WHASLEY FERREIRA DUARTE

PRODUÇÃO DE METABÓLITOS POR DIFERENTES LEVEDURAS NA ELABORAÇÃO DE FERMENTADOS E DESTILADOS DE FRUTAS

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para a obtenção do título de Doutor.

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LAVRAS – MG
2011

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RESUMO

A utilização de frutas na produção de bebidas fermentadas e destiladas tem sido assunto de estudos em diversas partes do mundo. Neste trabalho, diferentes frutas (cacau, cupuaçu, gabiroba, jabuticaba, framboesa e umbu) e diferentes leveduras *Saccharomyces* foram avaliadas na produção de bebidas fermentadas, sendo a jabuticaba também utilizada para a produção de bebida destilada. Para caracterização das bebidas, técnicas cromatográficas (*high performance liquid chromatography* ou HPLC, *high performance liquid chromatography diode array detection* ou HPLC-DAD, *gas chromatography* ou GC, *gas chromatography – mass spectometry* ou GC-MS e *pulsed flame photometric detector* ou PFPD) e análises sensoriais (*quantitave descriptive analysis* ou QDA e escala hedônica) foram empregadas. Na avaliação dos frutos tropicais (cacau, cupuaçu, gabiroba, jabuticaba e umbu) para a produção de bebidas fermentadas constatou-se que a levedura *S. cerevisiae* UFLA FW 1162 foi eficiente na fermentação das polpas, resultando em bebidas com boa aceitação sensorial e características peculiares quanto à composição de voláteis (93 compostos), como a presença de compostos terpenoicos (mentol, limetol e linalol, entre outros). A jabuticaba, além do potencial para produção da bebida fermentada, foi também empregada com sucesso na produção de uma bebida destilada. Na produção do fermentado de framboesa, das 16 leveduras avaliadas, a cepa *S. cerevisiae* UFLA FW 15 apresentou melhores concentrações de compostos voláteis aromáticos, como 3-metil-1-butanol, butirato de etila, decanoato de etila, acetato de metilbutila e 3-mercapto-1-hexanol, dentre outros e também melhores resultados na análise sensorial.

Palavras-chave: metabólitos, leveduras, cromatografia gasosa, cromatografia líquida, bebidas alcoólicas, frutas.

ABSTRACT

The use of fruits in the production of both fermented and distilled beverages has been the subject of studies in many parts of the world. In this work, different fruits (cocoa cupuaçu, gabiroba, jaboticaba, umbu and raspberry) and different *Saccharomyces* were evaluated in the production of fermented beverages; jabuticaba was also used to produce a distilled beverage. To the characterization of the beverages, chromatographic techniques (HPLC, HPLC-DAD, GC, GC-MS and PFPD) and sensory analysis (QDA and hedonic scale) were used. In the assessment of tropical fruits (cocoa, cupuaçu, gabiroba, jaboticaba and umbu) for production of fermented beverages, the strain *S. cerevisiae* UFLA FW 1162 was efficient to ferment the pulp of fruits resulting in beverages that showed good acceptability and has peculiar characteristics of the composition of volatiles (93 compounds), such as the presence of terpenics compounds (menthol, limetol, linalool, among others).The jaboticaba was successfully employed in the production of both fermented and distilled beverage. In the production of raspberry wine, among 16 evaluated yeasts, *S. cerevisiae* UFLA FW 15 was the strain that produce wine with best concentrations of volatile aromatic compounds such as 3-methyl-1-butanol, ethyl butyrate, ethyl decanoate, methylbutyl acetate, 3-mercapto-1-hexanol, among others, besides showing better results in sensory analysis.

Keywords: yeast, metabolites, gas chromatography, liquid chromatography, alcoholic beverages, fruits.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Os alimentos fermentados estão entre os primeiros alimentos consumidos pelos seres humanos. Isto não se deu porque os primeiros seres humanos planejaram ou tiveram a intenção de fazer a fermentação dos alimentos, mas sim devido ao fato de a fermentação ser o simples e inevitável resultado quando a matéria-prima alimentar é deixada em estado de não preservação. A produção de bebidas fermentadas é realizada pela humanidade há mais de 5.000 anos. Entre 4000 e 3000 a.C., os egípcios já produziam cerveja em grande quantidade. O vinho é uma das mais antigas bebidas fermentadas produzida pelo homem e estudos indicam que nas regiões em que hoje estão Turquia, Egito e Irã, no período entre 8500 e 4000 a.C., já se produzia vinho (HUTKINS, 2006).

Entre as bebidas fermentadas produzidas a partir da polpa de frutas, o vinho de uva é a produzida em maior quantidade. No entanto, atualmente, diversas frutas vêm sendo utilizadas. Banana (AKUBOR et al., 2003), cajá (DIAS; SCHWAN; LIMA, 2003), jabuticaba (CHIARELLI; NOGUEIRA; VENTURINI FILHO, 2005; DUARTE et al., 2010a), cacau (DIAS et al., 2007; DUARTE et al., 2010a), laranja (CORAZZA; RODRIGUES; NOZAKI, 2001), abacaxi (MUNIZ et al., 2002), gabiroba (DUARTE et al., 2009), cagaita (OLIVEIRA et al., 2011), papaia (LEE et al., 2010a,2010b), kiwi (SOUFLEROS et al., 2001) têm sido utilizadas com sucesso, resultando na produção de bebidas com boa aceitação sensorial.

A utilização de frutas na produção de bebidas surge como uma alternativa para o emprego do excesso de produção, no caso de frutas comercialmente cultivadas, como uma nova forma de exploração de frutas nativas não comercialmente cultivadas, podendo gerar recursos econômicos, principalmente nas regiões de ocorrência das fruteiras. Além disso, representa uma alternativa de exploração de ecossistemas ameaçados, como o cerrado brasileiro (DUARTE et al., 2009).

A produção de bebidas alcoólicas como alternativa para uso de excesso de produção foi relatada por Reddy e Reddy (2005). Estes autores relataram o emprego do excesso da produção de mangas na elaboração de uma bebida fermentada. Lee et al. (2010a) utilizaram papaya para produzir uma bebida fermentada, demonstrando que o excedente de produção desta fruta de fácil degradação apresenta-se como material viável para uso na produção de bebidas. No caso de frutas não comercialmente exploradas, Duarte et al. (2009) demonstraram que a gabiroba apresenta potencial para uso na produção de fermentado de frutas, o que pode encorajar a implantação de mecanismos de exploração do cerrado brasileiro, visando à redução da degradação deste bioma.

O presente trabalho foi realizado com os seguintes objetivos: i) avaliar o potencial de diferentes frutas para a produção de bebidas fermentadas e destiladas; ii) avaliar diferentes cepas de *Saccharomyces* como culturas iniciadoras na fermentação para a produção de bebidas a partir de polpa de frutas e iii) identificar os diferentes metabólitos produzidos por leveduras na fermentação para a produção de fermentados de frutas utilizando técnicas cromatográficas como HPLC, HPLC-DAD, GC, GC-MS e PFPD.

2 REFERENCIAL TEÓRICO

2.1 Frutas, fermentados e destilados de frutas

A produção mundial de frutas vem aumentando gradativamente nos últimos anos. No Brasil, embora a produção média anual tenha sofrido uma redução de aproximadamente 3% nos últimos 3 anos, a elevada produção de frutas tem colocado o país na terceira posição, no ranking dos maiores produtores de frutas do mundo, atrás apenas da China e da Índia (FOOD AND AGRICULTURE ORGANIZATION ON THE UNITED NATIONS – FAO, 2011). Com o aumento na produção, em muitos casos, observa-se também aumento nas perdas. Novas formas de aproveitamento dos excedentes de produção, como a elaboração de bebidas fermentadas e destiladas, surgem como boa alternativa para a redução de perdas e o aumento da rentabilidade econômica.

As frutas, de modo geral, apresentam, em sua constituição, compostos, como açúcares (glicose e frutose), em quantidades suficientes para serem utilizados pelos microrganismos, em especial leveduras, produzindo bebidas fermentadas com características peculiares. Além de açúcares, frutos também apresentam em sua composição uma série de compostos aromáticos voláteis. No entanto, para a maioria dos frutos ainda não foi realizada uma caracterização dos compostos aromáticos.

Uma melhor caracterização dos compostos voláteis presentes nos frutos pode permitir a elaboração maneiras mais eficientes de processamentos, de modo que se mantenha um elevado grau qualidade de aroma e sabor no produto final (FRANCO; SHIBAMOTO, 2000). Algumas frutas, como a laranja (KELEBEK et al., 2009) e a framboesa (DUARTE et al., 2010b,2010c), apresentam propriedades antioxidantes, sendo esta mais uma característica que faz com que sejam cada vez mais exploradas para a produção de bebidas.

Em suco e vinho de laranja, um total de 13 compostos fenólicos foi identificado e quantificado, incluindo ácidos hidroxibenzoico (2), ácidos hidroxicinâmicos (5), flavanonas (6). Hesperidina, narirutina e ácido ferúlico foram os mais abundantes compostos fenólicos em suco e vinho de laranja cujas atividades antioxidantes foram mensuradas utilizando-se a técnica de DPPH (2,2-difenil-1-picrilhidrazil). Constatou-se que a capacidade antioxidante do suco de laranja foi maior que aquela mensurada no vinho de laranja (KELEBEK et al., 2009). No vinho de framboesa, Duarte et al. (2010b) identificaram e quantificaram compostos antioxidantes (ácido clorogênico, ácido ferrúlico e ácido *p*-cumárico), demonstrando que, após a fermentação da polpa, a bebida final ainda apresentava potencial antioxidante.

No Brasil, a legislação define fermentado de fruta como uma bebida com graduação alcoólica de 4% a 14% em volume, a 20^{o}C, obtida pela fermentação alcoólica do mosto de fruta sã, fresca e madura de uma única espécie, do respectivo suco integral ou concentrado, ou polpa, que poderá, nestes casos, ser adicionado de água. Já o destilado, ou aguardente de frutas, é definido como a bebida com graduação alcoólica de 36% a 54% em volume, a 20^{o}C, obtida de destilado alcoólico simples de fruta ou pela destilação de mosto fermentado de fruta (BRASIL, 2009)

Em diversas partes do mundo, um crescente número de trabalhos tem sido desenvolvido com a utilização de frutas na produção de fermentados ou “vinhos de frutas” e destilados. Dentre as frutas utilizadas, podem-se citar manga (KUMAR; PRAKASAM; REDDY, 2009; REDDY; REDDY, 2005), papaya (LEE et al., 2010a, 2010b, 2010c), laranja (CORAZZA; RODRIGUES; NOZAKI, 2001; DA PORTO et al., 2003; SELLI; CABAROGLU; CANBAS, 2003; SELLI, 2007; SELLI et al., 2008; KELEBEK et al., 2009), gabiroba

(DUARTE et al., 2009, 2010a), kiwi (SOUFLEROS et al., 2001), melão (GÓMEZ-HERNÁNDEZ; ÚBEDA; BRIONES, 2008), acerola (SANTOS et al., 2005), cupuaçu (DUARTE et al., 2010a); caja (DIAS; SCHWAN; LIMA, 2003), cacau (DIAS et al., 2007; DUARTE et al., 2010a), jabutica (CHIARELLI; NOGUEIRA; VENTURINI FILHO, 2005; DUARTE et al., 2010a; DUARTE et al., 2011), cagaita (OLIVEIRA et al., 2011) e framboesa (DUARTE et al., 2010b,2010c).

Em países tropicais, como o Brasil, durante todo ano, há grande produção e oferta de frutas para serem consumidas frescas ou para uso na indústria de alimentos na produção de geleias, sucos, sorvetes e doces. Contudo, grandes quantidades ainda são desperdiçadas durante os períodos de pico de colheita, devido à rápida deterioração pós-colheita, causada por altas temperaturas e umidade, manuseio precário e procedimentos de armazenamento deficientes (DUARTE et al., 2009). Além das frutas cultivadas comercialmente, algumas frutas nativas também são empregadas na produção de bebidas. No Brasil, frutas nativas encontradas no cerrado, como gabiroba (DUARTE et al., 2009), cagaita (OLIVEIRA et al., 2011) e umbu (DUARTE et al., 2010a) , têm sido empregadas com sucesso na produção de vinhos de frutas. Similarmente às fruteiras do cerrado, frutas da Amazônia, como cupuaçu, apresentam potencial para uso na produção de vinhos de frutas (DUARTE et al., 2010a).

Na utilização das frutas para a produção de bebidas fermentadas, algumas correções, como teor de açúcares e sais nutritivos para as leveduras, são necessárias para a obtenção de um produto final de qualidade (CORAZZA; RODRIGUES; NOZAKI, 2001; SANTOS et al., 2005). Soufleros et al. (2001) constataram que o uso de kiwi para produção de vinho resultou em uma bebida com quantidades inferiores de compostos aromáticos quando comparado ao vinho de uva, entretanto, na análise sensorial verificou-se uma boa aceitação.

Estes autores utilizaram enzimas pectinolíticas para correção do mosto, o que resultou em uma elevada concentração de metanol na bebida final.

Para uma mesma espécie de fruta, características inerentes a cada cultivar ou variedade resultam em bebidas com aspectos químicos e sensoriais distintos. Reddy e Reddy (2005), concluíram que a partir de 6 diferentes variedades de manga, foi possível produzir um vinho com características de sabor e aroma semelhantes aos do vinho uva. Baseado no baixo custo de produção, os autores relataram ainda que, a manga se mostrou um bom substrato para produção de vinho e o seu aproveitamento sob forma de bebida fermentada pode contribuir para economia daqueles países produtores desta fruta.

Além das correções realizadas na polpa ou suco das frutas para posterior fermentação, em muitos trabalhos (DIAS et al., 2007; LEE et al., 2010c), autores tem verificados que a levedura utilizada na fermentação é um dos fatores que mais influencia a qualidade final da bebida produzida. Na produção de vinho de papaya, Lee et al. (2010c), avaliando o uso de leveduras *Saccharomyces cerevisiae* e *Williopsis saturnus*, constataram que o uso de culturas puras ou culturas mistas das leveduras avaliadas resultaram em vinhos de papaya distintos entre si, principalmente aquele produzido com cultura mista, cuja complexidade de compostos aromáticos foi superior aos vinhos produzidos com culturas puras de *S. cerevisiae* e *Williopsis saturnus*. Na avaliação de três diferentes *S. cerevisiae*, Dias et al. (2007) relataram que, em uma avaliação prévia, a estirpe codificada como UFLA CA1183 apresentou melhor performance de fermentação na polpa de cacau, sendo então utilizada para a produção de vinho de cacau. Ainda segundo estes autores, a bebida de cacau apresentou valores de metanol, álcoois superiores, acetaldeído e ésteres próximos àqueles encontrados em vinho de uva. Com os resultados do trabalho, os autores concluíram que o uso da polpa de cacau na produção de vinho é uma nova e viável alternativa para a utilização do fruto de cacau.

Diversas frutas têm sido utilizadas para a produção de bebidas destiladas. Dentre elas, podem-se citar melão (GÓMEZ-HERNÁNDEZ; ÚBEDA; BRIONES, 2008), koumaro (SOUFLEROS; MYGDALIA; NATSKOULIS, 2005), laranja (DA PORTO et al., 2003), marula (FUNDIRA et al., 2002), amora preta e groselha preta (GONZÁLEZ et al., 2010), pêra (GARCÍA-LLOBODANIN et al., 2008), ameixa e cereja (SCHEHL et al., 2005), framboesa e medronho (GONZÁLEZ et al., 2011). Assim como observado para os fermentados ou vinhos de frutas, os destilados produzidos a partir de diferentes frutas apresentam características peculiares, principalmente a composição de voláteis aromáticos. Tešević et al. (2009) identificaram 84 compostos voláteis no destilado de cereja de cornalina, sendo os ácidos graxos de cadeia linear, etil ésteres de ácidos com cadeias C_6-$C1_8$, limoneno, 2-feniletanol e 4-etilfenol os compostos mais abundantes. Considerando-se os resultados obtidos a partir da caracterização da bebida, os autores concluíram que a composição de voláteis do destilado de cereja cornalina assemelha-se a outros destilados alcoólicos.

O efeito positivo ou negativo dos compostos voláteis na qualidade do destilado depende das concentrações destes compostos na bebida. García-Llobodanin et al. (2008) utilizaram suco de pera concentrado e suco de pera natural para a produção do destilado e observaram que o aumento na concentração de compostos voláteis não necessariamente influencia positivamente a qualidade da bebida. Metanol e furfural exercem efeito negativo na qualidade da bebida. Entretanto, compostos como acetaldeído, lactato de etila e lactato de metila passam a exercer efeito negativo na qualidade do destilado, quando suas concentrações são aumentadas. Para minimizar os efeitos negativos de elevadas concentrações de alguns compostos voláteis no destilado, Duarte et al. (2011) o fracionaram em três porções, “cabeça”, “coração” e “cauda”, sendo a fração coração aquela correspondente à bebida com características desejáveis para consumo.

A espécie *Campomanesia pubescens* (DC) O. Berg é popularmente conhecida por gabiroba ou guabiroba. O gênero *Campomanesia* é representado por árvores e arbustos, podendo ser encontrado do norte da Argentina até Trindade e desde a região costeira brasileira até os Andes no Peru, Equador e Colômbia (LANDRUM, 1986). O nome *Campomanesia* é uma homenagem ao naturalista espanhol Rodrigues de Campomanes e a palavra "gabiroba" tem suas raízes na língua tupi-guarani e significa casca amarga (CARVALHO, 2002).

As plantas são pouco exigentes quanto ao tipo de solo e os frutos amadurecem nos meses de setembro a novembro, apresentando formato redondo, de coloração que varia do verde-escuro ao verde-claro e amarelo, exalando aroma adocicado e bastante agradável. Comumente são consumidos em algumas regiões de ocorrência sendo também utilizados para produção de sorvetes, sucos, doces e picolés. As espécies do gênero *Campomanesia* se destacam como potencial recurso alimentar da avifauna e do homem (VALLILO; BUSTILLOS; AGUIAR, 2006). Os frutos são empregados na alimentação humana, sendo consumidos *in natura*, na forma de doces, sucos, licores, sovertes, geleias e picolés (CARVALHO, 2002).

A jabuticaba é nativa do Brasil, originária da região centro-sul, podendo ser encontrada desde o estado do Para até o Rio Grande do Sul. Mas é nos estados de São Paulo, Rio de Janeiro, Minas Gerais e Espírito Santo que ocorrem maiores produções. Dentre as espécies atualmente conhecidas, destaca-se a *Myrciaria cauliflora* (DC) Berg (jabuticaba-paulista ou jabuticaba-ponhema ou jabuticaba-assu) e a *Myrciaria jabuticaba* (Vell) Berg (jabuticaba-sabará) que produzem frutos apropriados tanto para a indústria como para o consumo *in natura*. É uma fruta tipicamente brasileira que, apesar de ser considerada apropriada tanto para consumo in natura como para a indústria, tem o comércio limitado devido à sua alta perecibilidade, que compromete a qualidade, principalmente o aspecto externo (BRUNINI; OLIVEIRA; SALADINI, 2004). A jabuticaba é utilizada para a produção de aguardente, compota, geleia, jeripoga (espécie de vinho artificial, de mais fácil preparo), vinagre e vinhos. A jabuticaba também é utilizada na fabricação de um extrato que serve como corante de vinhos e vinagres, substituindo flores de sabugueiro, malva e papoulas, que são importadas (MANICA, 2000).

O umbuzeiro (*Spondias tuberosa* L.) é uma fruteira nativa de regiões semiáridas do nordeste brasileiro. Os frutos apresentam pH de 2,2 e 14,8^{o} Brix, com variações em função das características climáticas da região de ocorrência (LIRA JÚNIOR et al., 2005). Atualmente, esses frutos são consumidos restritamente na região nordeste do Brasil, principalmente na forma *in natura* ou preparados como refresco e sorvete (FOLEGATTI et al., 2003).

O cupuaçu (*Theobroma grandiflorum* Schum.) é nativo dos estados do Maranhão e Pará, sendo umas das mais populares frutas da região amazônica. A polpa é utilizada para produção de suco, sorvete, licor, geleia, balas e outros produtos (VENTURIERI, 1993). Os frutos são coletados quando caem no solo e a extração da polpa deve ser realizada até cinco dias depois, de modo a se evitar a perda da qualidade. Para a extração da polpa, utiliza-se, geralmente, o método manual, sendo esta uma etapa trabalhosa, pois a polpa encontra-se aderida à semente (VILLACHICA, 1996).

O cacau (*Theobroma cacao* L.) é originário das Américas do Sul e Central e é conhecido em todo o mundo devido ao uso de suas amêndoas na produção de chocolate. No Brasil, durante muitos anos, o cacau ocupou lugar de destaque na economia de alguns estados, principalmente o da Bahia (DIAS et al., 2007). A polpa apresenta sabor adocicado, tem baixo teor de compostos fenólicos e é muito aromática. O teor de sólidos solúveis da polpa é de aproximadamente 20%, o pH é 3,20 e a acidez total titulável é de 1%

(SCHWAN; SOUZA; MENDONÇA, 2000). Com base em suas características, a polpa pode ser utilizada em processos industriais para a produção de novos produtos, como geleias (SCHWAN, SOUZA; MENDONÇA, 2000; SCHWAN; WHEALS, 2004).

A framboesa (cv. Meeker) tem polpa com teor de sólidos solúveis (grau Brix) de aproximadamente 14,5 e pH de 3,6. Essas e outras características fazem da framboesa uma fruta com potencial para uso na produção de vinho. A framboesa é popular principalmente devido às suas características nutricionais, *flavour* e seus importantes benefícios à saúde advindos dos elevados teores de polifenóis e antioxidantes, com ação anticarcinogênica e efeitos contra doenças do coração (WEBER; HAI-LIU, 2002). A framboesa é produzida principalmente em países de clima temperado, no entanto, atualmente, no Brasil, a produção desta fruta tem aumentado em regiões mais frias, como Campos do Jordão, no estado de São Paulo.

2.2 Vinho

Vinho é uma bebida obtida pela fermentação alcoólica do mosto simples de uva sã, fresca e madura. O mosto simples de uva é o produto obtido pelo esmagamento ou prensagem da uva sã, fresca e madura, com a presença ou não de suas partes sólidas (BRASIL, 1988). Ainda segundo Brasil (1988), os vinhos podem ser classificados conforme a classe (de mesa, leve, fino, espumante, frisante, gaseificado, licoroso e composto), a cor (tinto, rosado, rosé ou clarete e branco) e o teor de açúcar (nature, extra-brut, brut, seco, meio seco, suave e doce).

2.2.1 Processo de produção

Hashizume (2001) definiu a vinificação como o conjunto de operações realizadas para transformar a uva em vinho. Ainda de acordo com este autor, o processo de vinificação pode variar conforme o tipo de vinho a ser produzido, podendo as operações comuns às diferentes vinificações ser resumidas em esmagamento e desengaçamento, sulfitagem, correções do mosto, inoculação de leveduras, remontagem e refrigeração, acompanhamento da fermentação e prensagem de bagaços. Outra maneira de listar as etapas que compõem o processo de vinifiação envolve as operações envolvidas na fabricação do vinho que compreendem a extração e o preparo do mosto, a fermentação alcoólica, a trasfega, a clarificação e a conservação (CORAZZA; RODRIGUES; NOZAKI, 2001). De forma semelhante e complementar, Fleet (1999) citou as mesmas etapas, acrescentando a fermentação malolática por bactérias do ácido lático como uma etapa opcional do processo de vinificação.

2.2.2.1 Extração do suco e preparo do mosto

Uma primeira etapa no processo de vinificação consiste no esmagamento das bagas da uva. O processo de esmagamento é realizado com o objetivo de romper as bagas, liberando o suco, de forma que as sementes e engaços não sejam esmagados. Esta operação visa também à obtenção de uma boa dissolução de matérias corantes e de taninos contidos na casca dilacerada (vinho tinto), além de provocar intensa aeração do mosto antes do início da fermentação, favorecendo o desenvolvimento das leveduras (HASHIZUME, 2001). Segundo Cataluña (1988), o processo de esmagamento tem como vantagem permitir que a maceração durante a fermentação seja mais eficiente, possibilitando a obtenção de vinhos mais tintos, quase completamente secos em curto prazo pela regularização da fermentação.

