee residues added to the culture medium.

Parameter	Coffee pulp (dry matter)	Coffee wastewater
Water content (%)	82.44	97.56
Total sugars (%)	9.7	1.21
Reducing sugars (%)	9.63	1.13
Non-reducing sugars (%)	0.07	0.08
Protein (%)	14.79	4.26
Lipids (%)	1.2	nd
Ash (%)	7.33	0.16
Crude fiber (%)	14.1	nd
Cellulose (%)	20.7	–
Hemicellulose (%)	3.6	–
Lignin (%)	14.3	–
Solubility (%)	6.19	–
Total pectin (g 100 g^{-1})	11.37	–
Soluble pectin (g 100 g^{-1})	0.7	–
Total phenolic (g 100 g^{-1})	2.62	0.022
Chemical oxygen demand (mg L^{-1})	–	10,259
Biochemical oxygen demand (mg L^{-1})	–	6,500
pH	–	5.25

nd: not detected.
Maximum permitted values according to State Council for Environmental Policy of Minas Gerais (COPAM): DQO = 180 mg/L, DBO = 60 mg/L.

The chemical composition of coffee residues can differ widely according to the altitude, crop variety, cultivation conditions, processing mode and stage of development when the coffee cherries were harvested (Elias, 1979). The presence of significant amounts of fermenting sugars, protein, lipids and mineral components (ash) indicated that these residues seem quite promising for biotechnological process.

3.2. Screening of eight yeast strains

All yeasts strains were able to grow and ferment the culture medium containing coffee pulp and coffee wastewater. The sugars (sucrose, glucose and fructose) content was almost totally consumed after 24 h of fermentation, except fermentations with *P. guilliermondi* UFLA CAF725 and *P. stipitis* NCYC 1541 that required a longer fermentation time (48 h) (Table 2). The total initial sugar concentration was 35.23 g/L. As a consequence of sugar consumption, the yeasts produced levels of ethanol ranging from 10.79 g/L (*P. anomala* UFLA CAF70) to 14.67 g/L (*H. uvarum* UFLA CAF76), with significant differences (Table 2). The ethanol content was higher (letter a in the Scott–Knott test) for the strains *S. cerevisiae* UFLA CA11, *T. delbruekii* UFLA CAF58, *C. tropicalis* CES-Y573, *H. uvarum* UFLA CAF76 and *P. stipitis* NCYC 1541, with concentrations ranging from 13.47 to 14.67 g/L. These strains also showed higher ethanol yield factors $Y_{p/s}$, higher conversion efficiencies (E_f) and higher ethanol productivities (Q_p) (Table 2). The last parameter was lower only for *P. stipitis* NCYC 1541. The strains *P. anomala* UFLA CAF70, *K. marxianus* UFLA CH1-1, *P. guilliermondi* UFLA CAF725 and *P. stipitis* NCYC 1541 were the most efficient in sugar consumption with values of *Conv* from 99.19% to 99.39% (Table 2). This result means that the last strains probably used more of their carbon source for either biomass production or other metabolites than for ethanol production.

H. uvarum UFLA CAF76, an apiculate yeast, was previously isolated from coffee fermentation (Vilela et al., 2010). A previous work published by our group (Duarte et al., 2013) showed that this strain was not able to produce significant amounts of ethanol from sugar cane juice; however this strain produced the highest ethanol concentration of 14.67 g/L (1.86% v/v) demonstrating its adaptation to ferment the substrate from which it was isolated. Previous studies reported that *H. uvarum* produced up to 6% v/v of ethanol in fermented beverages, and it can tolerate ethanol up to concentrations of 4–7% (Moreira et al., 2011). *H. uvarum* UFLA CAF76 had the highest value for the ethanol yield factor $Y_{p/s}$ of 0.42 g/g, thus it converted sugar to ethanol more efficiently, based on the theoretical maximum value of 0.51 g/g. Furthermore, this strain was the most efficient producer of ethanol per unit time with a Q_p of 0.61 g/L h. Although *H. uvarum* UFLA CAF76 had a slightly lower percentage of sugars consumed (*Conv*) of 98.86% (letter *b* in the Scott–Knott test), the E_f value was the highest with 82.57% (Table 2), indicating that this yeast was able to convert more of the consumed sugar into ethanol than the other ones. These results showed that *H. uvarum* UFLA CAF76 had a good performance in the fermentation process; hence it was selected for the subsequent step of this work.

Since most studies for ethanol production from agroindustrial residues used *S. cerevisiae*, the results found here are quite interesting, seeing that the fermentation performance was better with *H. uvarum* UFLA CAF76 than with *S. cevevisiae* UFLA CA11. These findings were probably due to *H. uvarum* UFLA CAF76 having been isolated from coffee fermentation during semi-dry processing (Vilela et al., 2010), hence it is adapted to the components and conditions present in this medium. This yeast is commonly found in fermentations of natural and pulped coffee (Vilela et al., 2010).

3.3. Volatile compounds profile of eight yeast strains in coffee residues fermentation

Another purpose of this work was to test the coffee residues for the production of aroma/flavor compounds that could be interesting for food, cosmetics or perfume industries. The GC-FID analysis of the samples, belonging to the uninoculated medium and the medium after the fermentations by the eight yeasts strains, allowed the identification of thirty-five compounds corresponding to six groups of volatile compounds, higher alcohols, acetates, ethyl esters, aldehydes, terpenes and volatile acids (Table 3). The highest concentration of higher alcohols was found in the fermentation

Table 2
Concentrations (g/L) of residual sugars and alcohols produced in fermented coffee residues and kinetics parameters for eight yeasts strains.

Yeasts	Compounds (g/L)					Parameters			
	Sucrose	Glucose	Fructose	Glycerol	Ethanol	$Y_{p/s}$ (g/g)	Q_p (g/L h)	E_f (%)	*Conv* (%)
S. cerevisiae UFLA CA11	0.04 ± 0.01	0.23 ± 0.00	0.24 ± 0.05	1.67 ± 0.13	13.47 ± 0.59^a	0.39^a	0.56^a	76.09^a	98.56^c
T. delbruekii UFLA CAF58	0.01 ± 0.00	0.23 ± 0.03	0.26 ± 0.01	1.68 ± 0.06	13.78 ± 0.29^a	0.40^a	0.57^a	77.79^a	98.61^c
P. anomala UFLA CAF70	nd	0.17 ± 0.06	0.09 ± 0.02	0.32 ± 0.26	10.79 ± 0.68^c	0.31^c	0.45^c	60.53^c	99.26^a
C. tropicalis UFLA CES-Y573	nd	0.16 ± 0.02	0.23 ± 0.00	1.38 ± 0.05	13.58 ± 0.08^a	0.39^a	0.57^a	76.42^a	98.89^b
K. marxianus UFLA CH1–1	nd	nd	0.28 ± 0.02	1.95 ± 0.01	12.39 ± 0.72^b	0.35^b	0.52^b	69.50^b	99.19^a
*H. uvarum*UFLA CAF76	0.02 ± 0.00	0.09 ± 0.02	0.29 ± 0.02	0.91 ± 0.09	14.67 ± 0.38^a	0.42^a	0.61^a	82.57^a	98.86^b
P. guilliermondi UFLA CAF725	nd	nd	0.22 ± 0.01	1.21 ± 0.09	12.57 ± 0.27^b	0.36^b	0.26^d	70.39^b	99.39^a
*P. stipitis*NCYC 1541	nd	nd	0.25 ± 0.06	1.25 ± 0.25	13.67 ± 0.62^a	0.39^a	0.28^d	76.65^a	99.29^a

Average ± SD, values followed by the same letter are not significantly different at the 0.05 level (Scott–Knott test) $Y_{p/s}$: ethanol yield factor; Q_p: ethanol volumetric productivity; E_f: conversion efficiency from sugars to ethanol; *Conv*: percentage of sugars consumed.

Table 3
Concentrations (μg/L) of volatile compounds in coffee residues fermented by eight yeast strains.