A prática de separação da ráquis (eixo da inflorescência) das bagas é denominada de "desengace". Esta separação é de grande importância para a qualidade do vinho, pois a presença da ráquis interfere negativamente na composição química do mosto, devido ao baixo teor de açúcares, acidez e elevado teor de potássio, podendo levar ao aparecimento de sabor amargo e sensação de adstringência nos vinhos tintos, devido à presença de taninos (HASHIZUME, 2001; MENEGUZZO; MANFROI; RIZZON, 2006). A presença do engaço pode levar a uma a redução do teor alcoólico do vinho de 0,2 a 0,4% pela água presente em sua constituição e pela absorção de parte do álcool pelo engaço (HASHIZUME, 2001). No entanto, Pato (1982) relatou que a adoção da prática de desengace apresenta vantagens e desvantagens, pois, no caso dos vinhos destinados a consumidores que têm preferência por uma bebida encorpada, corada e taninosa, a não retirada do engaço será desejada. Já para os vinhos suaves e delicados, o desengace se faz necessário.

A utilização do anidrido sulfuroso (SO_2) como agente antimicrobiano que vem sendo realizada durante séculos. Primeiramente usado pelos egípcios e posteriormente pelos romanos, o SO_2 era inicialmente usado para limpeza das ânforas. O seu uso na fermentação possibilitou melhoria significativa por causa do seu efeito inibitório no crescimento de leveduras e bactérias indesejáveis na fermentação (ROMANO; SUZZI, 1993).

O anidrido sulfuroso pode ser empregado em diferentes formas, como vapor gerado pela combustão de enxofre, anidrido sulfuroso puro (líquido) e metabissulfito de potássio ($K_2S_2O_5$). O emprego sob forma de vapor é o método mais antigo de uso e, hoje, encontra-se praticamente abandonado. Sob a forma líquida, o anidrido sulfuroso é amplamente utilizado em diversos países do mundo, sendo obtido sob condição de pressão e engarrafado em cilindros de aço.

O metabissulfito de potássio é comumente utilizado em pequenas indústrias devido à sua facilidade de uso. Trata-se de um sal branco que, teoricamente, rende 57% do seu peso em SO_2 (HASHIZUME, 2001). Segundo Romano e Suzzi (1993), as funções exercidas pelo SO_2 são necessárias para a obtenção de um vinho de boa qualidade. Estas funções são, de acordo com Hashizume (2001), as seguintes:

- efeito dissolvente, facilitando o efeito da dissolução da cor e dos polifenóis;
- efeito antioxidante, por receber o oxigênio do ar, protegendo o mosto e o vinho;
- efeito antioxidásico, destruindo a enzima oxidase, catalisadora da oxidação;
- efeito inibitório, inibindo o crescimento de microrganismos indesejáveis, como bactérias e algumas leveduras.

Constantí et al. (1998) verificaram que o uso de SO_2 inibiu o crescimento de leveduras como *Candida stellata* e *Hanseniaspora uvarum* e outras não *Saccharomyces*, pois estas somente estiveram presentes nos primeiros dias de fermentação quando não se utilizou o SO_2. Ainda segundo os mesmos autores, além da inibição de não *Saccharomyces*, o uso do SO_2 permitiu um rápido desenvolvimento de *Saccharomyces cerevisiae*. A ação inibitória do SO_2 sobre a população de leveduras não *Saccharomyces* também foi confirmada por Kling et al. (1998), em cujo trabalho, em comparação com mosto não sulfitado, não foram encontradas *Zygosaccharomyces* e *Kloeckera apiculata* quando se fez o emprego de 20 mg L^{-1} de SO_2 no mosto. O SO_2 apresenta também efeito inibitório eficiente sobre bactérias ácido-láticas. A forma de atuação deste composto sobre essas bactérias ainda não está bem esclarecida e estudos mais detalhados são necessários (ROMANO; SUZZI, 1993).

O emprego de SO_2 na vinificação pode levar a alterações nas características da bebida final. Garde-Cerdán e Ancín-Azpilicueta (2007) encontraram diferenças significativas na concentração de etil-hexanoato entre vinhos produzidos com SO_2 e vinhos produzidos sem adição de SO_2. Variações também foram encontradas no conteúdo total de álcoois. Kling et al. (1998) encontraram diferenças na avaliação sensorial das bebidas ao avaliarem o efeito do uso de SO_2 em fermentações inoculadas e não inoculadas.

Chaptalização é o termo usado para designar a operação de correção do mosto na qual se adiciona açúcar ao suco da uva (HASHIZUME, 2001). Esta prática vem sendo utilizada desde o século XVIII e, antes do uso de açúcar, a correção era feita com adição de mel (CATALUÑA, 1988). O açúcar a ser utilizado deve ser de boa qualidade e deve ser previamente diluído em pequena quantidade do próprio mosto.

A chaptalização é efetuada entre o segundo e o terceiro dia após iniciada a fermentação, juntamente com a remontagem, facilitando assim a homogeneização (MENEGUZZO; MANFROI; RIZON, 2006). Hashizume (2001) afirma que a chaptalização deve ser realizada em única vez na fase tumultuosa da fermentação, ou seja, quando o mosto apresenta metade do açúcar não transformado. Rizzon e Miele (2005), avaliando o uso de açúcar mascavo e de glicose de milho para chaptalização, concluíram que estes não substituem a sacarose comercial no processo. A correção é realizada considerando-se que a adição de 17 g de açúcar por litro resultará em um acréscimo de 1^o GL (Gay Lussac). Esta consideração é válida para fermentações cuja temperatura de processamento é baixa, o que não é comum na vinificação em tinto. Para condições de temperatura mais elevada, deve-se utilizar 18 g por litro para acréscimos de 1^o GL na bebida final (HASHIZUME, 2001).

A qualidade do vinho depende da composição do mosto e da tecnologia empregada na vinificação. Na vinificação, o processo de clarificação exerce influência direta nas qualidades organolépticas do vinho (MOZAZ et al., 1999).

A prática de utilização de substâncias que possibilitam a remoção de partículas que provocam turbidez ao vinho é denominada colagem (HASHIZUME, 2001). Agentes clarificantes e outras técnicas têm sido amplamente empregadas ao longo do tempo, para evitar a obtenção de vinhos escurecidos (LÓPEZ et al., 2001).

As substâncias utilizadas na colagem (colas) são comumente agrupadas em substâncias albuminoides, substâncias gelatinosas e substâncias minerais (PATO, 1982). Cosme, Silva e Laureano (2008) citam que, atualmente, diversos produtos são utilizados no processo de colagem. Os mais comumente empregados são bentonite, gelatina, caseína, caseinato de potássio, albumina de ovo, ictiocolas e, mais recentemente, algumas proteínas vegetais. Estes autores encontraram diferenças entre a composição fenólica de vinho branco submetido à ação de diferentes agentes clarificantes. López et al. (2001) estudaram a ação do carvão ativado associado a outros agentes clarificantes, como bentonite, caseína, albumina, gelatina e caseinato de potássio e verificaram que a constituição de compostos voláteis nos vinhos foi diferente para as diferentes combinações dos agentes clarificantes em conjunto com o carvão ativado.

A bentonite destaca-se como um dos agentes colantes mais utilizados. Esta é uma cola mineral que apresenta em sua constituição montmorilonita (silicato de alumínio), cuja capacidade de intumescimento é elevada e a presença de cargas negativas lhe confere forte poder de adsorção, sendo empregada com eficiência no combate à turvação proteica de vinhos (HASHIZUME, 2001). Catarino et al. (2006) relataram que o uso de bentonite pode influenciar a composição do vinho no que ser refere à presença de metais na bebida. Estes autores encontraram diferentes teores de minerais, como sódio, magnésio, alumínio, potássio, ferro e cobalto, entre outros, em vinho submetido à clarificação com bentonite, confirmando a influência do processo de clarificação na qualidade final.

2.2.2.2 Trasfega

O ato de transferir o vinho de um recipiente para outro, de forma a possibilitar a eliminação da borra depositada, é denominada trasfega (CATALUÑA, 1988; MENEGUZZO; MANFROI; RIZZON, 2006). Segundo Hashizume (2001), a remoção desta borra se faz necessária porque ela pode conter microrganismos e ser local de ocorrência de reações químicas que provocam alterações no vinho, resultando em odor desagradável ao mesmo tempo em que deprecia a bebida.

A realização desta prática se faz necessária para a obtenção de vinho de qualidade. A trasfega sem arejamento é comumente empregada para aqueles vinhos cuja acidez volátil têm tendência a aumentar, mas apresentam boa qualidade. A trasfega com aeração deve ser empregada no caso de vinhos que apresentam características de gás sulforoso presente em excesso, prejudicando assim seu paladar e cheiro. A realização da trasfega com passagem do vinho pelo sulfurador é uma prática antiga e atualmente pouco empregada. O número de trasfegas a serem feitas é dependente do tamanho das pipas (MENEGUZZO; MANFROI; RIZZON, 2006). A realização da primeira trasfega deve ocorrer por volta de uma semana após termino da fermentação e com aeração (HASHIZUME, 2001; PATO, 1982). Se a deposição de borra ainda for verificada após a realização da primeira trasfega, faz-se necessária a realização de uma segunda trasfega, 45-60 dias após a primeira (HASHIZUME, 2001).

2.2.2.3 Filtração

A clarificação do vinho é completa com a realização da colagem e, posteriormente, a filtração, que consiste na passagem do vinho por elementos filtrantes com porosidade reduzida de forma a torná-los brilhantes e cristalinos (HASHIZUME, 2001). Os diferentes tipos de filtros existentes para vinhos são normalmente aqueles cujo princípio é a tamisação ou adsorção. A filtração por tamisação é geralmente empregada para vinhos turvos com impurezas de grande dimensão, enquanto os filtros com funcionamento por adsorção são empregados para vinhos já quase limpos, os quais se pretende tornar mais límpidos e brilhantes (HASHIZUME, 2001). Segundo Meneguzzo, Manfroi e Rizzon (2006), os filtros podem ser classificados em três tipos:

- filtro de placa: este tipo de filtro é constituído de três tipos de placas. As primeiras são grandes e retêm as partículas de maiores tamanhos; o segundo tipo são as placas intermediárias, de porosidade variável e que recebem o vinho previamente filtrado pelas placas grandes; as últimas são as placas esterilizantes utilizadas pouco antes do engarrafamento;

- filtro de membrana: este tipo de filtro é instalado logo antes da enchedora de garrafas e é constituído de ésteres de celulose e uma camada de pré-filtragem com porosidade variável. Sua finalidade é a remoção de leveduras e bactérias.

2.2.2.4 Atesto

Atesto é o termo que se refere ao ato de preencher o espaço vazio do recipiente com o vinho. Este espaço vazio é, normalmente, formado devido à evaporação do vinho, uma vez que o armazenamento é, na maioria das vezes, realizado em recipientes de madeira (HASHIZUME, 2001). Segundo Pato

(1982), esse procedimento deve ser adotado logo após a última trasfega, a intervalos de 20 dias, para vasilhas de madeira. A taxa de evaporação varia de acordo com as condições de temperatura a que estão sujeitos os locais de armazenamento. Meneguzzo, Manfroi e Rizzon (2006) complementam citando que o atesto deve ser realizado semanalmente, dependendo do tamanho do recipiente.

A não permanência do espaço vazio pelo atesto impede o desenvolvimento de microrganismos aeróbios que podem causar danos ao vinho. O atesto deve ser realizado com cuidado de forma que o vinho usado deve apresentar a mesma qualidade daquele que está na pipa, evitando, assim, que todo o recipiente seja contaminado (HASHIZUME, 2001; MENEGUZZO; MANFROI; RIZZON, 2006)

2.3 Leveduras selecionadas

Atualmente, na fermentação para a produção de bebidas, têm se utilizado leveduras selecionadas, de modo a se obter fermentações mais rápidas, confiáveis, com redução dos riscos de ocorrência de contaminações bacterianas e fermentações lentas (VALERO et al., 2005). Como principais vantagens, o uso de leveduras selecionadas permite rápido início da fermentação, baixo risco de contaminação, melhor uniformidade nas taxas de fermentação, baixa competição por nutrientes, maior rendimento da bebida, baixas concentrações de açúcares residuais e manutenção das qualidades sensoriais da bebida (BERNADI et al., 2008; CAMPOS et al., 2010).

Desde o início dos anos 1980 até os dias atuais, *S. cerevisiae* ou “levedura do vinho” vem sendo extensivamente utilizada como iniciadora no processo de fermentação (VALERO et al., 2005). Segundo Guimarães et al. (2006), o uso de *S. cerevisiae* é uma estratégia que possibilita a manutenção da qualidade e assegura a reprodutibilidade das características do vinho. O uso de estirpes isoladas a partir de determinadas regiões constitui um fator interessante, pois essas estirpes apresentam elevada adaptação às condições climáticas e o vinho produzido, normalmente, possui características peculiares que são associadas às regiões produtoras específicas.

O uso de cepas selecionadas de *S. cerevisiae* na produção de vinhos e destilados de frutas tem sido realizado por diversos pesquisadores (DIAS et al., 2007; GONZÁLEZ et al., 2010; LEE et al., 2010b, 2010c), demonstrando a viabilidade e a eficiência do uso de culturas iniciadoras na produção de novas bebidas fermentadas e/ou destiladas.

Na produção do vinho de uva, o uso de leveduras selecionadas é uma prática já bem consolidada. De acordo com Nurgel et al. (2002a,2002b), a utilização de leveduras selecionadas na fermentação do mosto de uva resultou em maiores taxas de fermentação com consequente redução do tempo de fermentação quando comparado ao tempo da fermentação espontânea. Além da redução no tempo de fermentação, o uso da levedura selecionada levou à obtenção de uma maior concentração de etanol ao término da fermentação e a bebida final produzida apresentou diferenças significativas quanto à composição de compostos formadores de aroma, embora para uma cultivar específica de uva ('Emir Grown'), não tenham sido observadas diferenças significativas na composição de voláteis.

2.4 Técnicas cromatográficas para análise de metabólitos microbianos

Cromatografia é definida como um método físico de separação no qual os componentes a serem separados são seletivamente distribuídos entre duas fases imiscíveis (uma fase móvel e outra estacionária). O processo cromatográfico é o resultado de repetidas sorções/desorções durante o movimento do analito ao longo da fase estacionária (NIESSEN, 2007).

A utilização da cromatografia se iniciou por volta de 1900, quando Ramsey obteve a separação de mistura de gases e vapores em adsorvente similar ao carvão e Michael Tswett (Михаи́л Семёнович Цвет) conseguiu a separação de pigmentos de plantas por cromatografia líquida. Tswett é tido como o "pai" da cromatografia, principalmente por ter sido o primeiro a utilizar o termo e descrever o processo cientificamente (MCNAIR; MILLER, 2009).

Em 1906, Tswett publicou dois trabalhos na Berichte der Deutschen Botanischen Gesellschaft, nos quais descreve em detalhes o método cromatográfico para separação de pigmentos de plantas e utiliza o termo cromatografia pela primeira vez. Em um dos trabalhos, Tswett escreveu uma das suas mais famosas frases: "Como raios de luz no espectro, os diferentes componentes de uma mistura de pigmentos, obedecendo a uma lei, são separados na coluna de carbonato de cálcio, podendo ser qualitativa e quantitativamente determinados. Eu chamo este preparado de cromatograma e o método correspondente de "método cromatográfico".

O termo cromatografia é composto por dois radicais gregos, *chroma* (cor) e *graphien* (escrever) e sua tradução literal significa "cor da escrita", que se refere à visualização de anéis multicoloridos separados na coluna. Outra interpretação para o termo se refere ao sobrenome de Tswett (Цвет) que, em russo, significa "cor". Segundo esta interpretação, o termo cromatografia na verdade poderia significar "a escrita de Tswett" (ETTRE, 2008).

Nos 25 anos seguintes às descobertas de Tswett, poucos estudos foram realizados. O grande avanço veio em 1930-1931, no laboratório de Richard Kuhn, seguido por Paul Karrer (Zurique) e László Zechmeister (Hungria) e muitos outros (ETTRE, 2008).

No ano de 1941, A.J.P. Martin e L.M. Synge publicaram um trabalho intitulado *A new form of chromatogram employing tow liquid phases. 1 – A theory of chromatography. 2 – Application to the micro-determination of the higher monoamino-acids in proteins* (Figura 1). Neste trabalho foi descrita a aplicação do novo tipo de cromatografia, a cromatografia líquido-líquido, em diversos monoaminoácidos e não somente a dois, como apresentado anteriormente (COLLINS, 1999). Este trabalho também forneceu as idéias que formariam a base para cromatografia gasosa (LANÇAS, 1993, 2009).

151. A NEW FORM OF CHROMATOGRAM EMPLOYING TWO LIQUID PHASES

1. A THEORY OF CHROMATOGRAPHY

2. APPLICATION TO THE MICRO-DETERMINATION OF THE HIGHER MONOAMINO-ACIDS IN PROTEINS

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Figura 1 Título do trabalho de Martin e Synge, publicado em 1941.

No início dos anos 1950, a cromatografia passou por um grande avanço com a introdução da cromatografia gasosa (GC). Até a primeira metade dos anos 1960, houve uma evolução rápida da cromatografia gasosa, enquanto na segunda metade daquela década, observou-se a introdução de uma moderna e sofisticada cromatografia líquida, mas ainda baseada nos princípios propostos por Tswett, no início do século XX (ETTRE, 2008). O século XX tem sido considerado "o século da cromatografia", por vários autores, pois esta técnica foi de grande importância no desenvolvimento de várias áreas das ciências físicas e biológicas durante todo o seu decorrer (COLLINS, 2009).

A classificação dos métodos cromatográficos pode mudar de acordo com o enfoque dado pelo autor. De acordo com Lanças (1993), os critérios mais comumente utilizados na classificação são: (1) quanto ao mecanismo de separação, (2) quanto à técnica empregada e (3) em relação ao tipo de fase utilizada. Ainda de acordo com o autor, a classificação mais popular considera o tipo de superfície na qual a separação ocorre: se em um tubo, a técnica é denominada "cromatografia em coluna"; se a separação ocorre em uma superfície plana (placa de vidro ou metal, papel de filtro), será denominada "cromatografia planar". Segundo Niessen (2007), a técnica é denominada após a fase móvel: cromatografia gasosa (GC), cromatografia líquida (LC) e cromatografia com fluído supercrítico (SFC). A classificação apresentada por Mcnair e Miller (2009) mostrada na Figura 2.

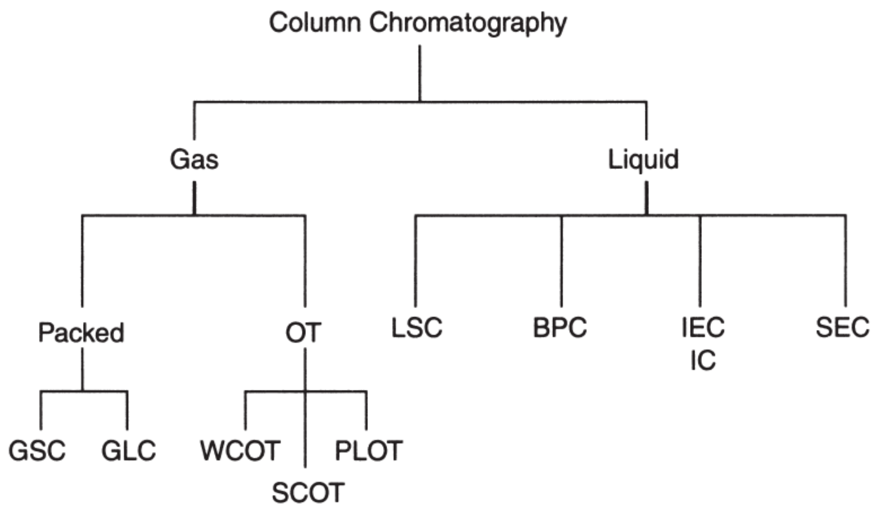

Figura 2 Classificação da cromatografia em coluna (MCNAIR; MILLER, 2009).

em que OP = coluna tubular aberta; GSC = cromatografia gás-sólido; GLC = cromatografia gás-líquido; WCOT = coluna tubular de parede revestida; PLOT = coluna tubular aberta com camada porosa; SCOT = coluna tubular aberta com suporte revestido; LSC = cromatografia líquido-sólido; BPC = cromatografia em fase ligada; IEC = cromatografia de troca iônica; SEC = cromatografia de exclusão por tamanho.

2.4.1 Cromatografia gasosa

A cromatografia gasosa (GC) é aquela na qual a fase móvel é um gás. Um dos mais importantes trabalhos sobre cromatografia gasosa foi publicado em 1952, por A.J.P. Martin e A.T. James. Rapidamente se descobriu que a técnica de GC era rápida, simples e aplicável para a separação de muitos compostos voláteis, principalmente para petroquímicos, para os quais a destilação era o método preferido de separação. As teorias descrevendo os processos foram rapidamente testadas, levando ao surgimento de novas e mais avançadas teorias. Paralelamente, a demanda por instrumentos fez surgir uma nova indústria que respondeu rapidamente desenvolvendo novos cromatógrafos (MCNAIR; MILLER, 2009).

O primeiro cromatógrafo gasoso foi apresentado na ACHEMA (Feira de Químicos), em Frankfurt, em 1952 e era constituído por uma fonte de gás de arraste, um sistema de injeção da amostra, uma coluna empacotada com sílica gel e um detector de condutividade térmica. O equipamento foi desenvolvido pela professora Erika Cremer, do Instituto de Físico-Química da Universidade de Innsbruck (ETTRE, 2008). Na Figura 3 é apresentado o esquema de um típico cromatógrafo gasoso.

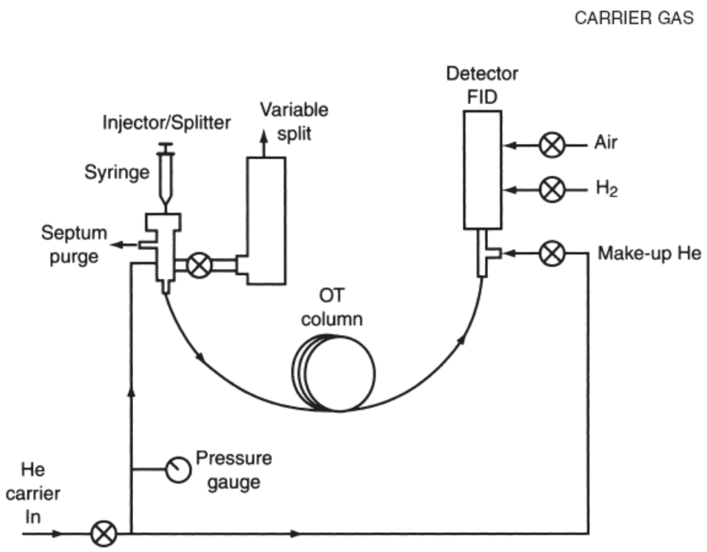

Figura 3 Esquema de um típico cromatógrafo gasoso (MCNAIR; MILLER, 2009).

Na cromatografia gasosa, a base para separação é a distribuição da amostra entre duas fases, a fase gasosa e a fase estacionária (LANÇAS, 1993). No funcionamento do processo, um gás de arraste flui a partir de um cilindro através da porta de injeção, coluna e detector. A amostra é introduzida na porta de injeção aquecida onde é vaporizada, sendo então carreada pela coluna. Após a coluna, a amostra e o gás de arraste passam através do detector. O detector mensura a quantidade de amostra e o sinal elétrico por ela gerado; o sinal elétrico é então enviado para o sistema de dados que gera o cromatograma (MCNAIR; MILLER, 2009).

A coluna cromatografia é considerada o “coração do sistema cromatográfico”, pois é nela que se dá a separação (LANÇAS, 1993). Colunas capilares são as mais comumente utilizadas em GC. Estas colunas são tubos abertos com um filme fino revestindo a parede interna. Os longos comprimentos destas colunas (até 100 m) possibilitam separações eficientes de amostras complexas. Estima-se que aproximadamente 90% das aplicações cromatográficas utilizam colunas capilares (MCNAIR; MILLER, 2009).

Os detectores afetam fortemente a informação obtida de uma análise cromatográfica e afetam também todo o desempenho do sistema. Dentre os detectores mais comumente utilizados nos últimos anos podem-se citar: ionização de chamas (FID), captura de elétrons (ECD), fotoionização (PID), absorção no infravermelho por transformada de Fourier (FT-IR), ionização por descarga pulsada de hélio (He-PDPID), chama fotométrica (FPD), emissão atômica (AED), termoiônico específico para fósforo e nitrogênio (TID), descarga luminescente (GDD), eletrocondutividade (ELCD), condutividade térmica (TCD), eletroanográfico (EAD), espectrometria de massas (MS), espectrometria de flourescência atômica (AFS) e plasma indutivamente acoplado (ICP) (EICEMAN et al., 2002).

A cromatografia gasosa tem sido utilizada com sucesso em diversas áreas, como clínica e forense, ambiental, alimentos e bebidas, *flavour* e fragrâncias, petroquímica e metabolômica (DORMAN et al., 2010).