No.	Compounds	Yeast strains (After fermentation)								
		Uninoculated medium	*S. cerevisiae* UFLA CA11	*P. anomala* UFLA CAF70	*T. delbriekii* UFLA CAF58	*C. tropicalis* UFLA CES-Y573	*K. marxianus* UFLA CH1–1	*H. uvarum* UFLA CAF76	*P. guilliermondi* UFLA CAF725	*P. stipitis* NCYC 1541
Higher alcohols (11)										
1	Methanol	120.39 ± 0.67	45.48 ± 5.44	39.55 ± 2.03	46.49 ± 0.14	50.75 ± 2.13	63.34 ± 2.53	126.66 ± 0.45	112.88 ± 0.05	127.73 ± 1.98
2	1-Propanol	nd	4.31 ± 0.65	4.20 ± 0.87	1.49 ± 0.00	5.52 ± 0.03	6.28 ± 0.00	18.80 ± 0.27	45.83 ± 0.23	19.51 ± 0.01
3	2-Methyl-1-propanol	nd	5.60 ± 0.76	23.11 ± 0.22	5.40 ± 0.26	29.16 ± 0.20	39.25 ± 0.18	84.81 ± 2.71	218.32 ± 0.72	105.96 ± 0.89
4	2-Methyl-1-butanol	1.51 ± 0.02	38.79 ± 0.44	124.40 ± 0.12	55.06 ± 0.21	61.28 ± 0.29	114.08 ± 1.62	222.27 ± 1.18	213.12 ± 0.83	243.25 ± 0.78
5	3-Methyl-1-butanol	nd	0.59 ± 0.03	nd	nd	nd	nd	nd	nd	61.32 ± 6.03
6	2-Heptanol	nd	nd	nd	0.65 ± 0.04	nd	nd	nd	nd	nd
7	1-Hexanol	nd	nd	nd	nd	nd	nd	nd	3.28 ± 0.61	nd
8	1-Butanol	6.32 ± 0.72	9.08 ± 1.48	4.95 ± 1.14	2.68 ± 0.06	1.52 ± 0.48	2.27 ± 0.37	18.72 ± 0.67	32.83 ± 0.19	8.57 ± 0.58
9	Trans-3-hexen-1-ol	6.46 ± 0.05	9.80 ± 1.04	4.99 ± 0.62	1.37 ± 0.14	1.64 ± 0.32	2.32 ± 0.25	19.28 ± 1.42	61.22 ± 0.77	8.04 ± 0.10
10	Furfuryl alcohol	nd	1.46 ± 0.23	1.58 ± 0.01	1.96 ± 0.07	2.04 ± 0.05	1.99 ± 0.07	3.62 ± 0.03	4.51 ± 0.49	5.95 ± 0.08
11	2-Phenylethanol	nd	6.50 ± 0.21	28.39 ± 4.85	11.77 ± 0.10	15.60 ± 0.34	15.06 ± 0.35	15.92 ± 0.13	20.89 ± 0.13	14.04 ± 0.27
	Total higher alcohols	134.68	121.61	231.17	126.87	167.52	244.60	510.08	712.87	594.36
Acetates (6)										
12	Isobutyl acetate	nd	nd	nd	nd	0.22 ± 0.02	nd	nd	nd	2.05 ± 0.07
13	Isoamyl acetate	nd	4.91 ± 0.59	73.46 ± 1.36	0.57 ± 0.05	1.52 ± 0.15	19.97 ± 0.85	nd	1.39 ± 0.19	12.82 ± 0.67
14	Ethyl acetate	12.71 ± 0.40	1.27 ± 0.10	nd	nd	nd	nd	10.84 ± 0.42	14.09 ± 0.60	9.26 ± 0.06
15	Propyl acetate	nd	nd	2.04 ± 0.17	nd	nd	0.83 ± 0.00	nd	nd	nd
16	Furfuryl acetate	4.81 ± 1.51	6.14 ± 0.91	1.45 ± 0.11	2.05 ± 0.11	2.84 ± 0.14	2.06 ± 0.49	10.37 ± 1.55	16.36 ± 0.49	4.48 ± 0.23
17	Phenylethyl acetate	5.78 ± 0.27	2.39 ± 0.14	6.95 ± 1.98	2.86 ± 0.23	2.58 ± 0.52	77.25 ± 0.49	26.66 ± 1.12	8.47 ± 0.32	38.53 ± 0.29
	Total acetates	23.30	14.71	83.91	5.49	7.17	100.11	47.87	40.31	67.13
Ethyl esters (1)										
18	Ethyl butyrate	nd	0.61 ± 0.02	nd	nd	nd	nd	nd	nd	3.32 ± 0.11
	Total ethyl esters	0.00	0.61	0.00	0.00	0.00	0.00	0.00	0.00	3.32
Aldehydes (4)										
19	Acetaldehyde	30.95 ± 0.18	10.46 ± 0.80	20.22 ± 0.01	20.93 ± 0.83	28.51 ± 0.09	21.23 ± 0.47	30.41 ± 0.52	52.64 ± 2.16	34.04 ± 2.70
20	Butyraldehyde	nd	4.43 ± 0.59	387.55 ± 7.86	5.53 ± 0.19	9.40 ± 0.11	295.43 ± 1.44	4.35 ± 0.15	22.05 ± 2.20	319.87 ± 0.25
21	Octanal	nd	nd	nd	2.48 ± 0.51	0.98 ± 0.05	nd	10.35 ± 0.36	42.57 ± 0.33	9.42 ± 0.56
22	Furfural	46.14 ± 0.93	nd	nd	nd	nd	nd	nd	nd	nd
	Total aldehydes	77.09	14.89	407.77	28.94	38.88	316.66	45.11	117.27	363.32
Terpenes (6)										
23	Linalool	6.82 ± 0.03	7.37 ± 0.67	9.80 ± 0.24	6.98 ± 0.14	6.86 ± 0.52	6.69 ± 0.37	9.26 ± 0.82	7.90 ± 0.04	9.58 ± 0.07
24	a-Terpeniol	2.32 ± 0.13	2.18 ± 0.27	1.92 ± 0.00	1.52 ± 0.06	1.39 ± 0.00	1.71 ± 0.13	4.32 ± 0.09	2.73 ± 0.02	4.46 ± 0.04
25	Verbenone	3.45 ± 0.15	2.97 ± 0.22	2.86 ± 0.20	3.01 ± 0.13	7.01 ± 1.82	nd	4.51 ± 0.48	3.51 ± 0.29	10.13 ± 0.89
26	b-Citronellol	3.89 ± 0.03	1.73 ± 0.31	22.73 ± 2.44	2.58 ± 0.40	11.34 ± 0.16	8.04 ± 1.03	29.86 ± 0.44	4.71 ± 0.25	9.46 ± 0.29
27	Geraniol	1.02 ± 0.31	nd	1.15 ± 0.13	0.84 ± 0.20	nd	1.15 ± 0.05	3.46 ± 0.32	nd	nd
28	Guaiacol	2.04 ± 0.07	1.10 ± 0.09	1.33 ± 0.26	1.08 ± 0.15	3.62 ± 0.39	nd	nd	nd	7.49 ± 0.52
	Total terpenes	21.54	15.35	39.80	16.00	30.23	17.59	51.40	18.85	41.12
Volatile acids (5)										
29	Isobutyric acid	2.13 ± 0.08	0.96 ± 0.08	3.02 ± 0.78	0.94 ± 0.03	1.35 ± 0.02	3.77 ± 0.15	3.09 ± 0.27	1.30 ± 0.28	nd
30	Butyric acid	nd	0.93 ± 0.03	nd	nd	nd	0.65 ± 0.04	nd	nd	8.78 ± 0.45
31	Octanoic acid	2.04 ± 0.11	1.29 ± 0.07	nd	nd	nd	2.38 ± 0.35	nd	nd	nd
32	Nonanoic acid	nd	nd	nd	nd	nd	0.56 ± 0.07	nd	nd	nd
33	Decanoic acid	1.58 ± 0.20	1.11 ± 0.38	nd	0.95 ± 0.13	nd	nd	2.51 ± 0.23	3.83 ± 0.13	8.75 ± 0.07
	Total volatile acids	5.75	4.29	3.02	1.89	1.35	7.36	5.60	5.13	17.53
Others (2)										
34	1,1-Dietoxyethane	nd	nd	1.48 ± 0.04	nd	nd	nd	nd	nd	nd
35	2,3-Butanedione	2.20 ± 0.42	5.97 ± 1.08	2.14 ± 0.24	1.36 ± 0.06	0.57 ± 0.01	0.60 ± 0.06	5.17 ± 0.88	4.13 ± 0.18	1.14 ± 0.12
	Total others	2.20	5.97	3.62	1.36	0.57	0.60	5.17	4.13	1.14