2.4.2 Cromatografia líquida

Desde o início da cromatografia líquida (LC), nos anos 1950, muitos avanços foram alcançados, sendo o principal impulsionador o tamanho das partículas constituintes da fase estacionária (MALDANER; JARDIM, 2009). Até o início dos anos 1970, a LC em coluna era praticada em tubos abertos em pressão ambiente ou condições de baixas pressões. Após a separação, as frações eram coletadas em tubos, o solvente era evaporado e os tubos eram pesados. A diferença de massa entre o peso do tubo após a evaporação do solvente e a do tubo original fornecia a massa eluída naquele tubo, o que possibilitava a construção de um gráfico em papel milimetrado, mostrando a separação encontrada (LANÇAS, 2009).

Nos últimos 40 anos, a cromatografia líquida de alta eficiência (CLAE) ou HPCL, do inglês *high performance liquid chromatography*, tem sido a técnica analítica mais desenvolvida, difundida e empregada em diversas áreas. A busca pelo aprimoramento da técnica visando análises mais rápidas sem comprometimento do desempenho tem sido o principal foco dos estudos em HPLC na última década. Para isso, a redução do tamanho das partículas da fase estacionária e das colunas foi a alternativa mais atrativa, porém, ficou limitada por um período, por causa da elevada pressão resultante desta concomitante redução, que não é compatível com os sistemas cromatográficos convencionais. Entretanto, o uso de partículas menores que 2 µm se tornou possível recentemente, com o desenvolvimento da cromatografia líquida de ultraeficiência (U-HPLC) (MALDANER; JARDIM, 2009).

O mecanismo básico de separação por LC é apresentado na Figura 4.

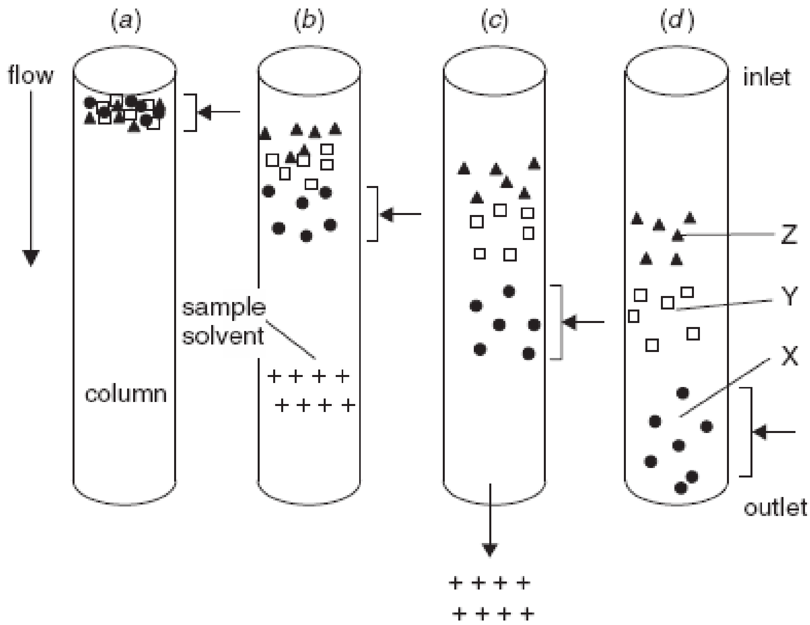

Figura 4 Separação hipotética por cromatografia líquida de três componentes em uma amostra (SNYDER; KIRLAND; DOLAN, 2010),

sendo: em (a) introdução da amostra na coluna; em (b) início do fluxo do solvente ou da fase móvel através da coluna, resultando no movimento das moléculas da amostra na coluna e separação parcial; em (c) continuação do movimento da fase móvel pela coluna, promovendo a separação dos componentes da amostra e em (d) componentes da amostra separados.

Os principais mecanismos de separação que ocorrem em cromatografia líquida são: (1) adsorção, (2) partição, (3) troca iônica e (4) exclusão por tamanho. Na adsorção utiliza-se uma fase estacionária polar (sílica ou alumina) e uma fase móvel apolar ou semipolar. Os componentes da amostra interagem com a fase estacionária de forma variável, de acordo com a sua polaridade e geometria, permitindo sua separação. Na partição, a separação se dá pela distribuição dos solutos entre a fase móvel e a fase estacionária (em forma de um filme colocado em um suporte sólido hidrofílico). A partição depende da solubilidade do analito nos dois líquidos. No mecanismo de separação por troca iônica, os compostos iônicos e muitos polares são separados em colunas cujas fases estacionárias são resinas trocadoras de íons. As resinas são compostas por materiais contendo excesso de cargas elétricas (positivas ou negativas) unidas à superfície das partículas da resina, compensadas por um número igual de íons livres de carga oposta (contraíons). Ao passar pela resina, uma solução que contenha íons de mesmo sinal que os contraíons, os novos contraíons do eluente, poderá deslocar os antigos, ocupando seu lugar e compensando cargas de sinal contrário na superfície da resina. Na exclusão por tamanho faz-se o uso de fase estaciónaria composta por materiais de porosidade controlada que funcionam com peneiras ou filtros. Moléculas de tamanho superior ao dos poros da fase estacionária não entram nos poros e passam mais rapidamente pela coluna, enquanto moléculas cujo tamanho é inferior ao tamanho dos poros irão penentrar nos poros da fase estacionária, demorando mais para serem eluídas (LANÇAS, 2009).

Na Figura 5 é apresentado um esquema de um sistema de HPLC, cujas partes componentes são: reservatório de fase móvel ou solvente, bomba, válvula de injeção, coluna e detector.

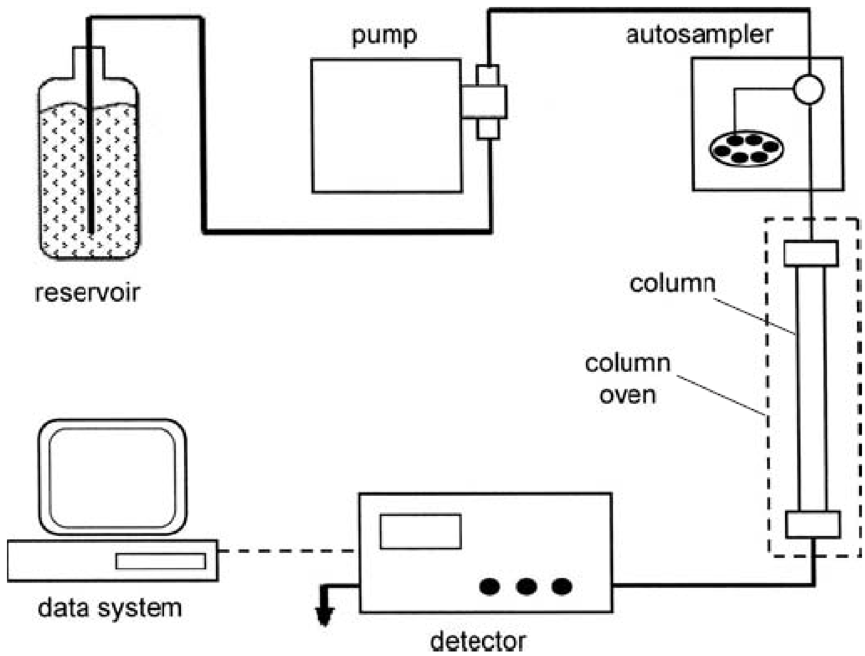

Figura 5 Representação esquemática de um sistema de HPLC (SNYDER; KIRLAND; DOLAN, 2010).

O reservatório constitui uma parte simples, mas essencial para o sistema. Um ou mais reservatórios podem ser utilizados, dependendo do sistema (separação isocrática ou com gradiente). A grande maioria dos reservatórios é confeccionada em vidro, sendo que em alguns casos utilizam-se outros tipos de materiais. Para alguns fabricantes de equipamentos existem modelos de reservatórios específicos (SNYDER; KIRLAND; DOLAN, 2010).

Em HPLC, a fase móvel é empurrada pelo sistema com o auxílio de bombas. As bombas utilizadas em HPLC são confeccionadas com materiais que conferem resistência e segurança. Várias partes são feitas de aço inoxidável, material que apresenta boa relação custo/benefício. Existem vários modelos de bomba, como as de pressão constante e as de volume constante (bombas do tipo seringa, bombas do tipo pistão reciprocante ou alternante) (LANÇAS, 2009).

Para a introdução da amostra no sistema, podem-se utilizar sistemas manuais de injeção. No entanto, em alguns casos, nos quais há necessidade de se injetar centenas de amostras por dia, necessita-se de uma injeção acurada e automática; nestes casos, o autoinjetor é utilizado.

O controle da temperatura da coluna exerce papel importante em HPLC. A influência da temperatura na separação sugere que mecanismos de controle são necessários. Os três tipos mais populares de aquecimento dos fornos para controle de temperatura são bloco, banho de ar e Peltier. No modelo tipo bloco, o aquecimento da coluna se dá pelo contato direto da mesma com uma fonte de calor. Geralmente, o calor é transferido de um bloco de alumínio no qual a coluna esta presa, sendo o calor fornecido por um aquecedor tipo cartucho. Em um sistema tipo banho de ar, o ar é utilizado para aquecimento, como ocorre na cromatografia gasosa. Neste tipo de forno, em função da menor eficiência do ar como condutor de calor, o equilíbrio da temperatura é mais demorado comparado ao aquecimento tipo bloco. Nos fornos tipo Peltier, além do aquecimento da coluna, é possível a manutenção da coluna em temperatura ambiente ou abaixo da temperatura ambiente (SNYDER; KIRLAND; DOLAN, 2010).

Os componentes da amostra separados pela eluição na coluna precisam ser detectados para posterior identificação e quantificação. O primeiro uso da detecção do efluente da coluna pelo índice de refração é atribuído a Tiselius, em 1940. Atualmente, diversos detectores têm sido utilizados, apresentando boa sensibilidade, fornecendo informações estruturais dos analitos e permitindo fácil quantificação (LANÇAS, 2009). O detector cromatográfico é um transdutor que converte uma propriedade física ou química de um analito eluído em um sinal elétrico que pode ser relacionado com a concentração do analito (SNYDER; KIRLAND; DOLAN, 2010).

Os detectores podem ser classificados de acordo com as propriedades medidas (detectores de propriedade do efluente e detectores de propriedade do soluto), com a forma de resposta (detectores diferenciais e detectores integrais), com o tipo de resposta (detectores sensíveis à concentração e detectores sensíveis ao fluxo de massa) e de acordo com a seletividade (detectores universais, detectores seletivos e detectores específicos) (LANÇAS, 2009). Segundo Snyder, Kirkland e Dolan (2010), os detectores mais comumente utilizados atualmente são UV-visível, fluorescência, eletroquímicos, condutividade, indíce de refração, espalhamento de luz, quirais e espectômetros de massas. Cada detector apresenta vantagens e desvantagens e, atualmente, não existe um detector ideal que reúna todas as características consideradas ideais, como alta sensibilidade, resposta a todos os solutos, não ser afetado por mudanças no fluxo e da temperatura, responda independentemente da fase móvel, não contribua para ampliação de picos extracoluna, ser confiável, de fácil uso, tenha uma resposta que aumente linearmente com a quantidade de soluto, seja não destrutivo para o soluto e forneça informações qualitativas do pico detectado.

2.5 Metabólitos microbianos - Compostos voláteis formadores de aroma

Etanol e gás carbônico constituem os dois principais compostos formados no processo de fermentação. Em menores quantidades, vários outros compostos são também produzidos na fermentação (LAMBRECHTS; PRETORIUS, 2000).

As leveduras promovem a conversão dos açúcares em produtos como etanol, glicerol, aldeídos, cetonas, ésteres e ácidos e estes compostos contribuem para formar as características de *flavour* do vinho (REED; PEPPLER, 1973). Dentre os compostos produzidos em maiores quantidades pelas leveduras na fermentação estão ácido acético, glicerol, ácido succínico e ácido lático (ANTONELLI et al., 1999).

2.5.1 Etanol

O etanol é o segundo composto mais abundante no vinho (RIBÉREAU-GAYON et al., 2006). Este composto é formado na via glicolítica em um mecanismo de duas reações. Na primeira reação, o piruvato é descarboxilado, produzindo acetaldeído e liberando CO_2. Em uma segunda reação, o acetaldeído é reduzido para produzir o etanol (LEHNINGER; NELSON; COX, 2006). A presença de etanol é essencial para reforçar as características sensoriais dos outros componentes do vinho, mas seu excesso pode interferir na percepção global do aroma e do sabor do vinho (SWIEGERS et al., 2005). O etanol determina a viscosidade (corpo) do vinho e atua como fixador de aroma (MINGORANCE-CARZOLA et al., 2003).

2.5.2 Glicerol

A origem do glicerol, um dos compostos mais abundantes no vinho, é a fermentação (RIBÉREAU-GAYON et al., 2006). Sua presença no vinho confere maior viscosidade, textura e doçura (ABBAS, 2006). Na fermentação, a produção do glicerol pelas leveduras se dá no início do processo, sendo considerado que sua produção ocorre com o consumo dos primeiros 50 g de açúcares (RIBÉREAU-GAYON et al., 2006). Este composto desempenha importante papel na viabilidade celular de leveduras com o fornecimento de precursores para a síntese de fosfolipídios, que são componentes das membranas celulares durante o período de crescimento da levedura, além da proteção osmótica das leveduras em condições de alta concentração de açúcar; contribuição para a manutenção do equilíbrio redox da célula e geração da energia (ATP) necessária para o crescimento celular (SWIEGERS et al., 2005).

2.5.3 Álcoois superiores

Vários álcoois com mais que dois átomos de carbono são produzidos durante a fermentação e são chamados de álcoois superiores (RIBÉREAU-GAYON et al., 2006). Os álcoois superiores constituem um grupo de compostos encontrados em grande número nas bebidas alcoólicas, nas quais exercem papel importante no aroma. Devido ao seu mecanismo de formação, são também chamados de álcoois de fusel e os principais encontrados nas bebidas são n-propanol, isobutanol, 2-feniletanol, álcool isoamílico e hexanol (BOULTON et al., 1998; GIUDICI; ROMANO; ZAMBONELLI, 1990; NYKÄNEN; SOUMALAINEN, 1983). A formação dos álcoois superiores pela ação das leveduras ocorre tanto diretamente, a partir da utilização de açúcares, quanto a partir de aminoácidos pela reação de Ehrlich (Figura 6) (RIBÉREAU-GAYON et al., 2006).

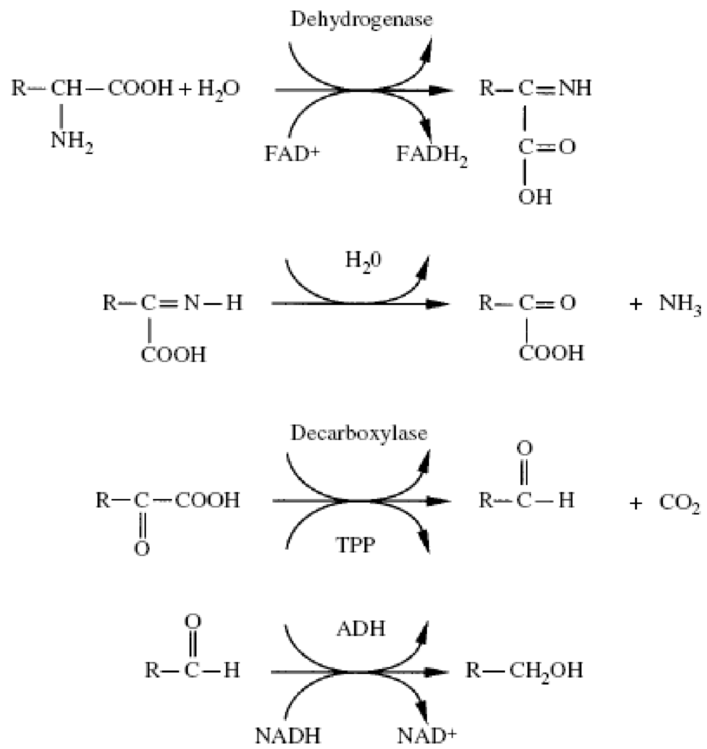

Figura 6 Biossintese de álcoois superiores a partir de aminoácidos de acordo com Ehrlich (RIBÉREAU-GAYON et al., 2006).

Na via catabólica de Ehrlich, primeiramente, o aminoácido é transaminado, originando um α-cetoácido em uma reação catalisada por uma aminotransferase. O α-cetoácido formado é convertido a aldeído pela ação de uma piruvato descarboxilase e o aldeído é posteriormente convertido ao álcool superior correspondente ao aminoácido em uma reação catalisada por uma enzima álcool desidrogenase (SWIERGERS; PRETORIUS, 2005).

2.5.4 Compostos carbonílicos – Aldeídos e cetonas

Alguns aldeídos contribuem para a formação de características de aroma e sabor, cuja sensação em análise sensorial está relacionada com descritores “maçãs”, “citros” e “castanhas”, além de serem associados à oxidação de vinhos (SWIERGERS; PRETORIUS, 2005). Estes compostos podem também estar associados ao sabor picante das bebidas (ETIÉVANT, 1991).

O principal composto carbonílico encontrado no vinho é o acetaldeído, cuja concentração pode variar entre 10 mg/L e 300 mg/L (SWIERGERS; PRETORIUS, 2005). O acetaldeído é formado durante a fermentação alcoólica, podendo também ser formado a partir de oxidação enzimática do etanol, degradação oxidativa de Strecker de aminoácidos, degradação de composto do lúpulo (cerveja) e auto-oxidação de ácidos graxos. Na via glicolítica, o acetaldeído é o último precursor do etanol e sua conversão é catalisada pela enzima álcool desidrogenase (NYKÄNEN; SOUMALAINEN, 1983; REED; PEPPLER, 1973; SWIERGERS; PRETORIUS, 2005).

Etanal é o mais importante composto carbonílico encontrado no vinho. Sua importância resulta das diversas formas pelas quais este composto pode ser formado, pela sua reatividade com dióxido de enxofre em baixas temperaturas e pelas suas propriedades organolépticas. Dentre os compostos com função cetona, diversos têm sido identificados no vinho como propanona, butanona e pentanona, sendo os mais importantes a acetoína e 2,3-butanediona (RIBÉREAU-GAYON et al., 2006).

2.5.5 Ésteres

Ésteres são compostos de grande importância para o aroma do vinho. Muitos são compostos secundários (originados na fermentação) e apresentam descritores aromáticos como “banana”, “abacaxi”, “maça”, “pera”, etc. (CLARKE; BAKKER, 2004). Os ésteres são formados pelas leveduras (Figura 7) durante a fermentação pela ação da acil-CoA, a qual tem grande importância na formação de ácidos orgânicos (BERRY; SLAUGHTER, 2003). A síntese dos ésteres envolve um ácido graxo, um álcool e uma CoA. A acetil-CoA presente na formação do acetato de etila é obtida pela descarboxilação oxidativa do piruvato, mas outras acil-CoA são formadas por reação de acilação da CoASH catalisada pela acil-CoA sintetase (SWIERGERS; PRETORIUS, 2005).

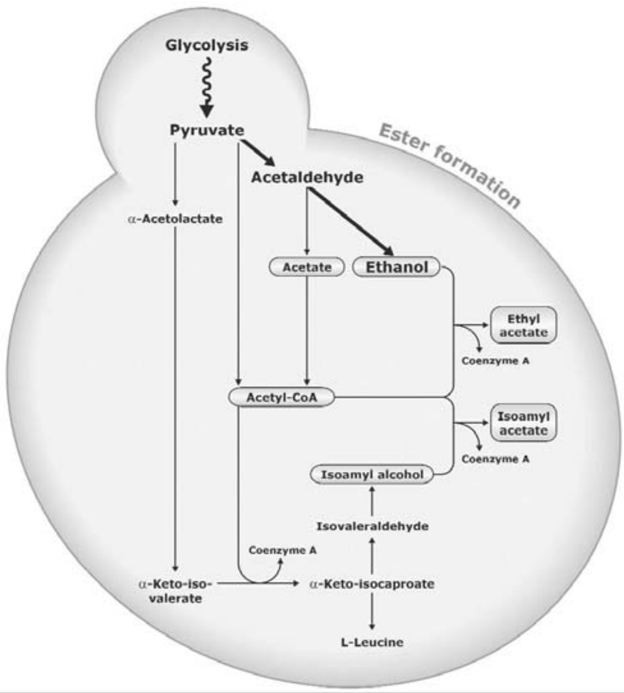

Figura 7 Representação esquemática da formação de acetato de etila e acetato de isoamila (SWIERGERS et al., 2005).

Os ésteres de maior importância aromática são acetato de etila, acetato de isoamila, acetato de isobutila, 2-feniletil acetato e caprato de etila (SWIERGERS et al., 2005). O acetato de etila é o éster predominante no vinho, produzido em pequenas quantidades por leveduras durante a fermentação. Durante o período de envelhecimento do vinho, pode ser produzido em grandes quantidades pela ação de bactérias acéticas (RIBÉREAU-GAYON et al., 2006).

2.5.6 Ácidos orgânicos

Os ácidos orgânicos são compostos de grande importância, pois têm influência sobre diversas propriedades organolépticas, como aroma, sabor e cor das bebidas alcoólicas. Estes compostos também estão relacionados ao controle da estabilidade microbiológica das bebidas (MATO; SUAREZ-LUQUE; HUIDOBRO, 2005; RIBÉREAU-GAYON et al., 2006). A contribuição dos ácidos pode ser negativa ou positiva para a qualidade do vinho, dependendo da concentração em que são encontrados. Estes compostos são divididos em ácidos voláteis e não voláteis. Os ácidos voláteis apresentam cadeia de carbono curta e, no vinho, o principal representante deste grupo é o ácido acético, cuja quantidade geralmente encontrada pode corresponder a 90% do conteúdo de ácidos voláteis (SWIEGERS et al., 2005). Ácidos com cadeia de carbono variando entre C3 e C16 são sintetizados pelas leveduras durante a fermentação alcoólica e têm influência sobre o aroma. O aroma das bebidas alcoólicas recebe interferência principalmente dos ácidos graxos de cadeia curta, como ácido isobutírico, ácido butírico, ácido propiônico, ácido isovalérico, ácido hexanoico, ácido octanoico e ácido caprico (ABBAS, 2006).

Na formação dos ácidos orgânicos, em uma primeira etapa, é formada acetil-CoA. Logo em seguida, ocorre a formação de um intermediário, N-carboxibiotinil, seguida pelo acoplamento do grupo carboxil com a acetil-CoA formando o malonil-CoA. Posteriormente, dois átomos de carbono vindos do malonil-CoA são adicionados em ciclos sucessivos a acil-CoA. Assim, os ácidos cuja cadeia apresenta número par de carbonos são formados. Para os ácidos que apresentam número ímpar de átomos tem-se a propanoil-CoA na etapa inicial, em lugar da acetil-CoA (LYNEN, 1972).

2.5.7 Compostos sulfurados

Diversos compostos sulfurados têm sido identificados em vinhos, desde simples tióis ou mercaptanos a complexos tiolactonas e terpenotióis. Estes últimos apresentam fortes efeitos aromáticos em baixas concentrações (CLARKE; BAKKER, 2004). De modo geral, compostos sulfurados são associados a descritores aromáticos desagradáveis, como“ovo podre”, “alho”, “cebola”, “couve” e “borracha”, podendo influenciar negativamente a qualidade do vinho. No entanto, alguns compostos sulfurados podem contribuir de forma positiva para o aroma do vinho com descritores como “maracujá”, “café” e “morango” (SWIEGERS et al., 2005). Leveduras podem produzir compostos voláteis sulfurosos (Figura 8), sendo as características genéticas e fisiológicas das leveduras determinantes da capacidade de liberação de tióis (SWIEGERS; PRETORIUS, 2007).

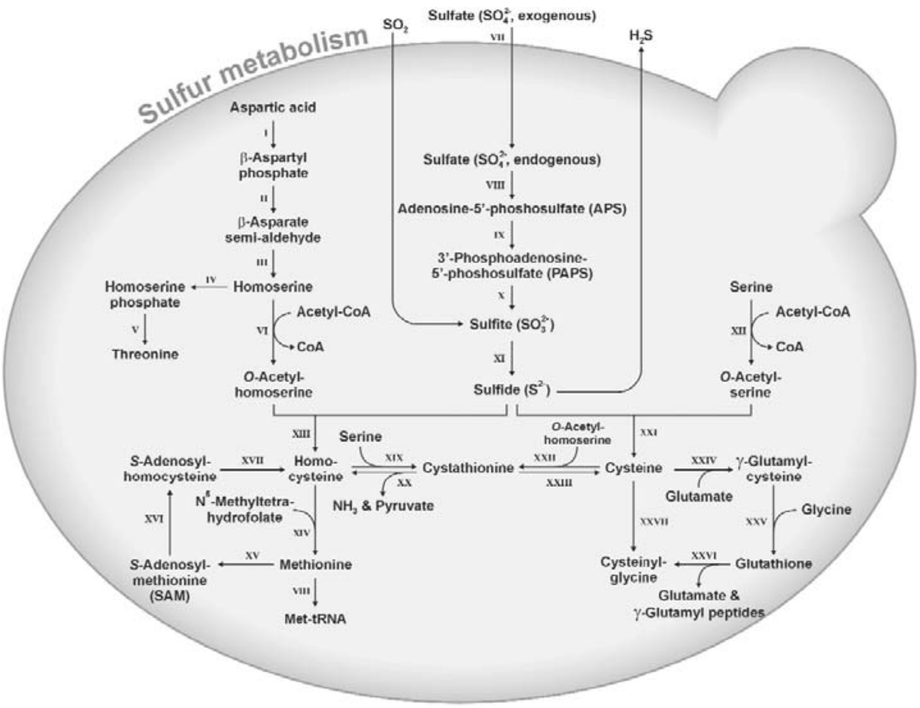

Figura 8 Representação esquemática do metabolismo de compostos sulfurados (SWIERGERS et al., 2005).

2.6 Otimização

Recentemente, diversos métodos estatísticos de *design* experimental têm sido empregados para otimização em bioprocessos. Dentre as diversas técnicas, a metodologia de superfície de reposta (RSM) é uma das mais eficientes para estudo de efeitos de variáveis, objetivando-se a determinação de condições ótimas em sistemas multivariáveis. A metodologia de superfície de resposta tem sido utilizada com sucesso na otimização de condições de fermentação (KUMAR; PRAKASAM; REDDY, 2009).

Nwabueze (2010) relatou que algumas técnicas estatísticas empregadas na otimização de processos são pouco confiáveis e irreprodutíveis. Para trabalhos de otimização, as técnicas de superfície de reposta fornecem resultados confiáveis que permitem uma eficiente otimização em bioprocessos. Diversos autores (DE LEÓN-RODRÍGUEZ et al., 2008; KUMAR; PRAKASAM; REDDY, 2009) têm relatado o uso da metodologia de superfície de resposta para otimização de condições de fermentação para a produção de bebidas fermentadas e destilada.

3 CONSIDERAÇÕES FINAIS

Os resultados obtidos neste trabalho demonstraram que a utilização de cacau, cupuaçu, gabiroba, jabuticaba, umbu e framboesa constitui uma alternativa viável para elaboração de novas bebidas fermentadas. A jabuticaba foi também empregada com sucesso na elaboração de uma bebida destilada.

Com o uso das técnicas de HPLC, HPLC-DAD, GC, GC-MS e PFPD foi possível caracterizar as bebidas, principalmente em termos de compostos aromáticos voláteis. Com esta caracterização disponibilizamos informações ainda pouco disponíveis na literatura, como por exemplo, para bebidas de frutos tropicais.

A partir dos resultados obtidos, foi possível observar que para uma fruta, leveduras diferentes levam à obtenção de bebidas finais com características químicas e sensoriais distintas. Quando uma mesma levedura foi utilizada para fermentação de diferentes frutas, as bebidas obtidas também apresentaram características diferenciadas. Com estes resultados concluímos que para cada fruta deve-se buscar cepas de leveduras capazes de fermentar a polpa produzindo uma bebida de qualidade.

As informações obtidas neste trabalho serão de grande valia para uso em trabalhos futuros com fermentação de frutas, tanto para elaboração de bebidas fermentadas quanto para elaboração de bebidas destiladas.

REFERÊNCIAS

ABBAS, C. A. Production of antioxidants, aromas, colours, flavours, and vitamins by yeast. In: QUEROL, A.; FLEET, H. (Ed.). **Yeast in food and beverages**. Berlin: Spring-Verlag, 2006. chap. 10, p. 285-334.

AKUBOR, P. I. et al. Production and quality evaluation of banana wine. **Plants Foods for Human Nutrition**, Netherlands, v. 58, n. 5, p. 1-6, Sept. 2003.

ANTONELLI, A. et al. Yeast influence on volatile composition of wines. **Journal of Agricultural and Food Chemistry**, Easton, v. 47, n. 3, p. 1139-1144, Mar. 1999.

BERNARDI, T. L. et al. *Saccharomyces cerevisiae* strains associated with the production of cachaça: identification and characterization by traditional and molecular methods (PCR, PFGE and mtDNA-RFLP). **World Journal of Microbiology and Biotechnology**, Oxford, v. 24, n. 11, p. 2705-2712, Nov. 2008.

BERRY, D. R.; SLAUGHTER, J. C. Alcoholic beverage fermentation. In: LEA, A. G. H.; PIGGOTT, J. R. (Ed.). **Fermented beverage production**. 2^{nd} ed. New York: Kluwer Academic, 2003. chap. 2, p. 25-38.

BOULTON, R. B. et al. **Principles and practices of winemaking**. New York: Springer, 1998. 604 p.

BRASIL. Decreto nº 6.871, de 4 julho de 2009. Regulamenta a lei nº 8918 de 14 de julho de 1994, que dispõe sobre a padronização, a classificação, o registro, a inspeção, a produção e a fiscalização de bebidas. **Diário Oficial [da] República Federativa do Brasil**, Brasília, 4 jul. 2009. Disponível em: em:<http://extranet.agricultura.gov.br/sislegis/action/detalhaAto.do?method=consultarLegislacaoFederal)>. Acesso em: 11 mar. 2011.

BRASIL. Lei nº 7678 de 8 novembro de 1988. Dispõe sobre a produção, circulação e comercialização do vinho e derivados da uva e do vinho, e dá outras providências. **Diário Oficial [da] República Federativa do Brasil**, Brasília, 8 nov. 1988. Disponível em: <http://extranet. agricultura.gov.br/sislegis-consulta/ consultarLegislacao.do? operacao =visualizar&id=189>. Acesso em: 28 mar. 2008.

BRUNINI, M. A.; OLIVEIRA, A. L.; SALANDINI, C. A. R. Influência de embalagens e temperatura no armazenamento de jabuticabas (*Myrciaria jabuticaba* (Vell) Berg) cv 'Sabará' **Ciência e Tecnologia de Alimentos**, Campinas, v. 24, n. 3, p. 378-383, jul./set. 2004.

CAMPOS, C. R. et al. Features of *Saccharomyces cerevisiae* as a culture starter for the production of the distilled sugar cane beverage cachaça in Brazil. **Journal of Applied Microbiology**, Oxford, v.108, n. 6, p. 1871-1879, June 2010.

CARVALHO, P. E. R. de. Gabiroba, a fruta do mato. **Globo Rural**, Rio de Janeiro, n. 203, set. 2002. Disponível em: <http://revistagloborural.globo.com/GloboRural/0,6993,EEC380084-1641,00.html>. Acesso em: 29 mar. 2008.

CATALUÑA, E. **As uvas e os vinhos**. 2. ed. Rio de Janeiro: Globo, 1988. 207 p.

CATARINO, S. et al. Release of contaminant elements from bentonites to wine: a contribuition to achieve a test solution. **Ciência e Técnica Vitivinícola**, Lisboa, v. 21, n. 1, p. 17-31, 2006.

CHIARELLI, R. H. C.; NOGUEIRA, A. M. P.; VENTURINI FILHO, W. C. Fermentados de jabuticaba (*Myrciaria cauliflora* Berg): processos de produção, características físico-químicas e rendimento. **Brazilian Journal of Food Technology**, São Paulo, v. 8, n. 4, p. 277-282, out./dez. 2005.

CLARKE, R. J.; BAKKER, J. **Wine flavour chemistry**. Oxford: Blackwell, 2004. 326 p.

COLLINS, C. H. I. Michael Tswett e o "nascimento" da cromatografia. **Scientia Chromatographica**, São Carlos, v. 1, n. 1, p. 7-21, 2009.

CONSTANTÍ, M. et al. Molecular analysis of yeast population dynamics: effect of sulphur dioxide and inoculum on must fermentation. **International Journal of Food Microbiology**, Amsterdam, v. 41, n. 3, p. 169-175, June 1998.

CORAZZA, M. L.; RODRIGUES, D. G.; NOZAKI, J. Preparação e caracterização do vinho de laranja. **Química Nova**, São Paulo, v. 24, n. 4, p. 449-452, jul./ago. 2001.

COSME, F.; SILVA, J. M. R. da; LAUREANO, O. Interactions between protein fining agents and proanthocyanidins. **Food Chemistry**, London, v. 106, n. 2, p. 536-544, Jan. 2008.

DA PORTO, C. et al. Analyses of orange spirit flavour by direct-injection gas chromatography-mass spectrometry and headspace solid-phase microextraction/GC-MC. **Flavour and Fragrance Journal**, Chichester, v. 18, n. 1, p. 66-72, Jan./Feb. 2003.

DE LEÓN-RODRÍGUEZ, A. et al. Optimization of fermentation conditions for the production of the mescal from *Agave salmiana* using response surface methodology. **Chemical Engineering and Processing**, Lausanne, v. 47, n. 1, p. 76-82, Jan. 2008.

DIAS, D. R. et al. Elaboration of a fruit wine from cocoa (*Theobroma cacao* L.). **International Journal of Food Science and Technology**, Amsterdam, v. 42, n. 3, p. 319-329, Mar. 2007.

DIAS, D. R.; SCHWAN, R. F.; LIMA, L. C. O. Metodologia para elaboração de fermentado de cajá (*Spondias mombin* L.). **Ciência e Tecnologia de Alimentos**, Campinas, v. 23, n. 3, p. 342-350, set./dez. 2003.

DORMAN, F. L. et al. Gas chromatography. **Analytical Chemistry**, Washington, v. 82, n. 12, p. 4775-4785, June 2010.

DUARTE, W. F. et al. Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jabuticaba and umbu. **LWT Food Science and Technology**, London, v. 43, n. 10, p. 1564-1572, Dec. 2010a.

DUARTE, W. F. et al. Fermentative behavior of *Saccharomyces* strains during microvinification of raspberry juice (*Rubus idaeus* L.). **International Journal of Food Microbiology**, Amsterdam, v. 143, n. 3, p. 173-182, Out. 2010b.

DUARTE, W. F. et al. Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production. **Journal of Industrial Microbiology and Biotechnology**, Hampshire, v. 36, n. 4, p. 557-569, Apr. 2009.

DUARTE, W. F. et al. Optimization of fermentations conditions for production of the jabuticaba (*Myrciaria caulifora*) spirit using the response surface methodology. **Journal of Food Science**, Chicago, 2011. Disponível em: <http://onlinelibrary.wiley.com/doi/10.1111/j.1750-3841.2011.02169.x/pdf>. Acesso em: 21 mar. 2011.

DUARTE, W. F. et al. Raspberry (*Rubus idaeus* L.) wine: yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds. **Food Research International**, Barking, v. 43, n. 9, p. 2303-2314, Nov. 2010c.

EICEMAN, G. A. et al. Gas chromatography. **Analytical Chemistry**, Washington, v. 74, n. 12, 2771-2780, June 2002.

ETIÉVANT, P. X. Wine. In: MAARSE, H. (Ed.). **Volatile compounds in food and beverages**. New York: CRC Press, 1991. chap. 16, p. 483-547.

ETTRE, L. S. **Chapters in the evolution of chromatography**. 1st ed. London: Imperial College, 2008. 473 p.
FLEET, G. H. Microorganisms in food ecosystems. **International Journal of Food Microbiology**, Amsterdam, v. 50, n. 1/2, p. 101-117, Sept. 1999.

FOLEGATTI, M. I. S. et al. Aproveitamento industrial do umbu: processamento de geléia e compota. **Ciência e Agrotecnologia**, Lavras, v. 2, n. 6, p. 1308-1314, nov./dez. 2003.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. **Databasecollection**: agricultural data. Disponível em: <http://faostat.fao.org/site/567/default.aspx. >. Acesso em: 11 mar. 2011.

FRANCO, M. R. B.; SHIBAMOTO, T. Volatile composition of some Brazilian fruits: Umbu-caja (*Spondias citherea*), Camu-camu (*Myrciaria dubia*), Araça-boi (*Eugenia stipitata*) and Cupuaçu (*Theobroma grandiflorum*). **Journal of Agricultural and Food Chemistry**, Easton, v. 48, n. 4, p. 1263-1285, Apr. 2000.

FUNDIRA, M. et al. Comparison of commercial enzymes for the processing of Marula pulp, wine and spirits. **Journal of Food Science**, Chicago, v. 67, n. 6, p. 2346-2351, Aug. 2002.

GARCÍA-LLOBODANIN, L. et al. Pear distillates: influence of the raw material used on final quality. **European Food Research Technology**, Berlin, v. 228, n. 1, p. 75-82, Nov. 2008.

GARDE-CERDÁN, T.; ANCÍN-AZPILICUETA, C. Effect of SO_2 on the formation and evolution of volatile compounds in wines. **Food Control**, Guildford, v. 18, n. 12, p. 1501-1506, Dec. 2007.

GIUDICI, P.; ROMANO, P.; ZAMBONELLI, C. A biometric study of higher alcohol production in *Saccharomyces cerevisiae*. **Canadian Journal of Microbiology**, Ottawa, v. 36, n. 1, p. 61-64, Jan. 1990.

GOMÉZ-HERNANDEZ, L. F.; ÚBEDA, J.; BRIONES, A. Characterisation of wines and distillated spirit from melon (*Cucumis melo* L.). **International Journal of Food Science and Technology**, Oxford, v. 43, n. 4, p. 644-650, Apr. 2008.

GONZÁLEZ, E. A. et al. Production and characterization of distilled alcoholic beverages obtained by solid-state fermentation of black mulberry (*Morus nigra* L.) and black currant (*Ribes nigrum* L.). **Journal of Agricultural and Food Chemistry**, Easton, v. 58, n. 4, p. 2529-2535, Feb. 2010.

GONZÁLEZ, E. A. et al. Solid-state fermentation of red raspberry (Rubus idaeus L.) and arbutus berry (Arbutus unedo L.) and characterization of their distillates. **Food Research International**, Barking, 2011. Disponível em: <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6V-529MV9R-1&_user=10&_coverDate=03%2F04%2F2011&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_searchStrId=1745886057&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=e8870e30fdfd5ac04866376457612068&searchtype=a.>. Acesso em: 21 mar. 2011. doi:10.1016/j.foodres.2011.02.032.

GUIMARÃES, T. M. et al. Isolation and characterization of *Saccharomyces cerevisiae* strains of winery interest. **Revista Brasileira de Ciências Farmecêuticas**, São Paulo, v. 42, n. 1, p. 119-126, Jan./Mar. 2006.

HASHIZUME, T. Tecnologia do vinho. In: AQUARONE, E. et al. (Ed.). **Biotecnologia industrial**: biotecnologia na produção de alimentos. São Paulo: E. Blücher, 2001. cap. 1, p. 21-68.

HUTKINS, J. W. **Microbiology and technology of fermented foods**. 1st ed. Oxford: Blackwell, 2006. 473 p.

KELEBEK, H. et al. Determination organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from cv. Kozan. **Microchemical Journal**, New York, v. 91, n. 2, p. 187-192, Mar. 2009.

KLING, T. H. et al. Selective effects of sulfur dioxide and yeast starter culture addition on indigenous yeast population and sensory characteristics of wine. **Journal of Applied Microbiology**, Oxford, v. 84, n. 5, p. 865-876, June 1998.

KUMAR, Y. S.; PRAKASAM, R. S.; REDDY, O. V. S. Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology. **International Journal of Food Science and Technology**, Oxford, v. 44, n. 11, p. 2320-2327, Nov. 2009.

LAMBRECHTS, M. G.; PRETORIUS, I. S. Yeast and its importance to wine aroma: a review. **South African Journal of Enology and Viticulture**, v. 21, p. 97-129, 2000. Special Issue.

LANÇAS, F. M. **Cromatografia em fase gasosa**. 1st ed. São Carlos: Acta, 1993. 254 p.

LANÇAS, F. M. **Cromatografia líquida moderna**. 1st ed. São Paulo: Átomo, 2009. 382 p.

LANDRUM, L. R. *Campomanesia*, *pimenta*, *Blepharocalyx*, *Legrandia*, *Acca*, *Myrrhinium*, *and Luma* (Myrtaceae). **Flora Neotropica Monograph**, New York, v. 45, p. 1-179, Nov. 1986.

LEE, P. R. et al. Effect of fusel oil addition on volatile compounds in papaya wine fermented with *Williopsis saturnus* var. *mrakii* NCYC2251. **Food Research International**, Barking, 2010a. Disponível em: <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6V-51S6X7J-6&_user=10&_coverDate=12%2F22%2F2010&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_searchStrId=1745888577&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=6eb35b444ecc30a20839dd91c2767e7a&searchtype=a>. Acesso em: 21 mar. 2011. doi:10.1016/j.foodres.2010.12.026.

LEE, P. R. et al. Evolution of volatile compounds in papaya wine fermented with three *Williopsis saturnus* yeasts. **International Journal of Food Science and Technology**, Oxford, v. 43, n. 10, p. 2032-2041, Oct. 2010b.

LEE, P. R. et al. Profile of volatile compounds during papaya juice fermentation by a mixed culture of *Saccharomyces cerevisiae* and *Williopsis saturnus*. **Food Microbiology**, London, v. 27, n. 7, p. 853-861, Oct. 2010c.

LEHNINGER, A. L.; NELSON, D. L.; COX, M. M. **Lehninger princípios de bioquímica**. 4. ed. São Paulo: Sarvier, 2006. 1202 p.

LIRA JÚNIOR, J. S. et al. Caracterização física e físico-química de frutos de cajá-umbu (*Spondias* spp.). **Ciência e Tecnologia de Alimentos**, Campinas, v. 25, n. 4, p. 757-761, out./dez. 2005.

LÓPEZ, S. et al. The use of activated charcoal in combination with other fining agents and its influence on the organoleptic properties of sherry wine. **European Food Research and Technology**, Heidelberg, v. 212, n. 6, p. 671-675, Apr. 2001.

LYNEN, F. Enzymes systems for fatty acids synthesis.**The Biochemical Journal**, London, v. 128, n. 1, p. 1-2, June 1972.

MALDANER, L.; JARDIM, I. C. S. F. O estado atual da arte da cromatografia líquida de ultra eficiência. **Química Nova**, São Paulo, v. 32, n. 1, p. 214-222, 2009.

MANICA, I. **Frutas nativas, silvestres e exoticas 1**: tecnicas de producao e mercado : abiu, amora-preta, araca, bacuri, biriba, carambola, cereja-do-rio-grande, jabuticaba. Porto Alegre: Cinco Continentes, 2000. 327 p.

MATO, I.; SUAREZ-LUQUE, S.; HUIDOBRO, J. F. A review of the analytical methods to determine organic acids in grape juices and wines. **Food Research International**, Barking, v. 38, n. 10, p. 1175-1188, Dec. 2005.

MCNAIR, H. M.; MILLER, J. M. **Basic gas chromatography**. 2^{nd} ed. New Jersey: J. Wiley, 2009. 230 p.

MENEGUZZO, J.; MANFROI, L.; RIZZON, L. A. Vinho, sistema de produção. **Embrapa Uva e Vinho**, Bento Gonçalves, ago. 2006. Disponível em: <http://www.cnpuv.embrapa.br/publica/sprod/VinhoTinto/index.htm >. Acesso em: 13 abr. 2008.

MINGORANCE-CARZOLA, L. et al. Contribution of different natural yeasts to the aroma of two alcoholic beverages.**World Journal of Microbiology & Biotechnology**, Netherlands, v. 19, n. 3, p. 297-304, Apr. 2003.

MOZAZ, S. R. et al. Influence of decantation of viura must on the cation content: evolution during wine fermentation and stabilization. **Food Research International**, Barking, v. 32, n. 10, p. 683-689, Dec. 1999.

MUNIZ, C. R. et al. Bebidas fermentadas a partir de frutos tropicais. **Boletim do Centro de Pesquisas e Processamento de Alimentos**, Curitiba, v. 20, n. 2, p. 309-322, jul./dez. 2002.

NIESSEN, W. M. A. **Liquid chromatography – Mass spectrometry**. 3^{rd} ed. Palm Beach: CRC Press, 2007. 600 p.

NURGEL, C. et al. Contribution by *Saccharomyces cerevisiae* yeasts to fermentation and flavour compounds in wines from cv. kalecik karasi grape. **Journal of the Institute of Brewing**, London, v. 108, n. 1, p. 68-72, Oct. 2002a.

NURGEL, C. et al. Influence of *Saccharomyces cerevisiae* strain on fermentation and flavor compounds of white wines made cv. Emir grown in Central Anatolia, Turkey. **Journal of Industrial Microbiology & Biotechnology**, Hampshire, v. 29, n. 2, p. 28-33, Apr. 2002b.

NYKÄNEN, L.; SOUMALAINEN, H. **Aroma of beer, wine and distilled alcoholic beverages**. Berlin: Springer, 1983. 424 p.

NWABUEZE, T. U. Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. **International Journal of Food Science and Technology***,* Oxford, v. 45, n. 9, p. 1768-1776, Sept. 2010.

OLIVEIRA, M. E. S. et al. Fruit wine produced from cagaita (*Eugenia dysenterica* DC) by both free and immobilized yeast cell fermentation. **Food Research International**, Barking, 2011. Disponível em: < http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6V-528909M-6&_user=10&_coverDate=02%2F26%2F2011&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_searchStrId=1745887445&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=12341c6174cb3865a3e435eeb806d926&searchtype=a>. Acesso em: 21 mar. 2011. doi:10.1016/j.foodres.2011.02.028

PATO, O. **O vinho**: sua preparação e conservação. 7. ed. Lisboa: Livraria Clássica, 1982. 433 p. (Coleção Técnica agrária).

REDDY, L. V. A.; REDDY, O. V. S. Production and characterization of wine from mango fruit (*Mangifera indica* L.). **World Journal of Microbiology & Biotechnology**, Netherlands, v. 21, n. 8/9, p. 1345-1350, Dec. 2005.

REED, G.; PEPPLER, H. J. **Yeast technology**. Westport: The Avi, 1973. 378 p.

RIBEREAU-GAYON, P. et al. **Handbook of enology. The chemistry of wine and stabilization and treatments**. Chichester: J. Wiley, 2006. 441 p.

RIZZON, L. A.; MIELE, A. Correção do mosto da uva Isabel com diferentes produtos na serra gaúcha. **Ciência Rural**, Santa Maria, v. 35, n. 2, p. 450-454, mar./abr. 2005.

ROMANO, P.; SUZZI, G. Sulfur dioxide and wine microrganisms. In: FLEET, G. H. (Ed.). **Wine microbiology and biotechnology**. London: Harwood Academic, 1993. chap. 13, p. 272-395.

SANTOS, C. S. et al. Elaboração e análise sensorial do fermentado de acerola (*Malpighia punicifolia* L.). **Brazilian Journal of Food Technology**, São Paulo, v. 10, n. 181, p. 47-50, mar. 2005.

SCHEHL, B. et al. Effect of the stone content on the quality of plum and cherry spirits produced from mash fermentations with commercial and laboratory yeast strains. **Journal of Agriculture and Food Chemistry**, v. 53, n. 21, p. 8230-8238, Sept. 2005.

SCHWAN, R. F.; SOUZA, S. M. M. de; MENDONÇA, M. A. S. **Caracterização de frutas nativas da America Latina**. Caçador: Sociedade Brasileira de Fruticultura, 2000. 48 p. (Série frutas nativas).

SCHWAN, R. F.; WHEALS, A. E. The microbiology of cocoa fermentation and its role in chocolate quality. **Critical Reviews in Food Science and Nutrition**, Boca Raton, v. 44, n. 4, p. 205-222, Jan. 2004.

SELLI, S.; CABAROGLU, T.; CANBAS, A. Flavour componentes of orange wine made from a Turkish cv. Kozan. **International Journal of Food Science and Technology**, Oxford, v. 38, n. 5, p. 587-593, June 2003.

SELLI, S. et al. Characterizaiton of most Odor-active volatiles of orange wine made from a Turkish cv. Kozan (*Citrus sinensis* L. Osbeck). **Journal of Agriculture and Food Chemistry**, Washington, v. 56, n. 1, p. 227-234, Dec. 2008.