nd: not detected.

with *P. guilliermondi* UFLA CAF725 (712.87 μg/L). Among these compounds 2-phenylethanol is industrially interesting because of its rose-like odor, the production of this compound was higher in the fermentations carried out with the strains *P. anomala* UFLA CAF70, *P. guilliermondi* UFLA CAF725 and *H. uvarum* UFLA CAF76. The strain *K. marxianus* UFLA CH1–1 had the highest concentration of acetates (100.11 μg/L). In relation to ethyl esters, ethyl butyrate was only produced by two strains *S. cerevisiae* UFLA CA11 and *P. stipitis* NCYC 1541 (Table 3). Acetates and ethyl esters are positive aromatic descriptors contributing with fruity notes (Salgado et al., 2012).

Terpenes are derived from the raw material and their concentration may increase through the enzymatic process during fermentation; they are considered positive aromas because they supply floral and fruity notes and have commercial interest (Salgado et al., 2012). The fermentation with *H. uvarum* UFLA CAF76 produced the highest concentration of terpenes, which increased from 21.54 μg/L (uninoculated medium) to 51.40 μg/L.

Volatile acids were the more abundant group in the medium fermented by *P. stipitis* NCYC 1541 (17.53 μg/L), which have odor descriptors of fatty- rancid, cheesy and sweaty. Furfural was the most abundant aldehyde in the uninoculated medium with

46.14 μg/L, this concentration enabled cell growth of all yeast strains. Besides, this compound was not detected in the medium after the fermentation by all yeasts. Therefore, furfural was likely either degraded during the fermentation or reduced into furfuryl alcohol and esterified to furfuryl acetate (Duarte et al., 2005). Furfural is undesirable because it can affect the specific growth rate of cells, the cell-mass yield on ATP and the volumetric and specific ethanol productivities, depending on its concentration in the fermentation medium (Palmqvist and Hahn-Hägerdal, 2000).