SELLI, S. Volatile constituents of orange wine obtained from moro orange (*Citrus sinensis* [L.] Osbeck). **Journal of Food Quality**, Wastport, v. 30, n. 4, p. 330-341, Aug. 2007.

SNYDER, L. R.; KIRKLAND, J. J.; DOLAN, J. W. **Introduction to modern liquid chromatography**. 3rd ed. New Jersey: J. Wiley, 2010. 912 p.

SOUFLEROS, E. H. et al. Instrumental analysis of volatile and other compounds of Greek kiwi wine: sensory evaluation and optimisation of its composition. **Food Chemistry**, London, v. 75, n. 4, p. 487-500, Dec. 2001.

SOUFLEROS, E. H.; MYGDALIA, S. A.; NATSKOULIS, P. Production process and characterization of the traditional Greek fruit distillate “Koumaro” by aromatic and mineral composition. **Journal of Food Composition Analysis**, San Diego, v.18, n. 7, p. 699-716, Nov. 2005.

SWIEGERS, J. H. et al. Yeast and bacterial modulation of wine aroma and flavour. **Australian Journal of Grape and Wine Research**, Adelaide, v. 11, n. 2, p. 139-173, Jun. 2005.

SWIERGERS, J. H.; PRETORIUS, I. S. Yeast modulation of wine flavour. **Advances in Applied Microbiology**, San Diego, v. 57, p. 131-175, 2005.

TEŠEVIĆ, V. et al. Characterization of volatile compounds of “Drenja”, an alcoholic beverage obtained from the fruits of cornelian cherry. **Journal of the Serbian Chemical Society**, Belgrade, v. 74, n. 2, p. 117-128, Feb. 2009.

VALERO, E. et al. Dissemination and survival of commercial wine yeast in the vineyard : a large-scale, three-years study. **FEMS Yeast Research**, New York, v. 5, n. 10, p. 959-969, July 2005.

VALLILO, M. I.; BUSTILLOS, O. V.; AGUIAR, O. T. Identificação de terpenos no óleo essencial dos frutos de *Campomanesia adamantium* (Cambessédes) O. Berg – Myrtaceae. **Revista do Instituto Florestal**, São Paulo, v. 18, p. 15-22, dez. 2006.

VENTURIERI, G. A. **Cupuaçu**: a espécie, sua cultura, usos e processamento. Belém: Clube do Cupu, 1993.108 p.

VILLACHICA, H. **Frutales y hortalizas promisorios de la Amazonia**. Lima: TCA, 1996. 367 p.

WEBER, C.; HAI-LIU, R. Antioxidant capacity and anticancer properties of red raspberry. In: THE INTERNATIONAL RUBUS AND RIBES SYMPOSIUM, 8., 2002, Dundee. **Proceedings**… Dundee: International Society for Horticultural Science, 2002. p. 451-455.

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ARTIGO 1 Production and characterization of different fruit wines from cacao, cupuassu, gabiroba, jabuticaba and umbu

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Production and characterization of different fruit wines from cacao, cupuassu, gabiroba, jabuticaba and umbu

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Abstract

The main aim of this work was to produce fruit wines from pulp of gabiroba, cacao, umbu, cupuassu and jaboticaba and characterize them using gas chromatography-mass spectrometry for determination of minor compounds and gas chromatography-flame ionization detection for major compounds. Ninety-nine compounds (C_6 compounds, alcohols, monoterpenic alcohols, monoterpenic oxides, ethyl esters, acetates, volatile phenols, acids, carbonyl compounds, sulfur compounds and sugars) were identified in fruit wines. The typical composition for each fruit wine was evidenced by principal component analysis and Tukey test. The yeast UFLA CA 1162 was efficient in the fermentation of the fruit pulp used in this work. The identification and quantification of the compounds allowed a good characterization of the fruit wines. With our results, we conclude that the use of tropical fruits in the production of fruit wines is a viable alternative that allows the use of harvest surpluses and other underused fruits, resulting in the introduction of new products into the market.

Keywords: fruit wine; gas chromatography–mass spectrometry; alcoholic beverages; aroma; tropical fruits; cacao; gabiroba; cupuassu; jaboticaba; umbu

1. Introduction

There is an abundance of exotic tropical fruits in Brazil with the potential to be used by the food industry. Different new uses and new methods for processing tropical fruits need to be developed to minimize production losses, generate more profits and promote the sustainable use of biomes, such as the *cerrado* (Brazilian savannah) and the Amazon forest. One possible use of these fruits is in the production of fruit wines (Dias, Schwan, Freire, & Serôdio, 2007; Duarte, Dias, Pereira, Gervasio, & Schwan, 2009).

There are many studies in the literature that demonstrate the feasibility of using fruits, such as cacao (Dias et al., 2007), gabiroba (Duarte et al., 2009), kiwi (Soufleros et al., 2001), cajá (Dias, Schwan, & Lima, 2003), mango (Reddy & Reddy, 2005) and orange (Selli, Canbas, Varlet, Kelebk, Prost, & Serot, 2008) to produce alcoholic beverages.

There are several Brazilian fruits with the potential for use in the production of wines. In this study, we investigated the following fruits for this purpose: cupuassu (*Theobroma grandiflorum* Schum.), umbu (*Spondias tuberosa* L.), gabiroba [*Campomanesia pubescens* (DC.) O. Berg], cacao (*Theobroma cacao* L.) and jaboticaba (*Myrciaria jaboticaba* Berg).

Cupuassu is a fruit native to the Brazilian states of Maranhão and Pará and is one of the most consumed fruits in that region. Some authors consider cupuassu one of the most promising fruits for commercialization among many others of the Amazon region (Quijano & Pino, 2007). The cupuassu pulp has an average pH of 3.4 and its sugar content is about 10.7° Brix. It is used to produce juice, ice cream, jams, liqueur, filling for chocolates, and other products.

Umbu is a fruit native to the semi-arid regions in the Brazilian northeast. It is consumed locally as fresh fruit, in juices and as ice cream. Umbu pulp has a pH of 2.2 and a sugar content of 14.8° Brix; these values may vary according the climate of the region of origin of the plant (Lira Júnior, Musser, Melo, Maciel, Lederman, & Santos, 2007).

Gabiroba is a fruit native to the western and southern Brazilian *cerrado*. This fruit has been rated as a potential food source for both domestic fowl and humans. Gabiroba is consumed fresh locally and is also used in the production of homemade ice cream, jams, juices and sweets. The pulp of the gabiroba has a pH of 4.1 and a sugar content of about 14° Brix; these values, combined with good pulp yields, allow for the use of gabiroba fruits in wine production (Duarte et al., 2009).

Cacao is known worldwide for its beans, which are used in the production of chocolate. The production and commercialization of cacao beans have long been the basis of the economy of some Brazilian states, especially Bahia (Dias et al., 2007). The pulp of the cacao fruit is a substrate rich in nutrients; it is a by-product of the processing of the fruit and can be used in the production of wines and other products (Schwan & Wheals, 2004).

The jaboticaba tree, also known as the "Brazilian grape tree," is a tree native to Brazil that belongs to the Myrtaceae family. Its fruits are purplish black, and their skin and pulp have a sweet taste and low acidity. Jaboticaba fruits are consumed fresh and in processed forms such as jams, juices and liqueurs.

Alcoholic fermentation leads to a series of byproducts in addition to ethanol. They include carbonyl compounds, alcohols, esters, acids and acetals, all of them influencing the quality of the finished product. The composition and concentration levels of the byproducts can vary widely (ng L^{-1} to hundreds of mg L^{-1}) (Plutowska & Wardencki, 2008). The use of selected yeast strains (usually *Saccharomyces cerevisiae*), can affect the wine composition and positively affect the wine quality. Although the number of publications about fruit wines has increased in recent years, the use of selected yeast and characterization of the composition of these beverages has not been detailed. The purpose of this study was to produce fruit wines from cacao, cupuassu, gabiroba, jaboticaba and umbu pulp and characterize them using gas chromatography-mass spectrometry (GC-MS) for determination of minor compounds and gas chromatography-flame ionization detection (GC-FID) for major compounds. Additionally, glycerol, ethanol, sugars and organics acids were detected by high-performance liquid chromatography (HPLC). It is expected that the determination of the compositions of these beverages will allow for better use of these fruits in the production of fruit wines.

2. Materials and methods

2.1. Must preparation

The fruit wines made from the tropical fruits were prepared according to Dias et al. (2007) and Duarte et al. (2009). The fruit pulps was diluted with a sucrose solution to adjust the sugar content to 16^{o} Brix, and the pH was adjusted to 4.5 with the addition of calcium carbonate. Pectinolytic enzyme preparations were added to facilitate juice clarification. Ultrazym AFP-L (Novozymes, Novo Nordisk Ferment Ltd, Fuglebakken, Denmark) was added to a concentration of 0.7 mL L^{-1}. Sulfur dioxide, in the form of potassium metabisulfite, was added up to a concentration of 200 mg L^{-1} to inhibit bacterial growth. Also, 1% bentonite was added to the must to facilitate the sedimentation of non-fermentable solids. The bentonite had been previously suspended in water to a concentration of 10% to aid its dispersion in the must.

2.2. Fermentation assays

Six fermentations were performed: five of them (cacao, cupuassu, gabiroba (I), jaboticaba and umbu) were inoculated with 10^8 cells mL^{-1} of *Saccharomyces cerevisiae* UFLA CA 1162 and the other one (gabiroba (NI)) were allowed to ferment spontaneously with the gabiroba pulp. All vinifications were carried out in 5 L flasks in a cold room at 22 ^{o}C and the fermentation was monitored by the daily measurement of Brix value, CO_2 and temperature. The fermentation was considered complete when the Brix level was stable. At the end of fermentation, the vats were transferred to a 10 ^{o}C incubator to aid the sedimentation of solid material from the fruits pulp. After 10 days at this temperature, the wine transfer was carried out with some aeration and the beverages were incubated at 10 ^{o}C for another 30 days. After that period, another transfer without aeration was carried out and the fruit wines were left for another 10 days at 10 ^{o}C, prior to filtration (Dias et al., 2007). The fruit wines were then filtered using cellulose filters and stored at 10 ^{o}C in glass bottles fully filled to avoid oxygen entrance. All assays were carried out in triplicate.

2.3. Analytical methods

2.3.1. Chemicals

1-Hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexenol, 2-pentanol, 3-methyl-3-butene-1-ol, 4-methyl-1-pentanol, 2-heptanol, 3-methyl-2-buten-1-ol, 3-methyl-1-pentanol, 3-ethoxy-1-propanol, 1-heptanol, ethyl propionate, ethyl butyrate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl pyruvate, ethyl lactate, ethyl octanoate, ethyl 3-hydroxybutanoate, diethyl malonate, ethyl 2-furoate, diethyl succinate, diethyl glutarate, diethyl malate, monoethyl succinate, triethyl citrate, propyl acetate, linalool, myrtenol, methyl salicylate, 4-vinylguaiacol, vanillin, 3,4,5-trimethoxyphenol, propanoic acid, 2-methyl butyric acid, 3-methyl butyric acid, heptanoic acid, octanoic acid, octanal, 6-methyl-5-hepten-2-one, nonanal, 3-(methylthio)-1-propanol, benzothiazole, N-(2-phenylethyl)acetamide, tyrosol, tetradecanoic acid, methanol, 2-phenylethanol, malic acid were purchased from Aldrich Chemistry (Munich, Germany). 1-Butanol, 1-pentanol, 2- ethyl-1-hexanol, 1-octanol, furfurol, 1-phenylethanol, ethylphenyl acetate, 2-phenylethyl acetate, 2-methylpropyl acetate, (*E*)-furan linalool oxide, (*Z*)-furan linalool oxide, (*E*)-pyran linalool oxide, (*Z*)-pyran linalool oxide, geranic acid, isobutyric acid, butyric acid, hexanoic acid, nonanoic acid, octanoic acid, hexadecanoic acid, 3- hydroxy-2-butanone, 2-furaldehyde, 2-phenoxyethanol, acetaldehyde, 1,1-diethoxyethane, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol were purchased from Fluka Analyticals (Seelze, Germany). Limetol, linalool hydrate, α-terpineol, 4-terpineol, ho-trienol, borneol, citronellol, geraniol, verbenone, δ-decalactone were purchased from Lluch (Barcelona, Spain). Menthol, benzyl alcohol, ethyl acetate, succinic acid, glucose and fructose were purchased from Sigma-Aldrich (Saint Luis, EUA) and acetic acid, ethanol, dichloromethane and sodium sulfate were purchased from Merck (Darmstadt, Germany).

2.3.2. Minor volatile components

Minor volatile components in the fruit wines were determined by extraction with dichloromethane according to the methods of Oliveira, Faria, Sá, Barros, & Araújo (2006), followed by analysis of the extracts by GC–MS using a Varian 3400 gas chromatograph equipped with a septum-equipped temperature programmable injector (SPI), and an ion-trap mass spectrometer (Varian Saturn II). Samples of 1 µL were injected into a capillary column (Factor Four VF-Wax_{MS} Varian, 60 m x 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at 124 kPa (18 psi). The detector was operated in the electron-impact mode (70 eV), and mass spectra were acquired by scanning over the mass/charge (m/z) range of 29-360 with an acquisition rate of 610 ms. The temperature of the injector (SPI) was programmed to run from 20ºC to 250ºC at

180ºC min^{-1} and was then maintained at 250ºC during the analysis. The oven temperature was held at 60ºC for 5 min, then programmed to run from 60ºC to 220ºC at 3ºC min^{-1} and was finally maintained at 250ºC for 25 min.

Volatile compounds were identified using Varian Saturn GC/MS software (Version 5.2) by comparing mass spectra and linear retention indices with those of authentic standard compounds injected under the same conditions. 4-nonanol was chosen as internal standard and added to each sample and standard to a final concentration of 305 μg L^{-1}. The 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.

2.3.3. Major volatile components

In order to identify the major volatile compounds, the beverages were analyzed directly without any previous treatment according to Fraile, Garrido, and Ancín (2000). A Chrompack CP-9000 gas chromatograph equipped with a Split/Splitless injector, a flame ionization detector, and a capillary column (50 m x 0.25 mm i.d., 0.2 μm film thickness; Chrompack) coated with CP-Wax 57 CB was used. The temperature of the injector and detector was set to 250ºC. The oven temperature was held at 50ºC for 5 min, then programmed to run from 50ºC to 220ºC at 3ºC min^{-1}, and then held at 220ºC for 10 min. Helium was used as the carrier gas at 125 kPa, with a split vent of 15 mL min^{-1}. Injections of 1 μL were made in the splitless mode (vent time, 15 s); 4-nonanol (internal standard) was added to the sample to a final concentration of 122.05 mg L^{-1}. The volatile compounds were identified by comparing the retention times of the samples with those of standard compounds. Quantification of volatile compounds was performed with Varian Star Chromatography Workstation software (Version

6.41) and expressed as 4-nonanol equivalents, after determining the detector response factor for each compound.

2.3.4. Organic acids, glycerol, ethanol and sugars

Ethanol, glucose, fructose, glycerol, and acetic, malic and succinic acids were quantified by HPLC, using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI), UV-visible detector (Jasco 870-UV-visible) and a 67H Chrompack column (300 x 6.5 mm) at 37ºC, using 5 mmol L^{-1} sulfuric acid as the eluent, at a flow rate of 0.4 mL min^{-1} and a sample volume of 20 μL.

2.4. Statistical analysis

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS) Release 17.0 for Windows (SPSS Inc., Chicago, IL). Principal component Analysis (PCA) was used to summarize the information in a reduced number of principal components.

2.5. Sensory evaluation

The final beverages were evaluated by 50 panellists, males and females, 18 and 55 years of age (staff and students of the Universities Unilavras and UFLA). The panelists were selected for participation on the basis of their preference for wines, interest, and availability. Randomized, refrigerated (10 ^{o}C) samples of 20-25 mL were served in clear, tulip-shaped glasses with a volume of 100 mL; these were marked with three digit random numbers and covered with plastic Petri dishes. Distilled water was provided for rinsing of the palate during the testing. Evaluations took place in the mornings between 9:00 and 10:00 a.m. and were conducted at room temperature (20-22 ^{o}C) under white light. The fruit wines were evaluated for appearance (clarity and color), aroma, taste, and general acceptability according to the hedonic scale (Dias et al., 2007).

3. Results and discussion

Characterization of the fruit wines produced from the pulps of the gabiroba, umbu, cupuassu, jaboticaba and cocoa revealed that a large number of compounds were present in these beverages. Eighty-three compounds were quantified by GC-MS, nine compounds by GC-FID and seven compounds by HPLC.

3.1. Minor volatile components

Table 1 lists the concentrations of the minor volatile compounds detected in the six fruit wines. GC-MS analysis allowed for the identification and quantification of eighty-three volatile compounds, including C6 compounds, alcohols, ethyl esters, acetates, mono-terpenic alcohols, monoterpenic oxides, volatile phenols, acids, carbonyl compounds, sulfur compounds and others compounds.

3.1.1. C_6 compounds

In this group, 1-hexanol and (*Z*)-3-hexen-1-ol were the two most often detected compounds (Table 1). However, some compounds were present in one fruit wine only, e.g., (*E*)-2-hexenol and (*E*)-3-hexen-1-ol were present only in the inoculated gabiroba (I) wine and cupuassu wine in concentrations of 1.8 µg L^{-1} and 2.1 µg L^{-1}, respectively.

3.1.2. Alcohols

This volatile fraction contained a large number of compounds, such as ethyl esters group. However, some alcohols were present in one only fruit wine, e.g., 2-heptanol in the cacao wine (6.8 μg L^{-1}), 3-ethoxy-1-propanol in the jaboticaba wine (0.6 μg L^{-1}) and 2-phenoxyethanol in the fruit wines produced from the gabiroba pulp (15.3 μg L^{-1} gabiroba (I) and 26.2 μg L^{-1} in the non-inoculated gabiroba (NI) wine). The cacao wine was the one that contained the greatest number of alcohols; only 3-ethoxy-1-propanol and 2-phenoxyethanol were not found in this fruit wine. The gabiroba wines (gabiroba (I) and gabiroba (NI)) showed, qualitatively, the same composition of alcohols (1-butanol, 1-pentanol+3-methyl-3-butene-1-ol, 3-methyl-1-pentanol, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, furfural, benzyl alcohol and 2-phenoxyethanol). The fact that one or more compounds were found exclusively in some of the fruit wines is probably directly related to the characteristics of the fruit used in the production of those fruit wines.

3.1.3. Ethyl esters

Esters were one of the most prevalent group, with a total of 16 compounds and ethyl esters were the compounds present in the highest concentrations. Diethyl succinate and ethyl lactate had the highest concentrations among the ethyl esters detected in the fruit wines (Table 1).

Ethyl esters are one of the most important groups of aroma compounds in wine, and their concentrations depend on several factors, such as yeast strain, fermentation temperature, aeration, and sugar content. These compounds contribute positively to the overall wine quality, and most of them have a mature flavor and fruity aroma that contribute to the ‘‘fruity’’ and ‘‘floral’’ sensory properties of wines (Perestrelo, Fernandes, Albuquerque, Marques, & Câmara, 2006).

As proposed by Noguerol-Pato, González-Barreiro, Cancho-Grande, & Simal-Gándara (2009), to evaluate the contributions of the esters to the aromas of the fruit wines, the odor activity values (OAV) of the esters were calculated as the ratios between the concentration of each compound and its odor threshold, as found in the literature (Guth, 1997; Ferreira, López, & Cacho, 2000). The contribution of ethyl butyrate in the flavor of the gabiroba (I) and cupuassu wines was evidenced by high OAVs of 6.5 and 6.2 for the cupuassu and gabiroba (I) wines, respectively. According to some authors, ethyl butyrate is characterized as having a fruity aroma, as papayas and apples (Czerny et al., 2008; Siebert et al., 2005; Meilgaard, 1975). Ethyl-3-methylbotanoate (fruity, sweet fruity) had OAVs of 15.9 and 4.6 for the cupuassu and gabiroba (I) wines, respectively, while ethyl hexanoate (fruity and green apple) had OAVs of 5.2 and 3.5 for the gabiroba (I) and cupuassu wines, respectively. The compounds with higher OAVs contribute to the aroma of the fruit wines to a greater extent.

3.1.4. Acetates

Acetates were found in small numbers in the fruit wines studied (Table 1). Compounds of this group such as hexyl acetate mixed with ethyl caprylate and ethyl caprate give an “apple-like” aroma; 3-methylbutyl acetate gives a banana-like aroma and 2-phenylethyl acetate gives a fruity and flowery flavor with a honey note (Rapp & Mandery, 1986). 3-methylbutyl acetate (banana) and 2-phenylethyl acetate (apple, honey and roses) were found in all fruit wines (Table 1). The gabiroba (NI) wine showed the highest OAV for 3-methylbutyl acetate (2.6) and the cocoa wine showed the highest OAV for 2-phenylethyl acetate (0.3). According to Perestrelo et al. (2006), acetates are the result of the reaction of acetylCoA with higher alcohols, which are formed through the degradation of amino acids or carbohydrates.

Table 1. Concentration of minor volatile compounds ($\mu g\ L^{-1}$) detected in the fruit wines by GC-MS; odor threshold and descriptors reported in literature. Data are presented as mean ± SD.

No	Compounds	LRI	Fruit wines						Oth	Descriptors
			Cacao	Cupuassu	Gabiroba (I)	Gabiroba (NI)	Jaboticaba	Umbu	($\mu g\ L^{-1}$)	
	C_6 compounds (4)									
1	1-Hexanol	1348	6.3±1.1	28.4±3.7	38.4±7.6	35.7±8.9	11.8±0.7	3.6±0.3	8000[b ∞]	-
2	(*E*)-3-hexen-1-ol	1358	ND	2.1±2.5	ND	ND	ND	ND	-	-
3	(*Z*)-3-hexen-1-ol	1379	5.6±0.6	17±1.1	43.5±3.4	48.5±3.1	16±0.8	ND	3.9[a *]	Lettuce-like[a]
4	(*E*)-2-hexenol	1400	ND	ND	1.8±0.1	ND	ND	ND	-	Bitter, green leaves[e]
	Alcohols (16)									
5	2-Pentanol	1112	168.7±32.2	ND	ND	ND	3.1±0.4	ND	-	-
6	1-Butanol	1173	7.8±1.3	97.1±12.9	13±1.5	15.6±0.9	15.6±1.5	4.8±0.2	590[a *]	Malty[a]; fusel,spirituos[c]
7	1-Pentanol +3-Methyl-3-butene-1-ol	1244	4.2±0.4	10.1±1.1	3.8±0.1	8.6±3.1	2.1±0.2	3.7±1	-	-
8	4-Methyl-1-pentanol	1309	6.8±0.8	7.1±1.1	4.1±0.9	1.5±0.4	6.3±0.5	8.9±0.4	-	-
9	2-Heptanol	1315	6.8±0.6	ND	ND	ND	ND	ND	-	Coconut[e]
10	3-Methyl-2-buten-1-ol	1317	15.8±2.4	125.9±14.1	ND	ND	ND	4.9±0.7	-	-
11	3-Methyl-1-pentanol	1322	14.1±1.7	14.5±1.7	7.2±0.9	3.6±0.2	13.7±0.4	22.8±1.4	-	-
12	3-Ethoxy-1-propanol	1369	ND	ND	ND	ND	0.6±0	ND	-	-
13	1-Heptanol	1449	8.2±0.4	4.6±2.8	6.9±0.3	7.7±0.4	2.3±0.7	21.4±1.3	-	Coconut, unpleasant[e]
14	2-Ethyl-1-hexanol	1486	19.8±1	8.4±1.1	24.1±1.1	76.2±7.2	12.6±0.8	10.3±0.5	-	-
15	1-Octanol	1552	2.7±0.4	5.5±3.8	3.5±3	3.1±0.4	6.8±0.6	2.2±0.6	900[e §]	Coconut, walnut, oily[e]
16	Furfurol	1658	29.1±4.1	38.6±3.3	11.6±3	12.5±1.4	7.4±0.2	20.2±0.9	1000[c*]	Moldy hay[d]
17	1-Phenylethanol	1812	83.1±9.3	2.4±0.7	ND	ND	ND	ND	-	-
18	Benzyl alcohol	1869	10.8±1.9	8.9±1.8	14.6±1.5	17.7±1.7	17.2±0.7	9.5±1.2	-	Almonds, bitter[e]
19	2-Phenoxyethanol	2144	ND	ND	15.3±0.9	26.2±4	ND	ND	-	-
20	Tyrosol	3008	33.9±10.1	29.5±1.8	ND	ND	ND	ND	-	Bitter, chemical[e]

Table 1 (Continued)

No	Compounds	LRI	Fruit wines						Oth	Descriptors
			Cacao	Cupuassu	Gabiroba (I)	Gabiroba (NI)	Jaboticaba	Umbu	(µg L^{-1})	
	Ethyl Esters (16)									
21	Ethyl propionate	971	7.3±1.2	16.4±2.4	55.7±2.8	52.5±9.1	23.4±1.9	ND	45[b ∞]	Fruity[c]
22	Ethyl butyrate	1032	17.7±2.4	129.2±16.1	124.1±6.5	20.2±1.7	12.8±0.9	9.4±2	20[b ∞]	Fruity[a c]; papaya, butter, sweetish, apple, perfumed[e]
23	Ethyl 2-methylbutanoate	1049	ND	5.3±0.2	11.6±4.6	8.8±3	1.5±0.5	ND	18[g ϕ]	Fruity[a]; sweet fruity[c]
24	Ethyl 3-methylbutanoate	1066	12.9±0.7	47.7±4.3	13.8±3.5	8.1±0.8	4.2±0.8	ND	3[g ϕ]	Fruity, blueberry-like[a]; sweet fruity[c]
25	Ethyl hexanoate	1234	32±4.6	48.9±5.8	73.3±0.3	18.8±11	10.6±1.1	24±3.4	14[g ϕ]	Fruity, green apple[e,c]
26	Ethyl pyruvate	1267	24.5±3.7	ND	15.3±1.2	1.2±0.4	43.6±1.2	8.9±0.7	-	Herbaceous, oil painting, forage[e]
27	Ethyl lactate	1338	205.4±32.4	255.6±40.3	98.2±10.5	56.6±1.8	407.1±7.8	99±6.7	157810[h ∞]	Strawberry, raspberry[e,c]
28	Ethyl octanoate	1434	5.4±0.8	9.3±3.5	130.6±3.3	9.8±2.7	2.3±0.2	0.9±0.2	5[g ϕ]	Apple[e]; sweet[c]
29	Ethyl 3-hydroxybutanoate	1512	28.7±4	69.6±7.1	74.3±6.2	88.7±5.9	47.3±1.1	35.6±2.6	-	-
30	Diethyl malonate	1574	ND	ND	ND	ND	5±0.5	ND	-	-
31	Ethyl 2-furoate	1618	ND	ND	41.7±2.2	11.6±1.5	2.5±0.7	ND	1600[g ϕ]	-
32	Diethyl succinate	1672	1747.2±108	546.2±20.9	367.2±18.1	29.4±1.4	2191.5±98	169.2±10.5	200000[h ∞]	-
33	Diethyl glutarate	1774	5.1±3.9	ND	1.2±0.2	ND	11.9±0.4	ND	-	-
34	Diethyl malate	2037	448.7±59.7	259±18.7	16.4±8	ND	172.9±6.2	14.6±2.3	-	-
35	mono-Ethyl succinate	2377	1062±91.5	358.6±51.6	90±16.3	ND	978.7±179	309.3±1.2	-	Sweat, sour, fruity[e]
36	Triethyl citrate	2461	23.1±4.5	7.1±1.5	ND	ND	75.3±4.1	ND	-	-

Table 1 (Continued)

No	Compounds	LRI	Fruit wines						Oth (µg L^{-1})	Descriptors
			Cacao	Cupuassu	Gabiroba (I)	Gabiroba (NI)	Jaboticaba	Umbu		
	Acetates (5)									
37	Propyl acetate	982	ND	ND	ND	7±1.2	ND	ND	-	Solvent, sweet, fragrant[e]
38	2-Methylpropyl acetate	1009	ND	ND	8.5±2	39.1±2.5	10.5±0.6	ND	-	Banana, fruity[c]
39	3-Methylbutyl acetate	1125	17.3±1.6	26±0.7	50.1±19.1	79.3±4.9	29.9±1.1	37.5±3	30[b ∞]	Banana[c]
40	Ethylphenyl acetate	1788	121.9±17.4	22.8±5	5.7±1.6	6±1.4	4.3±0.2	ND	-	-
41	2-Phenylethyl acetate	1810	62.2±11.1	58±3.4	18±9.4	26.8±8.4	37.9±2.8	26.1±4.5	250[b ∞]	Apple, honey, roses, sweet[e]; flowery[c]
	Monoterpenic alcohols (10)									
42	Limetol	1113	ND	3.2±0.2	ND	ND	ND	ND	-	-
43	Linalool	1541	8.5±1.5	182.6±4.1	185.7±23.9	201±17.1	17.7±3.7	10.9±0.1	25.2[g ϕ]	Citruslike, bergamot[a]
52	(*E*)-Furan linalool oxide	1436	297.4±31.8	40.6±10	5±0.2	ND	22.3±1.1	ND	-	-
53	(*Z*)-Furan linalool oxide	1464	161.8±8.3	60.6±14.8	3±0.1	7.2±1.3	27.3±6.4	0.8±0.1	-	-
54	(*E*)-Pyran linalool oxide	1732	3±1.1	22±11.5	ND	ND	ND	ND	-	-
55	(*Z*)-Pyran linalool oxide	1756	35.2±4	6.5±1.1	ND	ND	ND	ND	-	-
56	Linalool hydrate	1967	ND	12.6±2.8	ND	ND	4.4±0.4	ND	-	-
57	Geranic acid	2347	ND	ND	ND	ND	ND	7.7±0.5	-	-
	Volatile phenols (4)									
58	Methyl salicylate	1770	ND	ND	2.9±0.3	ND	ND	ND	-	-
59	4-Vinylguaiacol	2192	ND	ND	ND	ND	ND	4.9±0.7	21[a *]	Clove-like, smoky[a]
60	Vanillin	2560	ND	ND	15.3±1.7	10.7±2.4	16.1±4	ND	65[d ϕ]	Vanilla-like, sweet[a]; vanilla[d]
61	3,4,5-Trimethoxyphenol	3060	ND	ND	ND	ND	ND	27.4±5.8	-	-

Table 1 (Continued)

No	Compounds	LRI	Fruit wines						Oth (µg L^{-1})	Descriptors
			Cacao	Cupuassu	Gabiroba (I)	Gabiroba (NI)	Jaboticaba	Umbu		
	Acids (11)									
62	Propanoic acid	1552	6.4±4.2	9.5±1.4	9.9±5.2	9.5±2.8	5.9±0.3	4.5±0.8	8100[h] ∞	Vinegar[c]
63	Isobutyric acid	1579	22.9±2.7	49.1±20.2	44.8±5.2	32.1±0.4	11.1±2.2	13.7±0.4	200000[b] ∞	Sweat, bitter[e]; cheese, rancid[c]
64	Butyric acid	1626	19.7±5.5	83±10.3	29±4.5	7.1±1.1	4±1.2	9.8±0.9	173[g] ϕ	Sweaty[a]; cheese, rancid[c]
65	2-Methyl butyric acid + 3-Methyl butyric acid	1667	143.8±17.5	334±50.7	110.0±9.9	123.6±13.1	18.8±0.6	31.8±2.8	3000[b] ∞+33[g] ϕ	Fruity, sweaty+Sweaty[a]; cheese[c]
66	Hexanoic acid	1841	540.9±68.9	630.3±60.8	241.2±45.4	77.1±5.6	150.5±16.7	392±35.1	420[g] ϕ	Fatty acids, vegetable oil[e]; cheese, sweaty[c]
	Carbonyl compounds (5)									
73	3-Hydroxy-2-butanone	1285	204.1±18.8	90.4±14.3	60±6.1	130.7±39.8	38.2±3.1	13.7±0.7	152600[h] ∞	Fuity, moldy, wood[e]
74	Octanal	1291	1.4±0.6	1.3±1.2	1.6±0	3.1±0.5	1.0±0.1	1.8±0.8	3.4[a] *	Citrus-like, green[a]
75	6-Methyl-5-hepten-2-one	1338	3.7±0.4	ND	1.1±0.2	1.1±0.1	ND	1.4±0.3	-	-
76	Nonanal	1396	4.9±3.9	3.3±1	2.5±0.6	3.9±1.2	2±0.5	4.9±0	2.8[a] *	Citrus-like, soapy[a]
77	2-Furaldehyde	1460	8.4±0.9	26.8±4	37±3.6	9.6±1.2	16.4±0.8	6.7±1.2	8000[d] *	Almonds[d]
	Sulfur (3)									
78	3-(Methylthio)-1-propanol	1715	71.7±11.1	205.4±21.1	32.7±3.5	7.3±3.2	17.7±1.5	29.9±4	36[a] *	Cooked potato-like[a]
79	2-Methyltetrahydrothiofeno-3-one	1533	ND	16.2±2.6	19.3±12.1	12±1.9	ND	ND	-	-
80	Benzothiazole	1962	11.5±1.8	4±1.2	3.2±0.8	6±1.2	6.2±0.9	6.9±0.7	-	-

Table 1 (Continued)

No	Compounds	LRI	Fruit wines						Oth (µg L^{-1})	Descriptors
			Cacao	Cupuassu	Gabiroba (I)	Gabiroba (NI)	Jaboticaba	Umbu		
	Other (3)									
81	Verbenone	1712	ND	ND	ND	ND	1.±0.3	ND	-	-
82	δ-Decalactone	2151	ND	13.2±2.6	ND	ND	ND	ND	31[a *]	Coconut-like[a]
83	N-(2-phenylethyl)acetamide	2585	35±5.5	15.6±1.6	27.2±6.1	29.2±6.5	40.5±3.7	26.4±3	-	-

LRI, linear retention index; I, inoculated gabiroba wine. NI, non-inoculated gabiroba wine. Oth, odor threshold. ND, not detected.
*Olfactory perception threshold in water; [∞] Olfactory perception threshold in hydro-alcoholic solution; [§] Olfactory difference threshold in beer;
[ϕ] Olfactory threshold in model wine.
[a] Czerny et al. (2008).
[b] Guth (1997).
[c] Siebert et al. (2005).
[d] Boidron, Chatonnet, and Pons (1988).
[e] Meilgaard (1975).
[f] Ribéreau-Gayon, Glories, Maujean, and Dubourdieu (2000).
[g] Ferreira, López, and Cacho (2000).

3.1.5. Acids

Short-chain fatty acids, such as isobutyric, butyric and isovaleric acids, are minor compounds in wines and their odor may be as strong as that of acetic acid; therefore, these acids can contribute significantly to the aromas of wines and spirits (Soufleros et al., 2001). The acids found to be present in the highest concentrations were octanoic and hexanoic acids. Among the fruit wines, the cacao wine had the highest concentration of octanoic acid (1149.2 μg L^{-1}) and the cupuassu wine had the highest concentration of hexanoic acid (630.3 μg L^{-1}) (Table 1). Despite the relatively high concentrations, all acids were present in quantities below their flavor threshold. Similar results have been reported for other wines (Perestrelo et al., 2006). The lowest concentrations of the octanoic ("fatty acids", "vegetable oil" and "rancid") and hexanoic ("fatty acids", "vegetable oil" and "cheese") acids were found in the gabiroba (NI) wine (Table 1).

3.1.6. Monoterpenics compounds

The monterpenic volatile fraction was comprised of ten monoterpenic alcohols and six monoterpenic oxides. As can be seen in Table 1, some compounds were found only in one fruit wine, such as limentol (cupuassu), ho-trienol and menthol (cacao), myrtenol (gabiroba (I) and gabiroba (NI)) and geranic acid (umbu). Some of these compounds may be used as markers of the fruit wine produced from a specific fruit. The monoterpenic compounds play an important role in the varietal flavor of the must and other fruit juices (Mateo & Jiménez, 2000). According to Peña et al. (2005) obtaining a "terpenic profile" is extremely useful for differentiating the genuinely monovarietals wines from those made by a mixture of some other varieties.

The monoterpene alcohols linalool, α-terpineol and geraniol were found in all fruit wines (Table 1). The highest OAVs for linalool were 7.4 and 8.0 for the gabiroba (I) and gabiroba (NI) wines, respectively. The monoterpene alcohol α-terpineol had an OAV of 1.1 in the umbu wine and an OAV of 0.8 in the cupuassu wine. Some of the monoterpene alcohols are among the most odoriferous compounds, especially linalool, *α*-terpineol, nerol, geraniol, citronellol and ho-trienol, which have a floral aroma reminiscent of rose essence. The olfactory perception thresholds of these compounds are rather low - as little as a few hundred micrograms per liter (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2000). (*E*)-pyran linalool oxide and (*Z*)-pyran linalool oxide were identified only in the cacao and cupuassu wines; the highest concentration of (*E*)-pyran linalool oxide was 22 μg L^{-1} (cupuassu) and the highest concentration of (*Z*)-pyran linalool oxide was 35.2 μg L^{-1} (cacao).

The results of the monoterpenes shown in Table 1 were further analyzed using PCA to obtain a more simplified view of the relationships among these compounds (Fig. 1). The first and second principal components explain about 61.4% and 26.8%, respectively, of the total variance. The results in Figure 1 show the formation of two groups. One of the groups is located on the positive part of the second factor, and includes the cacao, cupuassu and jaboticaba wines. The other group is closely related to the negative part of the axis, and includes the gabiroba (I), gabiroba (NI) and umbu wines. The umbu, gabiroba (I) and gabiroba (NI) wines were characterized by α-terpineol and linalool. In the other group, jaboticaba, cacao and cupuassu wines were correlated with (*Z*)-furan linalool oxide and (*E*)-furan linalool oxide.

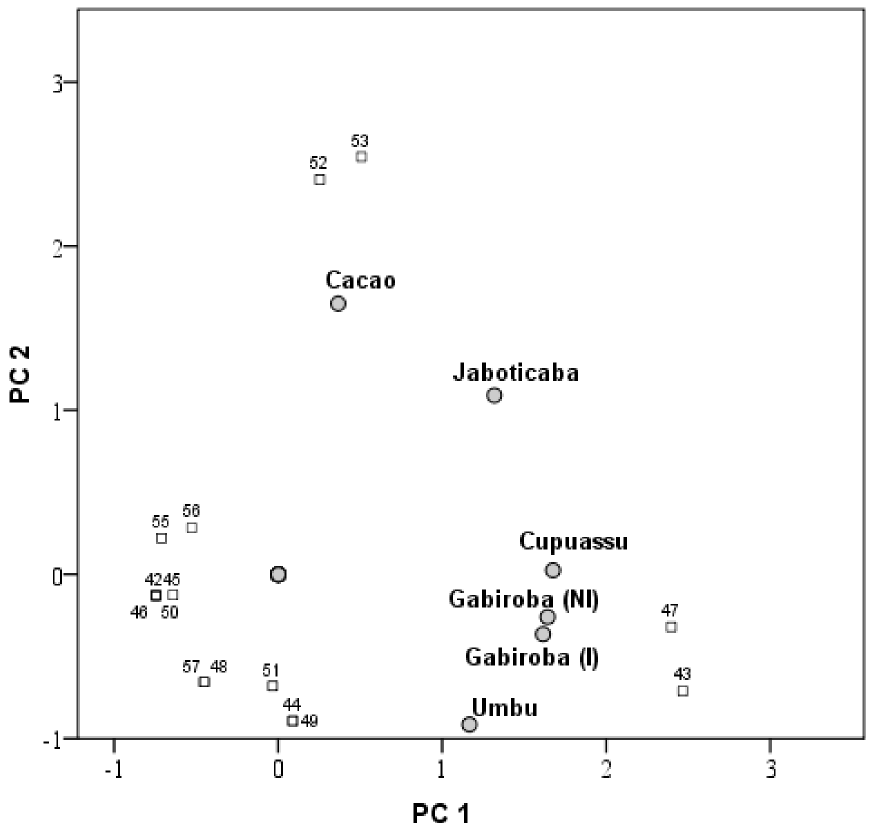

Figure 1. Principal component analysis (PCA) of monoterpenics compounds in fruit wines by GC/MS. I: inoculated gabiroba wine; NI: non-inoculated gabiroba wine. The volatilecompounds numbers are referred in Table 1.

3.1.7. Other compounds

Other groups with fewer compounds were also identified, such as carbonyl compounds (five), volatile phenols (four) and sulfur compounds (three).

Although these compounds were present in smaller numbers, they contributed to the aroma of the fermented beverages. For example, sulfur compounds, which comprise a structurally diverse class of molecules with a wide range of aromatic notes, may be considered detrimental to wine quality (Anocibar Beloqui & Bertrand, 1995). The volatile phenols could originate from *p*-coumaric and ferulic acids via decarboxylation (Perestrelo et al., 2006). 3,4,5-trimethoxyphenol and 4-vinylguaiacol were found only in the umbu wine (Table 1). 4-Vinylguaiacol contributes to “clove-like” and “smoky” odors (Czerny et al., 2008).

3.1.8. Multivariate statistical analysis of minor volatile compounds

The results obtained for the minor volatile compounds shown in Table 1 were submitted to PCA to obtain a more simplified view of the relationships among the volatile compounds analyzed. The results are shown in Fig. 2.

The first (PC 1) and second (PC 2) principal components explain 70.9% and 18.7%, respectively, of the total variance.

A plot of the results (Fig. 2) shows the formation of two groups. One of the groups is located on the positive part of the second factor, and includes the gabiroba (I) and gabiroba (NI) wines; the other group is closely related to the negative part of the axis, and includes the cacao, cupuassu, jaboticaba and umbu wines. Component 2 allowed for the differentiation of the wines produced from gabiroba pulp from the wines produced from the cacao, cupuassu, jaboticaba and umbu pulps.

The non-inoculated gabiroba (NI) and, to a lesser degree, inoculated gabiroba (I) wines were mainly associated with linalool, 2-methyl butyric acid + 3-methyl butyric acid and 3-hydroxy-2-butanone. The cacao, cupuassu, jaboticaba and umbu wines were associated with ethyl lactate, diethyl succinate, diethyl malate, mono-ethyl succinate and hexanoic acid.

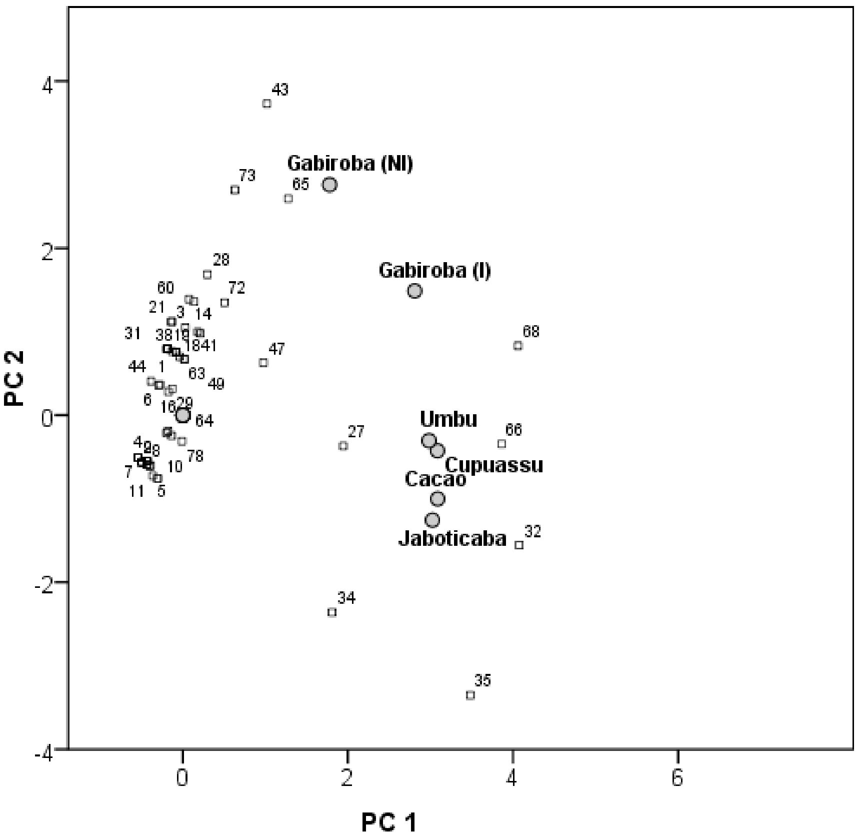

Figure 2. Principal component analysis (PCA) of minor volatile compounds in fruit wines by GC/MS.I: inoculated gabiroba wine; NI: non-inoculated gabiroba wine. The volatilecompounds numbers are referred in Table 1.

3.2. Major volatile components

Table 2 lists the concentrations of the major volatile compounds detected in the six fruit wines. Nine compounds were quantified: acetaldehyde, 1,1-diethoxyethane, ethyl acetate, methanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol.

Statistical analysis of the concentrations of the major volatile compounds in all fruit wines, using Tukey's test, showed significant differences in the concentrations of all compounds assayed.

The higher alcohols were found in the greatest number in all fruit wines. 3-methyl-1-butanol was markedly the most abundant higher alcohol (Table 2). The umbu wine had a higher concentration (261.3 mg L^{-1}) of 3-methyl-1-butanol, above the perception threshold. Thus, its sensorial contribution of a "malty", "alcohol" and "harsh" odor was expected. According to Tukey's test, there were no significant differences in 3-methyl-1-butanol concentrations among the cacao, jaboticaba and gabiroba (I) wines (Table 2).

The 2-phenylethanol is an aroma carrier and its presence may contribute to the floral nuance of wines (Wondra & Berovic, 2001). The aroma character of this compound changes with its oxidation from rose to a hyacinth bouquet. Further oxidation produces esters with a fine honey nose. The cacao wine had the highest concentration of 2-phenylethanol (99.7 mg L^{-1}) and the gabiroba (I) wine had the lowest concentration (15.8 mg L^{-1}) (Table 2). In our study, the cupuassu wine had the highest concentration of 1-propanol (36 mg L^{-1}), about 5 times higher than that found in the cacao wine, which was the one with the lowest concentration of this compound.

Table 2. Concentration of major volatile compounds (mg L^{-1}) detected in the fruit wines by GC-FID; odor threshold and descriptors reported in literature. Data are presented as mean ± SD.

Compounds	Fruit wines							
	Cacao	Cupuassu	Gabiroba (I)	Gabiroba (NI)	Jaboticaba	Umbu	Oth (µg L^{-1})	Descriptors
Acetaldehyde	$28^{(c)}\pm9.3$	$15.4^{(ab)}\pm0.4$	$45.3^{(bc)}\pm9.2$	$8.3^{(ab)}\pm4.5$	$16.4^{(ab)}\pm1.5$	$5.1^{(a)}\pm0.6$	$25^{a\,*}$	Fresh, green[a]
1,1-Diethoxyethane	$16^{(c)}\pm1.3$	$3^{(a)}\pm0.2$	$3.3^{(a)}\pm0.3$	$2.6^{(a)}\pm0.2$	$7.2^{(b)}\pm1.5$	ND	$50^{b\,\infty}$	
Ethyl acetate	$189.5^{(b)}\pm63.9$	$27.2^{(a)}\pm2.5$	$13.2^{(a)}\pm1.3$	$105.9^{(ab)}\pm0.1$	$54.7^{(a)}\pm5.1$	$89.6^{(ab)}\pm11.8$	$7500^{b\,\infty}$	Solvent, fruity[e]; nail polish[d]
Methanol	$195^{(c)}\pm42.7$	$137.7^{(abc)}\pm2$	$57.2^{(a)}\pm4.8$	$86.8^{(ab)}\pm0.5$	$181^{(c)}\pm7.8$	$144.9^{(bc)}\pm26.4$	-	-
1-Propanol	$7.2^{(a)}\pm1.6$	$36^{(c)}\pm1.7$	$9.7^{(a)}\pm1.3$	$17.9^{(b)}\pm2.4$	$18.1^{(b)}\pm0.1$	$21.6^{(b)}\pm0$	$750^{e\,\S}$	-
2-Methyl-1-propanol	$24.3^{(a)}\pm0.1$	$58.5^{(b)}\pm0.1$	$32.5^{(a)}\pm4.7$	$77.9^{(c)}\pm1.4$	$34.5^{(a)}\pm1.5$	$101.7^{(d)}\pm7.6$	$550^{a\,*}$	Malty[a]
2-Methyl-1-butanol	$26.1^{(b)}\pm0.5$	$35.8^{(c)}\pm0.9$	$24.1^{(b)}\pm2.8$	$23.4^{(ab)}\pm0.9$	$16.4^{(a)}\pm0.04$	$56.8^{(d)}\pm3.4$	$1200^{a\,*}$	Malty, solvent-like[a]
3-Methyl-1-butanol	$113.6^{(abc)}\pm5.2$	$141.5^{(c)}\pm0.8$	$103.4^{(ab)}\pm9.6$	$133^{(bc)}\pm2.8$	$80.8^{(a)}\pm1.5$	$261.3^{(d)}\pm17.4$	$220^{a\,*}$	Malty[a]
2-Phenylethanol	$99.7^{(c)}\pm28.1$	$65^{(bc)}\pm0.5$	$15.8^{(a)}\pm0.7$	$52.2^{(ab)}\pm3.1$	$29.3^{(ab)}\pm1.4$	$41.6^{(ab)}\pm5.8$	$140^{a\,*}$	Flowery, honey-like[a]

I, inoculated gabiroba wine. NI, non-inoculated gabiroba wine. Oth, odor threshold. ND, not detected.*Olfactory perception threshold in water;

$^{\infty}$ Olfactory perception threshold in hydro-alcoholic solution; § Olfactory difference threshold in beer; Values identified by the same letters are not significantly different at a significance level of 0.05 (Tukey's test).