Fig. 1 shows the Principal Component Analysis (PCA) performed on the data from Table 3, it indicates the relationship between the composition of the volatile compounds and the yeast strains. PCA reduces the dimensionality of a data set consisting of a large number of interrelated variables, while retaining as much as possible of the variation present in the data set. This is achieved by transforming to a new set of variables, the principal components (PCs), which are uncorrelated and ordered so that the first few retain most of the variation present in the original variables. The first two principal components, PC1 and PC2, accounted for 55.35% of total variance (34.61 and 20.71%, respectively). The PC1 enabled the differentiation between the yeasts strains (Fig. 1b). On the positive side of PC1 and the negative side of PC2, the strains *H. uvarum* UFLA CAF76 and *P. guilliermondi* UFLA CAF725 were correlated with higher concentrations of octanal (21), 1-butanol (8), furfuryl acetate (16), trans-3-hexen-1-ol (9), ethyl acetate (14), acetaldehyde (19), 2-methyl-1-propanol (3) e 1-propanol (2) (Fig. 1a, b). *P. stipitis* NCYC 1541 was positively characterized in PC1 and PC2 by butyric acid (30), isobutyl acetate (12), ethyl butyrate (18), 3-methyl-1-butanol (5) and verbenone (25). On the negative side of PC1 and the positive side of PC2, the strains *H. uvarum* UFLA CAF70 and *K. marxianus* UFLA CH1–1 were correlated mainly with isoamyl acetate (13) and propyl acetate (15). The uninoculated medium is located at negative values of PC1 and PC2, characterized by the contribution of furfural (Fig. 1a, b).

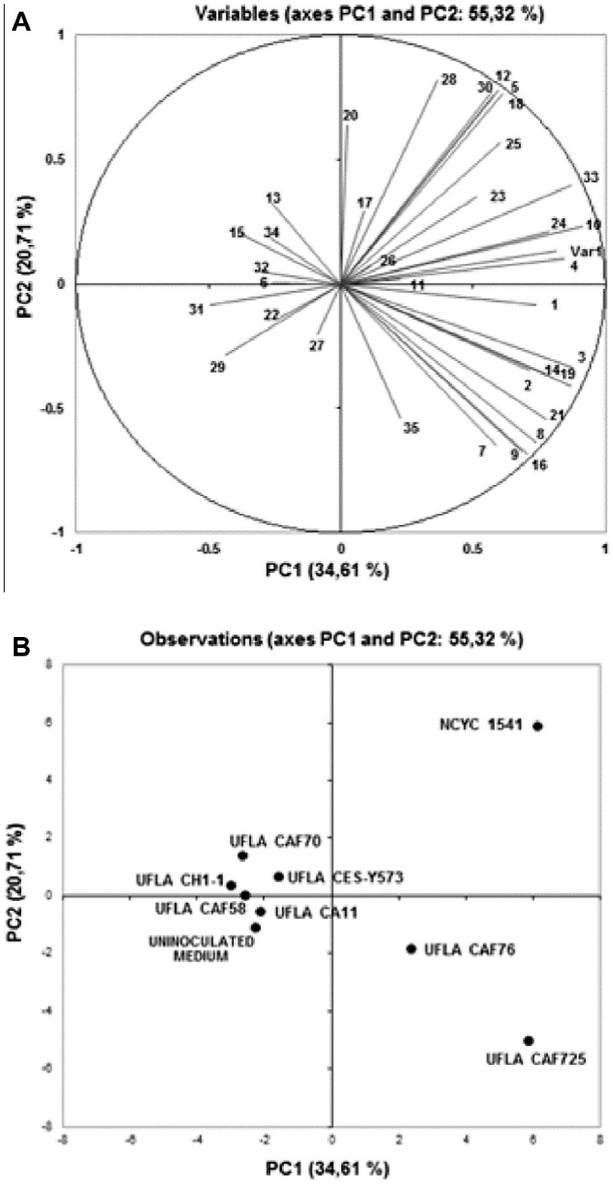

Fig. 1. Principal component analysis of volatile compounds (A) in coffee residues fermented by eight yeast strains (B). The numbers 1–35 correspond to the volatile compounds detailed in Table 3.

3.4. Evaluation of the effect of the culture medium composition and the inoculum size on fermentation performance

Three variables were tested for the improvement of fermentation performance with the yeast *H. uvarum* UFLA CAF76. The variables were coffee pulp concentration, yeast extract concentration and inoculum size (Table 4).

After 24 h of fermentation the higher ethanol concentrations were achieved in the assays 5, 6, 9 and 10 with values ranging from 20.39 to 21.02 g/L (letter a in the Scott–Knott test), they also had the best productivities (Q_p). These assays contained the higher concentration of coffee pulp (12%), with yeast extract varying between 1 and 2 g/L, as well as, the inoculum size (0.3 and 2 g/L). Therefore, the higher coffee pulp concentration and the addition of yeast extract enhanced the ethanol production. Regarding the ethanol yield ($Y_{p/s}$) and the efficiency (E_f), the assays 4 to 12 showed the higher values, varying within the range of 0.459–0.479 g/g and 90.3–93.8%, respectively (Table 4).

The selection of the best assay was made according to economic aspects. For 1 or 2 g/L of yeast extract, there were no significant differences among the values of each parameter, hence to reduce costs in the process 1 g/L can be chosen. Likewise, the parameters were not influenced by the inoculum size, since there were no significant differences between the assays when coffee pulp and yeast extract concentrations were kept constant. Thus, the lower concentration of inoculum (0.3 g/L) may be used for optimizing time and lowering the costs. Therefore, assay 5 was selected for the fermentation in 1 L. The assay 5 had a high ethanol concentration, high ethanol yield ($Y_{p/s}$), high productivity (Q_p) and high conversion efficiency (E_f) with values of 20.39 g/L, 0.474 g/g, 0.849 g/L h and 92.89%, respectively (Table 4). These values were greater than those achieved in the screening step by the same yeast (*H. uvarum* UFLA CAF76), which was probably due to the addition of yeast extract and increase of coffee pulp to 12%, which in turn increased the sugar content, improving the fermentation parameters.

The ethanol yield obtained with assay 5 was greater than that reported in previous studies using different coffee residues as substrate. For example, Mussatto et al. (2012) achieved 11.7 g/L of ethanol from 54 g/L of initial sugars, in a fermentation of spent coffee ground hydrolysates. A mixture of coffee pulp and mucilage submitted to acid hydrolysis and fermented with commercial baker's yeast showed an ethanol yield of 25.44 g/L, from 64.40 g/L of total sugars (Navia et al., 2011). For coffee residue waste, Choi et al. (2012) found that fermentation with *S. cerevisiae* KCTC 7906, produced 15.3 g/L of ethanol from 40.2 g/L of fermentable sugars, after a popping pretreatment and enzymatic hydrolysis.

Table 4
Results of ethanol production and kinetics parameters after 24 h of fermentation in different medium compositions and inoculum sizes of *H. uvarum* UFLA CAF76.

Assays	Variables				Parameters			
	Coffee pulp	Yeast extract (g/L)	Inoculum size (dry weight g/L)	Ethanol (g/L)	$Y_{p/s}$ (g/g)	Q_p (g/L h)	E_f (%)	Conv (%)
1	12	0	0.3	13.14 ± 0.14^c	0.419^b	0.547^c	82.104^b	74.348^d
2	12	0	2	15.57 ± 0.30^b	0.436^b	0.649^b	85.420^b	82.061^c
3	6	0	0.3	15.15 ± 0.10^b	0.446^b	0.631^b	87.532^b	99.028^a
4	6	0	2	15.16 ± 0.08^b	0.475^a	0.631^b	93.232^a	99.171^a
5	12	1	0.3	20.39 ± 0.25^a	0.474^a	0.849^a	92.887^a	95.356^b
6	12	1	2	20.67 ± 0.26^a	0.459^a	0.861^a	90.029^a	97.280^a
7	6	1	0.3	15.70 ± 0.11^b	0.479^a	0.654^b	93.883^a	97.510^a
8	6	1	2	15.78 ± 0.08^b	0.467^a	0.657^b	91.587^a	97.775^a
9	12	2	0.3	21.02 ± 0.16^a	0.471^a	0.876^a	92.362^a	97.384^a
10	12	2	2	20.93 ± 0.86^a	0.472^a	0.872^a	92.483^a	97.694^a
11	6	2	0.3	15.62 ± 0.44^b	0.466^a	0.651^b	91.443^a	97.946^a
12	6	2	2	16.01 ± 0.13^b	0.474^a	0.667^b	92.995^a	98.285^a

Values followed by the same letter are not significantly different at the 0.05 level (Scott–Knott test).
$Y_{p/s}$: ethanol yield factor; Q_p: ethanol volumetric productivity; E_f: conversion efficiency from sugars to ethanol; *Conv*: percentage of sugars consumed.

3.5. Fermentation under optimized conditions

The fermentation in 1 L of medium was performed with 12% of coffee pulp, 1 g/L yeast extract and 0.3 g/L (dry weight) of inoculum of *H. uvarum* UFLA CAF76. In this fermentation, after 24 h, the ethanol yield factor ($Y_{p/s}$) was the highest in all stages of this study, with a value of 0.48 g/g. Consistent with $Y_{p/s}$, the E_f value was also higher (94.11%), as well as, the *Conv* value (96.55%). The ethanol productivity (Q_p) was 0.55 g/L h. These values are better than those found in other studies of ethanol production from different raw materials of coffee. For example, fermentation of spent coffee grounds hydrolysates by *S. cerevisiae* RL-11 yielded an $Y_{p/s}$ of 0.26 g/g, Q_P of 0.49 g/L h, and E_f of 50.2% (Mussatto et al., 2012). Another study reported that fermentation of wet coffee pulp hydrolysate by *S. cerevisiae* achieved an ethanol yield of 0.46 g/g and showed an efficiency of 40% (Shenoy et al., 2011). Fermentation of coffee residue waste treated with enzymatic hydrolysis obtained a maximum ethanol yield of 89.9% (Choi et al., 2012). Kwon et al. (2013) after fermentation with *S. cerevisiae* found ethanol yields of 0.46 g/g for hydrolyzate of spent coffee grounds and 0.43 g/g for hydrolyzate of lipids from extracted coffee grounds.