[a] Czerny et al. (2008).

[b] Guth (1997).

[e] Meilgaard (1975).

The higher alcohols could be synthesized by yeast through either an anabolic pathway from glucose or a catabolic pathway from the corresponding amino acids (valine, leucine, *iso*-leucine and phenylalanine). Consequently, higher alcohols are released to the medium as secondary products of yeast metabolism and are responsible for the secondary aroma of wines (Noguerol-Pato et al., 2009).

The cacao and jaboticaba wines had the highest contents of methanol, (195 mg L^{-1} and 181 mg L^{-1}, respectively), but no significant differences in methanol concentrations were found among the cacao, jaboticaba, cupuassu and umbu wines. Methanol is a toxic alcohol commonly found in wines; consequently its concentration must be measured. It is formed from the enzymatic hydrolysis of the methoxy groups of pectin during fermentation, and its content depends on the extent to which the solids - especially the skins, which have high pectin content - are macerated (Peinado, Moreno, Muoz, Medina, & Moreno, 2004). Therefore, the differences in the concentrations of methanol between the fruit wines could be related to the pectin content of each fruit.

Acetaldehyde was the major aldehyde compound found in the fruit wines. At low levels, it gives a pleasant fruity aroma to wines, but in higher concentrations, it has a pungent, irritating odor (Miyake, & Shibamoto, 1993). The concentration of acetaldehyde in the umbu wine was 5.1 mg L^{-1}, the lowest concentration found in any of the fruit wines. There were no significant differences in the concentrations of acetaldehyde among the cupuassu wine (15.4 mg L^{-1}), jaboticaba (16.4 mg L^{-1}) and gabiroba (NI) (8.3 mg L^{-1}) wines. The highest concentration of acetaldehyde was found in the gabiroba (I) wine (45.3 mg L^{-1}) (Table 2). According to Perestrelo et al. (2006), aldehydes are formed from unsaturated fatty acids. Also, they can be considered as products of lipoxygenase catalysis.

Ethyl acetate is another compound whose presence may adversely affect the quality of wine due to its unpleasant flavor in high concentrations. On the other hand, at very low concentrations (50–80 mg L^{-1}), it has a positive impact on the flavor (Tešević et al., 2009). The concentration of this compound varied significantly among the fruit wines. The cacao wine had the highest concentration of ethyl acetate (189.49 mg L^{-1}), about 15 times higher than that found in the gabiroba (I) wine (Table 2).

3.3. Organic acids, glycerol, ethanol and sugars

The most important acids with regard to the acidity of wines are tartaric, malic, citric, lactic and succinic acids. However, several others acids can be present in wines. Most of them are organic acids, though inorganic acids may also be present in small quantities. Acidity is another important factor, since it contributes both directly and indirectly to the quality of wines (Clarke, & Bakker, 2004).

Malic, succinic and acetic acids were identified in the fruit wines. Succinic acid had the highest concentrations, ranging from 2.3 g L^{-1} (cupuassu wine) to 6.1 g L^{-1} (gabiroba (NI) wine) (Table 3). Succinic acid is a common by-product of the alcoholic fermentation of yeast; it is the major carboxylic acid formed during fermentation. It has been reported that this acid gives an unusual salty, bitter taste to wine (Coulter, Godden, & Pretorius, 2004).

The gabiroba (NI) wine had the highest concentrations of acetic acid (Table 3). This fact could be associated with the presence of non-saccharomyces yeast in spontaneous fermentations that normally produce larger amounts of acetic acid. The inoculated gabiroba (I) and non-inoculated gabiroba (NI) wines were the only ones in which the concentration of acetic acid was higher than 1 g L^{-1} (Table 3). Acetic acid is the most important volatile acid (Clarke, & Bakker, 2004). When acetic acid is present in high concentrations (> 0.7 g L^{-1}), the wine has a pronounced vinegar odor and taste.

Table 3. Concentrations (g L^{-1}) of acids, glycerol, ethanol and residual sugars detected in fruit wines by HPLC.

Compound	Fruit wines					
	Cacao	Cupuassu	Gabiroba (I)	Gabiroba (NI)	Jaboticaba	Umbu
Malic acid	0.29±0.03	1.76±0.10	0.07±0.04	1.60±0.04	0.62±0.03	0.10±0.04
Succinic acid	3.94±0.11	2.32±0.13	6.03±0.30	6.12±0.25	5.11±0.19	3.18±0.20
Acetic acid	0.37±0.10	0.14±0.07	1.45±0.11	1.62±0.10	0.78±0.15	0.65±0.03
Glycerol	7.14±0.40	6.54±0.30	5.35±0.51	6.11±0.85	7.56±0.38	7.69±0.54
Glucose	3.43±0.57	1.97±0.27	ND	0.65±0.08	0.06±0.02	2.41±0.38
Fructose	0 ND	0.17±0.05	ND	0.96±0.07	ND	ND
Ethanol	64.16±1.96	40.56±0.45	57.49±0.29	50.59±0.51	57.21±0.76	49.24±0.70

I: inoculated gabiroba wine; NI: non-inoculated gabiroba wine.

The presence of malic acid is also important in wines, because it is directly related to the acidity of the wines. Since malic acid contains two carboxylic acid groups, it releases more protons to the solution, increasing the acidity. The cupuassu and gabiroba (I) wines had the highest (1.7 g L^{-1}) and lowest (0.1 g L^{-1},) concentrations of succinic acid, respectively.

All of the fruit wines had similar glycerol contents, except the gabiroba (I) wine, which had the lowest concentration of this compound (5.3 g L^{-1}) (Table 3). Although a lower concentration of glycerol have been found in wine gabiroba (I), this fact probably will not have great influence on the differentiation between gabiroba wine and other fruit wines, because according to Lubbers, Verret, & Voilley (2001) glycerol did not change the relative volatility of aroma compounds in the range of 5 to 20 g L^{-1} in model wine and the increase of the amount of glycerol from 5.3 to 17.3 g L^{-1} in a white wine did not produce a detectable effect in the perceived aroma.

Residual sugars (glucose and fructose) were present in all fruit wines and in concentrations lower than 5 g L^{-1}, which characterizes the fruit wines as dry wines. Residual amounts of sugars, such as glucose and fructose, in a finished wine primarily determine its perceived sweetness or dryness (Clarke, & Bakker, 2004).

Ethanol is the major component of wine and determines the viscosity (body) of the wine while also acting as a fixative. The ethanol yield depends on the initial total sugar concentration in the fruit, which is measured as the total dissolved sugar concentration in the liquid must (Tešević et al., 2009). The initial sugar concentration in the must was adjusted to produce wines with low ethanol contents. The highest concentration of ethanol (8.1%) was found in the cacao wine (Table 3). The ethanol concentrations in the umbu and gabiroba (I) wines were approximately 7.2%. In the jaboticaba and gabiroba (NI) wines, ethanol concentrations were approximately 6% (Table 3).

3.4. Sensory evaluation

The fruit wines were subjected to sensory analysis to assess its acceptance. Table 4 presents percentage of acceptance attributed to each beverage by 50 untrained tasters. For all attributes assessed the beverages showed greater acceptance (at least 50%). The differences in sensory analysis found among these six beverages analyzed here might be the result of the different chemical compounds compositions of these final products (Tables 1-3). It was observed (Table 4) that in general, the acceptability attribute showed highest values for cacao (70%) and umbu (68%). Cacao and umbu wines also showed the highest percentage of acceptance for aroma, 73% and 74% for cacao and umbu, respectively. These results can be associated with the beverages composition.

Table 4. Percentage of the fruit wines acceptance in sensory analysis

Fruit wine	Appearance	Aroma	Taste	General acceptability
Cacao	63	67	68	70
Cupuassu	56	62	58	63
Gabiroba (I)	63	65	60	54
Gabiroba (NI)	69	71	54	60
Jaboticaba	52	70	61	56
Umbu	63	78	57	68

Data represents the grade attributed by tasters (50 untrained panelists) considering at least point 6 (liked slightly) until point 9 (liked extremely).

As shown in Fig. 2 these wines showed concentrations of ethyl esters such as ethyl lactate, diethyl succinate, diethyl malate, and mono-ethyl succinate. The ethyl esters group makes a positive contribution to the general quality of wine being responsible for their "fruity" and "floral" sensory properties (Perestrelo et al., 2006). The fruit wines gabiroba (I) and gabiroba (NI) had a lower percentage of acceptances (Table 4) when aroma and flavor attributes were observed. In the Fig. 2, these wines were characterized by compounds as 2-methyl butyric acid þ 3-methyl butyric acid and 3-hydroxy-2-butanone that might have influenced the wine aroma. The lower taste acceptance of wines gabiroba (I) and gabiroba (NI) could be associated with high concentration of acetic acid found in these wines (Table 3), which gave particular organoleptic characteristics reminiscent of vinegar and nail varnish, generally considered undesirable in wines, and reducing their quality (Clarke & Bakker, 2004).

4. Conclusions

Our results revealed that the fruit wines produced using pulps of cacao, cupuassu, gabiroba, jaboticaba and umbu fruits presented several compounds that are also found in other types of wines, such as fruit and grape wines. The fact that these fruit wines had a composition similar to other beverages demonstrated that these fruits have the potential to be used to produce fermented beverages. Furthermore, the major components found in the fruit wines (alcohols, monoterpenics compounds and ethyl esters) contributed to the formation of aromas which could be characterized as fruity, green apple, banana, sweet, citrus, citronella, vanilla, roses and honey. It was concluded that pulp of cacao, cupuassu, gabiroba, jaboticaba and umbu could be used to produce fruit wines with acceptable organoleptic characteristics. The typical volatile composition of minor compounds of each fruit wine, especially of the gabiroba wine, was evidenced by principal component analysis. Additionally, the yeast used for inoculation, *Saccharomyces cerevisiae* UFLA CA 1162 resulted in good must fermentation, especially with regard to the ethanol content, which ranged from 40.5 g L^{-1} (cupuassu) to 64.2 g L^{-1} (cacao). This variation could be attributed to differences in the pulp composition, which might be also responsible for the quality and quantity of volatile compounds in the final alcoholic beverages.

Acknowledgments

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References

Anocibar Beloqui, A., & Bertrand, A. Study of sulfur compounds in wine: preliminary results. (1995). *Italian Journal of Food Science*. *7*(3), 279–288.

Boidron, J. N., Chatonnet, P., & Pons, M. (1988). Influence du bois sur certaines substances odorantes des vins. *Connaissance de la Vigne et du vin*. 22(4), 275–294.

Clarke, R. J., & Bakker, J. (2004). Volatile components. *Wine Flavour Chemistry* (pp. 120–188). Oxford: Blackwell Publishing Ltd.

Coulter, A. D., Godden, P. W., & Pretorius, I. S. (2004). Succinic acid-how is it formed, what is its effect on titratable acidity, and what factors influence its concentration in wine? *The Australian & New Zealand Wine Industry Journal*. *19*(6), 16−20, 22−25.

Czerny, M., Christlbauer, M., Christlbauer, M., Fischer, A., Granvogl, M., Hammer, M., et al. (2008). Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *European Food Research and Technology*. *228*(2), 265-273.

Dias, D. R., Schwan, R. F., & Lima, L. C. (2003). Metodologia para elaboração de fermentado de cajá (*Spondias mombin* L.). *Ciência e tecnologia de alimentos*. *23*(3), 342−350.

Dias, D. R., Schwan, R. F., Freire, E. S., & Serôdio, R. D. (2007). Elaboration of a fruit wine from cocoa (*Theobroma cacao* L.) pulp. *International Journal of Food Science & Technology*. *42*(3), 319−329.

Duarte, W. F., Dias, D. R., Pereira, G. V. M., Gervásio, I. M., & Schwan, R. F. (2009). Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production. *Journal of industrial microbiology & biotechnology*. *36*(4), 557−569.

Etievant, P. (1991). Wine. In *Volatile Compounds in Foods and Beverages*. New York: Dekker Inc.

Ferreira, V., López, R., & Cacho, J. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*. *80*, 1659–1667.

Fraile, P., Garrido, J., & Ancín, C. (2000). Influence of a Saccharomyces selected strain in the volatile composition of Rosé wines. Evolution during fermentation. *Journal of Agricultural and Food Chemistry*, *48*(5), 1789–1798.

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal Agricultural and Food Chemistry*. *45*(8), 3027–3032.

Lira Júnior, J. S., Musser, S., Melo, A., Maciel, S., Lederman, I. E., & Santos, V. F. (2005). Caracterização física e físico-química de frutos de cajá-umbu (*Spondias* spp.). *Ciência e Tecnologia de Alimentos*. *25*(4), 757−761.

Lubbers, S., Verret, C., & Voilley, A. The effect of glycerol on the perceived aroma of a model wine and white wine. *Lwt-Food Science and Technology*. *34*(4), 262−265.

Mateo, J. J., & Jimenez, M. (2000). Monoterpenes in grape juice and wines. *Journal of Chromatography A*. *881*(1-2), 557−567.

Meilgaard, M. C. (1975). Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. *MBAA Technical Quarterly*. *12*, 151–168.

Miyake, T., & Shibamoto, T. (1993). Quantitative analysis of acetaldehyde in foods and beverages. *Journal of Agricultural and Food Chemistry*. *41*(11), 1968–1970.

Noguerol-Pato, R., González-Barreiro, B., Cancho-grande, B., & Simal-Gándara, B. (2009). Quantitative determination and characterisation of the main odourants of Mencía monovarietal red wines. *Food Chemistry*. *117*(3), 473-484.

Oliveira, J. M., Faria, M., Sá, F., Barros, F., & Araújo, I. M. (2006). C_6-alcohols as varietal markers for assessment of wine origin. *Analytica Chimica Acta*. *563*(1), 300–309.

Peinado, R. A., Moreno, J. A., Muñoz, D., Medina, M., & Moreno, J. (2004). Gas chromatographic quantification of major volatile compounds and polyols in wine by direct injection. *Journal of Agricultural and Food Chemistry*. *52*(21), 6389–6393.

Peña, R. M., Barciela., J., Herrero, C., & García-Martín, S. (2005). Optimization os solid-phase microextraction methods for GC-MS determination of terpenes in wine. *Journal of the Science of Food and Agriculture. 85*(7): 1227−1234 (2005).

Perestrelo, R., Fernandes, A., Albuquerque, F., Marques, J., & Camara, J. (2006). Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. *Analytica Chimica Acta*. *563*(1-2), 154–164.

Plutowska, B., & Wardencki, W. (2008). Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review. *Food Chemistry*. *107*(1), 449−463.

Quijano, C., & Pino, J. (2007). Volatile compounds of copoazú (*Theobroma grandiflorum* Schumann) fruit. *Food Chemistry*, *104*(3), 1123−1126.

Rapp, A., & Mandery, G. (1986). Wine aroma. *Experientia. 42,* 873–884.

Reddy, A., & Reddy, S. (2005). Production and characterization of wine from mango fruit (*Mangifera indica* L). *World Journal of Microbiology & Biotechnology*. *21*(8-9), 1345–1350.

Ribéreau-gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2000). Varietal aroma. *Handbook of enology. The chemistry of wine and stabilization and treatments* (Vol. 2, pp, 187-206). New York: John Wiley and Sons Ltd.

Schwan, R. F., & Wheals, A. E. (2004). The microbiology of cocoa fermentation and its role in chocolate quality. *Critical Reviews in Food Science and Nutrition*. *44*(4), 205–222.

Selli, S., Canbas, A., Varlet, V., Kelebek, H., Prost, C., & Serot, T. (2008). Characterization of the most odor-active volatiles of orange wine made from a Turkish cv. Kozan (Citrus sinensis L. Osbeck). *Journal of agricultural and food chemistry*. *56*(1), 227−34.

Siebert, T. E., Smyth,. H. E, Capone, D. L., Neuwöhoner, C., Pardon, K. H., Skouroumounis, G. K., et al. (2005). Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Analytical and Bioanalytical Chemistry*. *381*(4), 937–947.

Soufleros, E. H., Pissa, P., Petridis, D., Lygerakis, M., Mermelas, K., Boukouvalas, G., & Tsimitakes, E. (2001). Instrumental analysis of volatile and other compounds of Greek kiwi wine, sensory evaluation and optimisation of its composition. *Food Chemistry*. *75*(4), 487−500.

Tešević, V., Nikićević, N., Milosavljević, S., Bajić, D.,Vajs, V., Vučković, I., et al. (2009). Characterization of volatile compounds of "Drenja", an alcoholic beverage obtained from the fruits of cornelian cherry. *Journal of the Serbian Chemical Society*. *74*(2), 117−128.

Wondra, M., & Berovic, M. (2001). Analyses of aroma components of Chardonnay wine fermented by different yeast strains. *Chemical Analysis*. *39*(2), 141−148.

ARTIGO 2 Fermentative behavior of *Saccharomyces* strains during microvinification of raspberry juice (*Rubus ideaus* L.)

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Fermentative behavior of *Saccharomyces* strains during microvinification of raspberry juice (*Rubus ideaus* L.)

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Abstract

Sixteen different strains of *Saccharomyces cerevisiae* and *S. bayanus* were evaluated in the production of raspberry fruit wine. Raspberry juice sugar concentrations were adjusted to 16ºBrix with a sucrose solution, and batch fermentations were performed at 22ºC. Various kinetic parameters, such as the conversion factors of the substrates into ethanol ($Y_{p/s}$), biomass ($Y_{x/s}$), glycerol ($Y_{g/s}$) and acetic acid ($Y_{ac/s}$), the volumetric productivity of ethanol (Q_p), the biomass productivity (P_x), and the fermentation efficiency (E_f) were calculated. Volatile compounds (alcohols, ethyl esters, acetates of higher alcohols and volatile fatty acids) were determined by gas chromatography (GC-FID). The highest values for the E_f, $Y_{p/s}$, $Y_{g/s}$, and $Y_{x/s}$ parameters were obtained when strains commonly used in the fuel ethanol industry (*S. cerevisiae* PE-2, BG, SA, CAT-1, and VR-1) were used to ferment raspberry juice. *S. cerevisiae* strain UFLA FW 15, which was isolated from fruit, displayed similar results. Twenty-one volatile compounds were identified in raspberry wines. The highest concentrations of total volatile compounds were found in wines from *S. cerevisiae* strains UFLA FW 15 (87435 μg/L), CAT-1 (80317.01 μg/L), VR-1 (67573.99 μg/L) and *S. bayanus* strain CBS 1505 (71660.32 μg/L). The highest concentrations of ethyl esters were 454.33 μg/L, 440.33 μg/L and 438 μg/L for *S. cerevisiae* strains UFLA FW 15, VR-1 and BG, respectively. Similar to the concentrations of ethyl esters, the highest concentrations of acetates (1927.67 μg/L) and higher alcohols (83996.33 μg/L) were produced in raspberry wine from *S. cerevisiae* strain UFLA FW 15. The maximum concentration of volatile fatty acids was found in raspberry wine produced by *S. cerevisiae* strain VR-1. We conclude that *S. cerevisiae* strain UFLA FW 15 ferments raspberry juice and produces a fruit wine with low concentrations of acids and high concentrations of acetates, higher alcohols and ethyl esters.

Keywords: *Saccharomyces cerevisiae*; alcoholic beverages; fermentation kinetics; raspberry; volatile compounds.

1. Introduction

Although grapes and apples have been widely applied to ferment beverages, the use of other fruits, such as cajá (Dias et al., 2003), cacao (Dias, et al., 2007), gabiroba (Duarte et al., 2009), kiwi (Soufleros et al., 2001), mango (Kumar et al., 2009; Reddy and Reddy, 2009) and orange (Selli et al., 2008), in the production of wine has been recently demonstrated.

Generally, fruits contain quantities of sugar that can be used by yeast during the fermentation process. In addition to the inherent characteristics of fruit (pH values, sugar contents and nitrogen contents), other factors must be taken into account during fruit wine production. The initial sugar concentrations, fermentation temperatures, SO_2 concentrations and specific yeast strains are key factors in determining successful fermentative processes of fruit wine (Dias et al., 2003, 2007; Duarte et al., 2009, 2010).

The raspberry, *Rubus idaeus* L., (cv Meeker) displays specific acid and sugar contents (pH 3.6 and 14.5 ºBrix) that make it suitable for fruit wine. Raspberries have high concentrations of polyphenolic phytochemicals, particularly flavonoids such as anthocyanin pigments, which give them their characteristic colour. The phytochemicals in raspberries may have significant antioxidant effects that protect against biological oxidations in mammalian cells (Weber and Liu, 2002).

In modern winemaking, specific yeast strains have been preferentially used to guarantee the desired quality of the product. Yeasts are the prominent organisms involved in wine production and determine several characteristics of the wine, including the flavour, by a range of mechanisms and activities (Fleet, 2003).

Since the beginning of the 1980s, the use of *Saccharomyces cerevisiae* yeast starters has been extensively applied in the industrial and homemade beverage production processes. Currently, most of the wine production processes rely on *S. cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations (Valero et al., 2005). Yeast starter cultures that are specifically selected for the winemaking process on the basis of scientifically verified characteristics typically complement and optimise the raw material quality and individual characteristics of the wine, creating a more desirable product (Romano et al., 2003). Generally, wines produced with selected yeasts have a higher quality than wines produced by spontaneous fermentation (Fleet and Heart, 1993).

Some reports described the characterisation of volatile compounds in raspberry fruits (Aprea et al., 2009; Malowicki et al., 2008). However, no published papers demonstrate the use of raspberry juice to produce alcoholic fermented beverages.

To the best of our knowledge, this is the first report that describes the use of raspberries to produce a novel alcoholic fermented beverage and includes the volatile characterisation of the final product.

The aim of this work was to study the fermentation characteristics of sixteen different *Saccharomyces cerevisiae* and *Saccharomyces bayanus* in raspberry juice by analysing the kinetics of fermentation and the volatile composition of the wines . The results of this study will facilitate the selection of yeast strains displaying the best performance in raspberry juice fermentations.

2. Materials and methods

2.1. Raspberry must

The raspberry ripe fruits (Meeker variety) were harvested in April/May of 2009 in the city of Vila Verde, North Portugal (41°39'7.55"N, 8°26'1.06"O). Fifty kilograms was obtained from the harvest in an area of 3 ha. Fruits were stored at 5°C until June/2009. Fruits were washed with tap water to remove residues of the plant, and then the pulp was manually extracted by mechanical pressure. The seeds and residues of the pulp were separated from the juice by centrifugation. In the raspberry pulp, the initial sugar concentration was generally 14.5ºBrix, and the pH was 3.6. The raspberry must was prepared according to the methods of Dias et al. (2007) and Duarte et al. (2009) with minor modifications. The raspberry juice was mixed with a sucrose solution (1:1 v/v) to adjust the sugar concentration to 16ºBrix. $CaCO_3$ was added to increase the pH to 4.0. To inhibit bacterial growth, sulphur dioxide, in the form of potassium metabisulphite, was added at concentrations of 100 mg/L.

2.2. Microorganisms

Fifteen *S. cerevisiae* strains and one *S. bayanus* strain were evaluated (Table 1). All of the yeast strains were from the microbial collection at the Microbial Physiology Laboratory/Department of Biology from the Federal University of Lavras (UFLA), Brazil. Some of the *S. cerevisiae* strains (VR-1, BG, SA, PE-2 and CAT-1) are used in Brazil for ethanol production, and others were isolated from cachaça fermentations, fruit wine fermentations and cassava fermentations (Table 1).

2.3. Inoculum preparation and calibration curves

Yeasts were grown in 250 mL Erlenmeyer flasks containing 100 mL of YPD (10 g/L of yeast extract (Merck), 20 g/L of peptone (Merck) and 20 g/L of glucose (Merck)).for 24 h at 28ºC and 200 rpm. After the 24 h incubation, the yeast cells were centrifuged (*RCF* = 4053) for 5 min at 20ºC and washed twice with sterile peptone water. The biomass obtained was inoculated into 100 mL of raspberry juice and incubated at 28°C for 36 h without agitation. After the incubation, the cells were separated by centrifugation (*RCF* = 4053) for 5 min at

20ºC and washed twice with sterile peptone water. The biomass pellet was re-suspended in 30 mL of sterile peptone water, and 15 mL were used to determine the dry weight at 105°C during 24 h. The remaining 15 mL were used for serial dilutions to determine the absorbance at 600 nm. Calibration curves were built by plotting the absorbance values against the dry weight values. The calibration curves were then used to determine the initial inoculum concentration and to monitor the yeast growth during the fermentation process.