It can be observed that our production of ethanol by fermentation of coffee pulp and wastewater was quite satisfactory in comparison to literature data, since all those studies used acid or enzymatic hydrolysis of raw materials. On the other hand, it can be note that all those authors used *S. cerevisiae* in the fermentation. This study found that *H. uvarum* UFLA CAF76 was able to produce ethanol from coffee residues in similar or better yields. Therefore, further research should be carried out on the potential of this strain for ethanol production from these residues. Coffee residues could be widely used for fermentation processes, since fermentable sugars are available in high concentration and high ethanol production may be achieved.

After the fermentation, sucrose, glucose and fructose remained in concentrations of 0.26 g/L, 0.53 g/L and 0.19 g/L, respectively. It had been reported that *H. uvarum* strains showed a fructophilic character, having a preferential consumption of fructose in respect to glucose (De Benedictis et al., 2011), which was confirmed with our results.

Table 5 shows the twenty-one volatile compounds found in coffee residues fermented with a pure culture of *H. uvarum* UFLA CAF76. Apiculate yeasts have been reported as producers of aroma compounds, mainly in winemaking (De Benedictis et al., 2011; Moreira et al., 2011). Here the volatile profile obtained was similar to that found in the screening stage, with a slight decrease in concentrations. Later, the most commercially interesting volatile compounds will be addressed.

The more abundant group was that with the higher alcohols; the oxygenation by stirring contributes to their production. One of the most interesting higher alcohols is the 2-phenylethanol, an aromatic alcohol with a rose-like odor widely used in perfumery, cosmetics and the food industry. This aroma compound was produced during the fermentation with *H. uvarum* UFLA CAF76 (14.56 μg/L) as well as phenylethyl acetate (16.42 μg/L), a derivative with rose, honey and apple odors. The ability of apiculate yeasts to produce these compounds was reported previously (Moreira et al., 2011). Currently, most consumers prefer natural food additives and since the 2-phenylethanol extracted from plants has a high price in the market (US$ 1,00 per kg), the microbial fermentation is a good and efficient alternative for its production in a relatively inexpensive way (Eshkol et al., 2009). The production cost could be even lower if the raw materials are agroindustrial residues. The 2-phenylethanol is produced naturally by various yeast strains from the catabolism of 2-phenylalanine via Ehrlich pathway (Longo and Sanromán, 2006). It had been reported that the presence of ethanol and 2-phenylethanol in the medium resulted in a synergistic inhibition, which reduced the tolerance of yeast to 2-phenylethanol and thus its production (Eshkol et al., 2009; Longo and Sanromán, 2006). Hence, in a further study with the purpose of producing 2-phenylethanol, this compound could be removed *in situ* with specialized resins to increase its production.

Another interesting group of aroma compounds for industry are the terpenes found mainly in plants, but fungi belonging to the ascomycetes and basidiomycetes also synthesize several important terpenes (Longo and Sanromán, 2006). These compounds were characterized by the production of linalool (sweet, fresh, citrus odor) and b-citronellol (fresh, rose-like odor) in concentrations of 11.61 μg/L and 9.50 μg/L, respectively. They are two of the most flavor-active compounds due to their low sensory threshold (Longo and Sanromán, 2006). Terpenes can be present as free molecules or non-aromatic glycosylated precursors, and non-*Saccharomyces* species can release enzymes (e.g., β-glucosidase) with the capacity to hydrolyze these precursors making their active aromatic forms, useful for flavor and fragrance industries (Maturano et al., 2012).

There are no reports in the literature addressing the value addition to agroindustrial residues using *H. uvarum*. However, most of the volatile compounds produced by *H. uvarum* UFLA CAF76 in fermented coffee residues have been identified by several authors in wine fermentation (De Benedictis et al., 2011; Moreira et al. 2011), indicating the potential of this yeast for natural production of aromas.

The amount of wastewater produced during coffee processing ranges from 1 to 15 liters per kg of coffee beans. The coffee-producing unit (Lavras, state of Minas Gerais – Brazil), from where

Table 5
Concentrations (μg/L) of volatile compounds in coffee residues fermented by *H. uvarum* UFLA CAF76.

No.	Compounds	Uninoculated medium	After fermentation
Higher alcohols			
1	Methanol	108.69 ± 1.95	98.24 ± 5.14
2	1-Propanol	nd	17.47 ± 1.04
3	2-Methyl-1-propanol	nd	57.46 ± 1.69
4	2-Methyl-1-butanol	nd	111.53 ± 2.48
5	1-Butanol	6.60 ± 0.51	9.11 ± 1.46
6	Trans-3-hexen-1-ol	7.74 ± 0.28	12.07 ± 0.55
7	Furfuryl alcohol	nd	2.33 ± 0.24
8	2-Phenylethanol	nd	14.56 ± 0.89
	Total higher alcohols	123.03	322.77
Acetates			
9	Ethyl acetate	14.91 ± 0.10	15.63 ± 0.49
10	Furfuryl acetate	1.29 ± 0.22	3.67 ± 0.36
11	Phenylethyl acetate	5.56 ± 0.49	21.98 ± 1.19
	Total acetates	21.76	41.28
Aldehydes			
12	Acetaldehyde	31.65 ± 2.08	30.44 ± 1.55
13	Butyraldehyde	nd	2.95 ± 0.43
	Total aldehydes	85.80	33.39
Terpenes			
14	Linalool	5.61 ± 0.04	17.22 ± 0.76
15	a-Terpeniol	2.75 ± 0.16	3.90 ± 0.42
16	Verbenone	1.85 ± 0.10	3.26 ± 0.65
17	b-Citronellol	2.74 ± 0.26	12.24 ± 0.66
18	Geraniol	1.54 ± 0.23	2.27 ± 0.20
	Total terpenes	15.12	33.89
Volatile acids			
19	Nonanoic acid	5.72 ± 0.19	3.20 ± 0.44
20	Decanoic acid	3.57 ± 0.10	6.17 ± 0.57
	Total volatile acids	15.18	9.37
Others			
21	2,3-Butanedione	0.88 ± 0.08	1.63 ± 0.26
	Total others	0.88	1.63

nd: not detected.

the wastewater used in this work was collected, uses in average 4 liters of water per kg of coffee cherries. Coffee pulp corresponds to 29% of residues generated from semi-dry coffee processing (Murthy and Madhava Naidu, 2012). According to the International Coffee Organization (ICO) the coffee production (2013) in Brazil was approximately 3 million tons produced in a cultivated area of 2.05 million hectares. Taking into account the Brazilian coffee yield of 1.4 ton/ha, the production of wastewater and coffee pulp is 5.6 m^3/ha and 0.4 ton/ha, respectively. The areas of coffee plantations in Brazil range from a few hectares to thousands of hectares. Considering the coffee production in Brazil, the amount of coffee pulp and wastewater generated and the estimated ethanol production; even for small coffee producers, the use of coffee residues with the process described in this study represents a new and interesting source of income for coffee producers. Besides ethanol, the production per hectare of others value-added compounds were estimated, e.g., 71.05 mg of 2-phenylethanol, 79,36 mg of phenylethyl acetate, 56.12 mg of linalool and 45.9 mg of b-citronellol. Although from an economic point of view the concentrations of volatile compounds were not too expressive, the obtained data sets precedent for new experiments to produce volatile compounds using coffee pulp and coffee wastewater.

On the other hand, phenolic compounds present in coffee pulp may produce lower absorption of amino acids and glucose, reduce food intake and lower digestibility of nutrients in ruminants, depending on the concentration (Waghorn and McNabb, 2003). These compounds were measured in the residual solid after the fermentation and a concentration of 0.13 g/100 g of dry material was found. Compared with the concentration in coffee pulp (2.62 g/100 g of dry matter), a substantial decrease of total phenolic compounds can be noted. This means that the fermentation process could detoxify the coffee pulp and this solid residue could have a possible application in animal feed.

This was an initial work to test the potential of coffee residues to produce value-added compounds, so there are many aspects that can be studied and improved on, as operational conditions (e.g. temperature, agitation, pH, oxygen availability, etc.), hydrolysis pretreatments and production of other value-added compounds (e.g. single-cell protein, phytochemicals, antioxidant compounds, enzymes, organic acids). This work proposes a low-cost fermentation process that could be of industrial value for coffee processing industries, generating extra incomes from value-added compounds. Likewise, this technology could be a proper alternative for waste disposal and recovery, minimizing the negatives effects to the environment.

4. Conclusions

Coffee residues have a large potential as raw material for bioethanol and volatile compounds production. *H. uvarum* UFLA CAF76 presented the best fermentation performance, which improvement was achieved with 12% w/v of coffee pulp, 1 g/L of yeast extract and 0.3 g/L of inoculum. Under these conditions, interesting results of ethanol yield ($Y_{p/s}$), productivity (Q_p) and efficiency (E_f) were obtained. The profile of aroma compounds was characterized by 2-phenylethanol, phenylethyl acetate, linalool and b-citronellol. *H. uvarum* demonstrated to be a quite promising yeast for fermentations from coffee residues, further research is necessary to elucidate and maximize their potential.

Acknowledgements

The authors would like to thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing financial support.

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