Table 1. Yeasts used in raspberry fruit wine production and their respective sources

Yeast	Source
VR-1	Fermenting sugar cane juice (bioethanol)
PE-2	Fermenting sugar cane juice (bioethanol)
6167 1A	DIPROVAL* – Andrea Caridi
BG	Fermenting sugar cane juice (bioethanol)
UFLA FW 1183	Fermenting fruit must
UFLA FW 1174	Fermenting fruit must
SA	Fermenting sugar cane juice (bioethanol)
UFLA CA 11	Fermenting sugar cane juice (cachaça)
UFLA FW 1185	Fermenting fruit must
UFLA FW 1187	Fermenting fruit must
UFLA CA 155	Fermenting sugar cane juice (cachaça)
UFLA FW 15	Fermenting fruit must
CAT-1	Fermenting sugar cane juice (bioethanol)
UFLA EU 60.1	Fermenting cassava
S. bayanus CBS 1505	DIPROVAL * – Andrea caridi
UFLA FW 1162	Fermenting fruit must

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2.4. Fermentation assays

The inoculum for the fermentations was prepared as described in section 2.3. After measuring the absorbance at 600 nm, the cell suspension volume was adjusted to obtain an inoculum of 1.5 g/L (dry weight) in raspberry must. The inoculum was used to inoculate aseptically 180 mL of raspberry must in 250 mL flasks fitted with side-arm port sealed with a rubber septum, and inocubated at 22°C without agitation. All samples were collected aseptically. The experiment was conducted in duplicate.

2.5. Fermentation monitoring

Samples were collected at 0, 4, 8, 12, 16, 24, 32, and 48 h to determine the concentrations of sugars, acetic acid, glycerol, ethanol, and biomass (dry weight). Fermentation activities were monitored by weight loss as an estimate of CO_2 production.

2.6. Chemical analysis

Ethanol, glycerol, acetic acid, and multiple sugars (sucrose, glucose and fructose) were quantified by high-performance liquid chromatography (HPLC) with a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI), a UV-visible detector (Jasco 870-UV-visible) and a 67H Chrompack column (300 × 6.5 mm). HPLC was performed at 37ºC using sulphuric acid (5 mmol/L) as the eluent at a flow rate of 0.4 mL/min with a sample volume of 20 µL (Duarte et al., 2010).

Volatile compounds were analysed after extraction with dichloromethane according to the methods of Oliveira et al. (2006). The extracts were analysed using the chromatographic conditions proposed by Duarte et al. (2010). Volatile compounds were tentatively identified by comparing the retention times between the compounds and the pure standard compounds. All of the samples were analysed in triplicate.

2.7. Evaluation of fermentation performance

To determine the fermentation performance, μ_{max} (maximum specific growth rate), μ_s (maximum specific rate of substrate consumption), and μ_p (maximum specific rate of product formation) were defined as:

$$[\mu_{max} = (1 / X) \times dX / dt]; [\mu_s = (1 / X) \times dS / dt]; [\mu_p = (1 / X) \times dP / dt]$$

where X represents biomass, S represents substrate and P represents product (ethanol). The derivates were calculated according to the method proposed by Le Duy and Zajic (1973). Besides the parameters that were mentioned previously, the conversion factors of the substrates into ethanol ($Y_{p/s}$), biomass ($Y_{x/s}$), glycerol ($Y_{g/s}$) and acetic acid ($Y_{ac/s}$), the volumetric productivity of ethanol (Q_p), the biomass productivity (P_x), and the conversion efficiency (E_f) were also calculated (Oliveira et al., 2004). The equations used in this work are presented below:

$$[Y_{p/s} = (P_f - P_i) / (S_i - S_f)]; [Y_{x/s} = (X_f - X_i) / (S_i - S_f)]; [Y_{g/s} = (g_f - g_i) / (S_i - S_f)];$$
$$[Y_{ac/s} = (Ac_f - Ac_i) / (S_i - S_f)]; [Q_p = (P_f - P_i) / t_f]; [P_x = (X_f - X_i) / t_f]$$

where P_i is the initial concentration of ethanol, P_f is the ethanol concentration at the of fermentation, S_i is initial substrate concentration, S_f is substrate concentration at the end of fermentation, X_i is initial biomass concentration, X_f is the biomass concentration at the of fermentation, g_i is initial glycerol concentration, g_f is glycerol concentration at the end of fermentation, Ac_i is the initial acetic acid concentration, Ac_f is the concentration of acetic acid at the end of fermentation. t_f is the total time of fermentation.

2.7. Sensory analyses

Raspberry wine produced with yeast UFLA FW 15 was subjected to sensory analyses by trained panelists. The evaluation of beverages by sensory analysis was done using QDA (quantitative descriptive analysis) methodology. During the analysis, the wine tasters indicated different descriptors (aroma) perceived and the intensity of each attribute was rated with a scale from 0 to 9.

2.8. Statistical analysis

Principal component analyses (PCA) were performed using the XLSTAT 7.5.2 software (Addinsoft's, New York, NY, USA). CO_2 production (dCO_2/dt) was calculated using the Origin Pro 8.0 software (OriginLab, Northampton, MA, USA). Analyses of the variance and the Scott-Knott test were performed with SISVAR 5.1 software (Ferreira, 2008).

3. Results and discussion

3.1. Yeast biomass growth and CO_2 production

Fig. 1 shows the growth profile of various yeasts during fermentations of raspberry juice. Based on the maximum biomass produced at the end of the fermentation process, four groups were created for simplicity. The first group contains yeasts showing a final biomass concentration lower than 10.00 g/L (Fig. 1A), while the second group (Fig. 1B) contains yeasts that have a biomass concentration between 10.30 g/L and 10.75 g/L. Final biomass values from 11.59 g/L to 11.91 g/L are depicted in the third yeast group (Fig. 1C), while the fourth group contains yeasts with a final biomass greater than 12 g/L (Fig. 1D).

Yeast strains CAT-1, VR-1 and *S. bayanus* CBS 1505 had the lowest biomass values (below 10 g/L) in the raspberry juice fermentation process (Fig. 1A). *S. cerevisiae* strains UFLA FW 1174, UFLA FW 1162, UFLA FW 1183 and UFLA CA 155 displayed superior growth abilities in raspberry juice as the final biomass concentrations were greater than 12 g/L. *S. cerevisiae* UFLA FW 1183 had the maximum biomass value of 13.40 g/L (Fig. 1D). *S. cerevisiae* UFLA FW 1162 showed lower biomass value that occurred mainly from 16 to 32 h, which indicates a lower fermentation rate at the beginning of the process. Despite this lower fermentation rate, UFLA FW 1162 reached biomass values similar to those obtained with the other yeasts at the end of the fermentative process. The observed differences in the yeast growth can be associated with their abilities to adapt to substrate. According to Ivorra et al. (1999), several factors, which include heat-shock, oxidative and osmotic stress, available nitrogen, and sugar and ethanol concentrations, affect yeast growth during fermentation. Yeasts with lower resistance to these factors have difficulties in the fermentation process, which ultimately leads to a reduction in their growth and survival rates and may result in lower fermentation efficiencies (Querol et al., 2003).

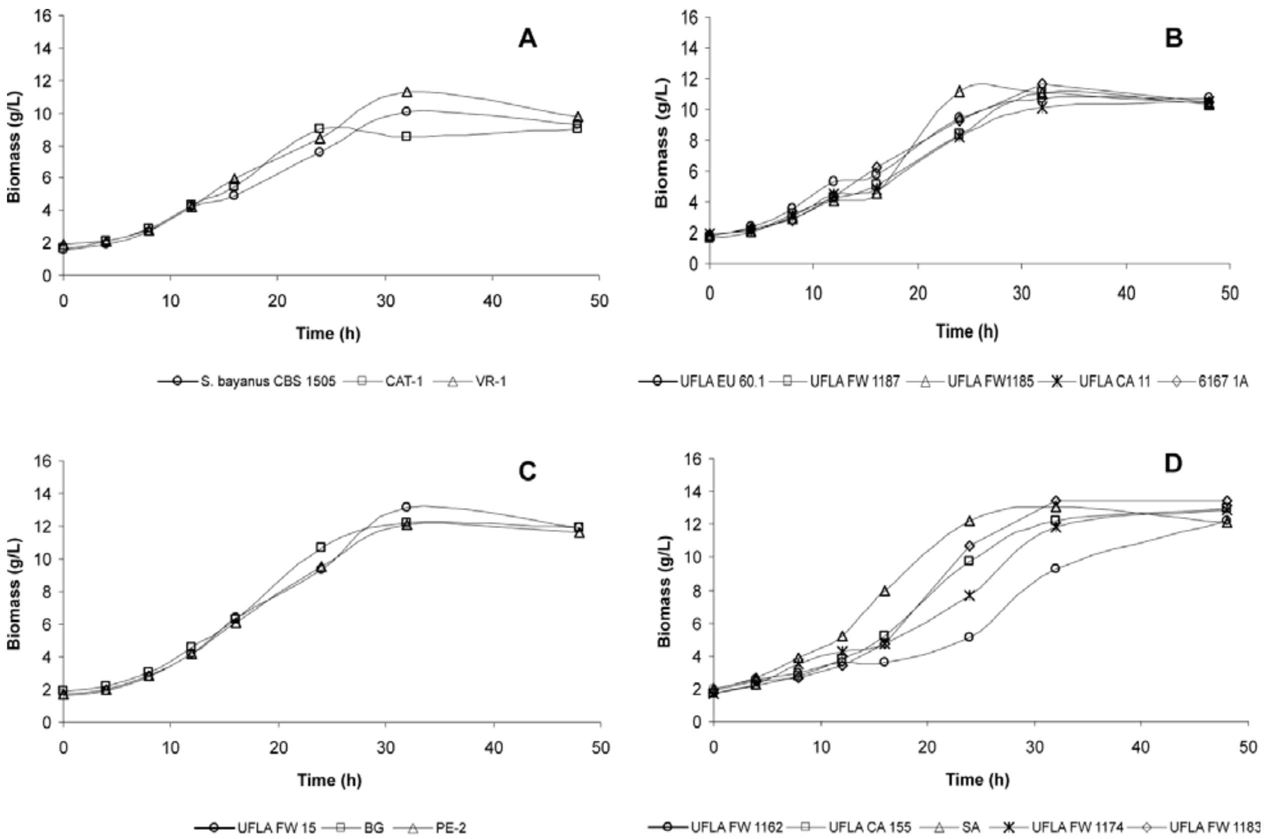

Figure 1. Yeasts biomass production during raspberry must fermentation.
A: Biomass < 10 g/L; **B:** Biomass from 10.30 g/L to 10.75 g/L; **C:** Biomass from 11.59 g/L to 11.91 g/L; **D:** Biomass > 12.00 g/L.

Fermentation monitoring was also based on the production rates of CO_2 (dCO_2/dt). The rates of CO_2 production ranged from 1.02 g/L/h to 2.07 g/L/h. Yeast strains UFLA FW 1162, SA and VR-1 showed distinct behaviours when compared to the other studied yeasts. The maximum CO_2 production rate (dCO_2/dt_{max}) of *S. cerevisiae* UFLA FW 1162 was obtained 24 h after fermentation began (Fig. 2A), which was later than the time points at which the dCO_2/dt_{max} occurred in other yeasts. Based on the two types of fermentation curves (the risk-free fermentation curve and the curve that indicates a risk of stuck fermentation) proposed by Dubois et al. (1996), the profile of CO_2 production displayed by *S. cerevisiae* UFLA FW 1162, which has a long lag phase, resembles a typical curve of stuck fermentation. The curves of the other yeasts are indicative of risk-free fermentations. In Fig. 2B, the SA yeast is distinctive because its dCO_2/dt_{max} occurred before 10 h of fermentation. *S. bayanus* CBS 1505 and *S. cerevisiae* UFLA EU 60.1, CAT-1, UFLA CA 155 and UFLA CA 11 were similar in their dCO_2/dt evolutions and the times that were needed to obtain the dCO_2/dt_{max} (Fig. 2C). Besides having a dCO_2/dt_{max} near 12 h of fermentation, *S. cerevisiae* VR-1 also displayed the highest dCO_2/dt_{max} in comparison to the other yeasts(Fig. 2D). Fermentation monitoring based on CO_2 production is a common practice in wine fermentations. The relationship that exists between the biomass curves and the CO_2 production rates allows the characterisation of three distinct phases in the fermentative process: the lag phase, which is characterised by a small release of CO_2; a second phase in which the maximum population is reached; and a third stationary phase that shows a continuous decrease in cellular activity (Bely et al., 1990).

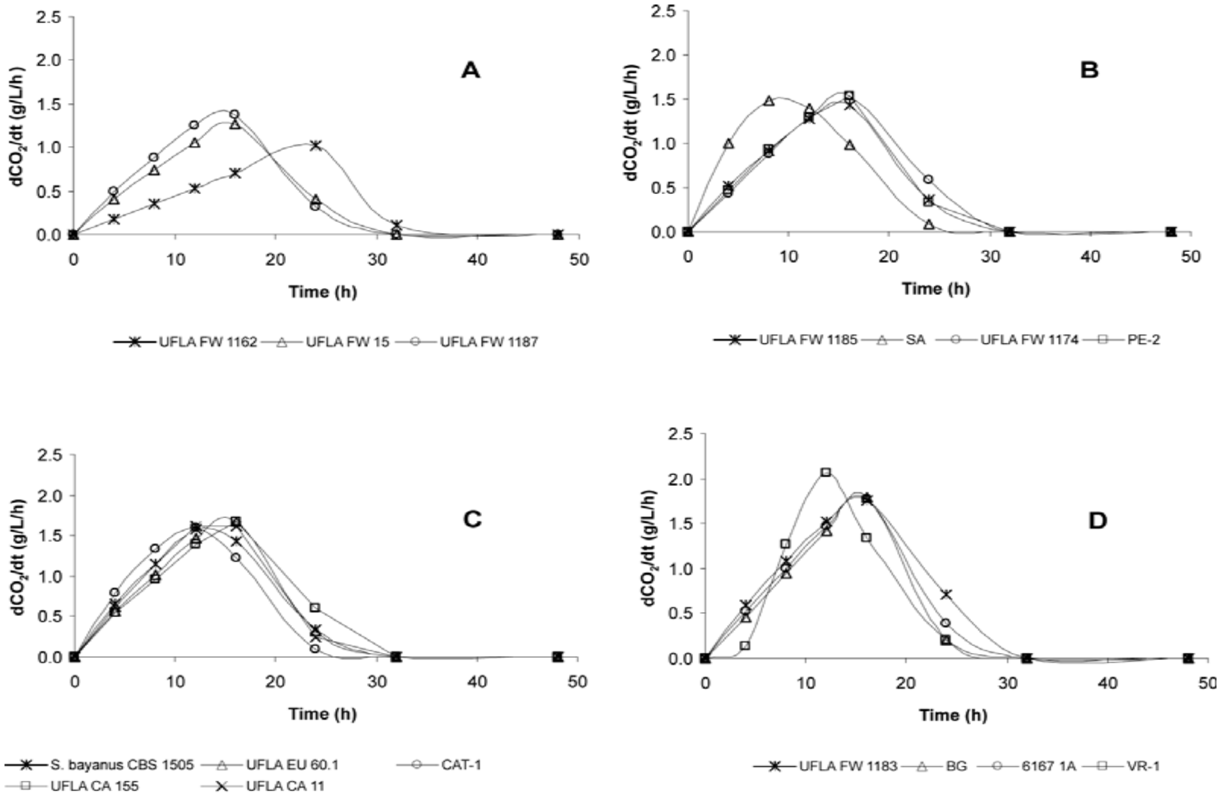

Figure 2. Rates of CO_2 (dCO_2/dt) production by yeast during raspberry must fermentation.
A: dCO_2/dt 1.02 g//L/h to 1.38 g//L/h; **B:** dCO_2/dt 1.44 g//L/h to 1.53 g//L/h; **C:** dCO_2/dt 1.57 g//L/h to 1.67 g//L/h; **D:** dCO_2/dt 1.76 g//L/h to 2.07 g//L/h.

3.2. Kinetic parameters

The maximum specific growth (μ_{max}) was obtained when UFLA FW 15 was used in the fermentation of raspberry juice. μ_{max} 0.119 (h^{-1}) obtained for UFLA FW 15 was close to those obtained for UFLA CA 1183 (0.115 h^{-1}), SA (0.104 h^{-1}), VR-1 (0.103 h^{-1}), UFLA CA 1187 (0.102 h^{-1}) and PE-2, CAT-1, EU 60.1 (0.101 h^{-1}). To maximum specific rates of ethanol production (μ_p) the highest values were 0.982 (h^{-1}) and 0.918 (h^{-1}) for yeast strains CAT-1 and UFLA CA 1174, respectively. The yeast strain UFLA CA 1185 showed the highest value for the maximum specific rates of substrate consumption (μ_s) (2.783 h^{-1}), followed by *S. bayanus* CBS 1505 (2.514 h^{-1}), UFLA FW 15 (2.399 h^{-1}) and CAT-1 (2.376 h^{-1}). The lowest values for μ_{max}, μ_p and μ_s were respectively 0.079 (h^{-1}), 0.453 (h^{-1}) and 1.021 (h^{-1}). These values were obtained when UFLA FW 1162 was used to ferment raspberry juice. . The positive correlation between the μ_{max} and ethanol yields indicates that the selection of yeast strains with high μ_{max} values will also result in high yields of ethanol (Oliveira et al., 2004).

The $Y_{p/s}$, $Y_{x/s}$, $Y_{g/s}$, $Y_{ac/s}$, and E_f parameters and the substrate conversions were grouped in Table 2. The values ranged from very low to very high based on the classifications that were previously proposed by Oliveira et al. (2004) with minor modifications.

In addition to an initially low biomass production (Fig. 1D) and a late dCO_2/dt_{max} (Fig. 2A), UFLA FW 1162 was the only yeast that grouped into the low level parameter "conversion" group in Table 2. All of the other yeasts displayed efficient conversions of the substrates, in which values greater than 99% were classified as very high. Fermentation efficiencies (E_f) ranged from 75.56% to 96.24%. Seven (43.75%) yeast strains were grouped into the high E_f level, 5 (31.25%) strains grouped into the low E_f level, 3 (18.75%) strains grouped into the medium E_f level and only VR-1, which displayed an E_f of

96.24%, was grouped into the very high level (Table 2). With respect to E_f levels, all of the yeast strains isolated from ethanol production grouped into the high and very high levels. Variations in the E_f levels can be justified by the Gay-Lussac equation for alcoholic fermentations, which established that under anaerobic conditions, each kg of glucose consumed produces 0.51 kg of ethanol. However, part of the carbon source is used in the generations of biomass, glycerol and volatile compounds (Soboncan and Glavic, 2000).

Fifty percent of the yeasts tested showed low and medium levels of the conversions of substrates into ethanol ($Y_{p/s}$). The remaining 50% of the yeasts grouped into high and very high $Y_{p/s}$ levels. The VR-1 strain was uniquely classified in the very high level (Table 2). The $Y_{p/s}$ values ranged from 0.38 g/g to 0.49 g/g. These values weresimilar to the values found in the literature about fermentations for cachaça production (Oliveira et al., 2004; Oliveira et al., 2009) and fermentations for ethanol production (Liang et al., 2008; Ribeiro and Horii, 1999). Although some yeast strains displayed low $Y_{p/s}$ values, all of the strains had $Y_{x/s}$ values were greater than 0.044 g/g and were grouped into high and very high levels, which confirms their ability to grow in raspberry juice. The values for the volumetric productivity of ethanol (Q_p) found in this study ranged from 1.156 g/L/h to 1.495 g/L/h for UFLA FW 1162 and 6167 1A strains, respectively.

Table 2. Average ranges of variation for the fermentation parameters levels of the yeast strains.

Parameters	Levels				
	Very low	Low	Medium	High	Very high
E_f (%)		*70.0–81.0* UFLA EU 60.1, UFLA CA 155, UFLA FW 1185, UFLA FW 1162, *S. bayanus* CBS 1505 (5)	*82.0–88.0* UFLA FW 1174, UFLA CA 11, UFLA FW 1187 (3)	*88.1–95.0* PE-2, 6167 1A, BG, UFLA FW 1183, SA, UFLA FW 15, CAT-1 (7)	*95.1–99.5* VR-1 (1)
Conversion (%)	*25.0–60.0*	*60.1–90.0* UFLA FW 1162 (1)	*90.0–97.0*	*97.1–99.0*	*99.1–100.00* VR-1, PE-2, 6167 1A, BG, UFLA FW 1183, UFLA FW 1174, SA, UFLA CA 11, UFLA FW 1185, UFLA FW 1187, UFLA CA 155, UFLA FW 15, CAT-1, UFLA EU 60.1, *S. bayanus* CBS 1505 (15)
$Y_{p/s}$ (g/g)		*0.380–0.419* UFLA CA 11, UFLA FW 1185, UFLA CA 155, UFLA EU 60.1 (4)	*0.420–0.450* UFLA FW 1174, UFLA FW 1187, UFLA FW 1162, *S. bayanus* CBS 1505 (4)	*0.451–0.490* PE-2, 6167 1A, BG, UFLA FW 1183, SA, UFLA FW 15, CAT-1 (7)	*0.491–0.510* VR-1 (1)
$Y_{x/s}$ (g/g)		*0.039–0.040*	*0.041–0.043*	*0.044–0.061* VR-1, UFLA FW 1185, UFLA FW 1187, CAT-1, *S. bayanus* CBS 1505, 6167 1A, UFLA EU 60.1, UFLA CA 11 (8)	*>0.061* PE-2, BG, UFLA FW 1174, UFLA FW 1183, SA, UFLA CA 155, UFLA FW 1162, UFLA FW 15 (8)

Table 2. (continued)

Parameters	Levels				
	Very low	Low	Medium	High	Very high
$Y_{g/s}$ (g/g)		*0.029–0.040* UFLA FW 1162 (1)	*0.041–0.050* 6167 1A, UFLA FW 1185, UFLA FW 1187, UFLA CA 155, UFLA FW 15, CAT-1, UFLA EU 60.1, *S. bayanus* CBS 1505 (8)	*0.051–0.080* VR-1, PE-2, BG, UFLA FW 1183, SA (5)	*0.081–0.120* UFLA FW 1174, UFLA CA 11 (2)

Numbers between brackets correspond to the number of strains in each range

The values for the conversion of substrates into acetic acid ($Y_{ac/s}$) were low and very low for all of the yeastsexcept strain VR-1 (Table 2). The lowest $Y_{ac/s}$ value was 0.003 g/g, while the highest value was 0.016 g/g. The production of acetic acid by the *S. cerevisiae* strains typically used in winemaking significantly varies during the fermentation process from as low as 100 mg/L to as high as 2 g/L (Radler, 1993). The production of high amounts of acetic acid by yeasts may be due to the hydrolysis of acetyl-coA (Zamora, 2008).

The conversion factor of substrates into glycerol ($Y_{g/s}$) ranged from 0.035 g/g to 0.085 g/g. Most of the yeasts had medium (0.041 g/g to 0.050 g/g) and high (0.051 g/g to 0.080 g/g) $Y_{g/s}$ values (Table 2). The maximum $Y_{g/s}$ values from different *S. cerevisiae* strains previously reported by Gomes et al. (2007) was 0.0266 g/g. Glycerol is formed by yeasts at the beginning of the fermentation and is generally produced with the first 50 g of fermented sugars. This period corresponds to the start of the glyceropyruvic fermentation. The only way that yeast can ensure the reoxidation of the $NADH^{+}/H^{+}$ coenzyme is by reducing dihydroxyacetone to glycerol (Ribéreau-Gayon et al., 2006).

The highest $Y_{g/s}$ values found in this work may result from the addition of SO_2 to the raspberry juice. According to Ribéreau-Gayon et al. (2006), SO_2 added to must can combine with the ethanol formed during the beginning of the fermentation and increase the glyceropyruvic fermentation rate and the overall amount of glycerol.

The results obtained from the kinetic parameter measurements were subjected to principal component analyses (PCA). Three initial principal components (PC) accounted for 76.77% of the total initial variance.

Fig. 3 shows the plot of the PCA for the first (PC1) and the second (PC2) principal components, which explains 35.31% and 26.83% of the total variance, respectively.

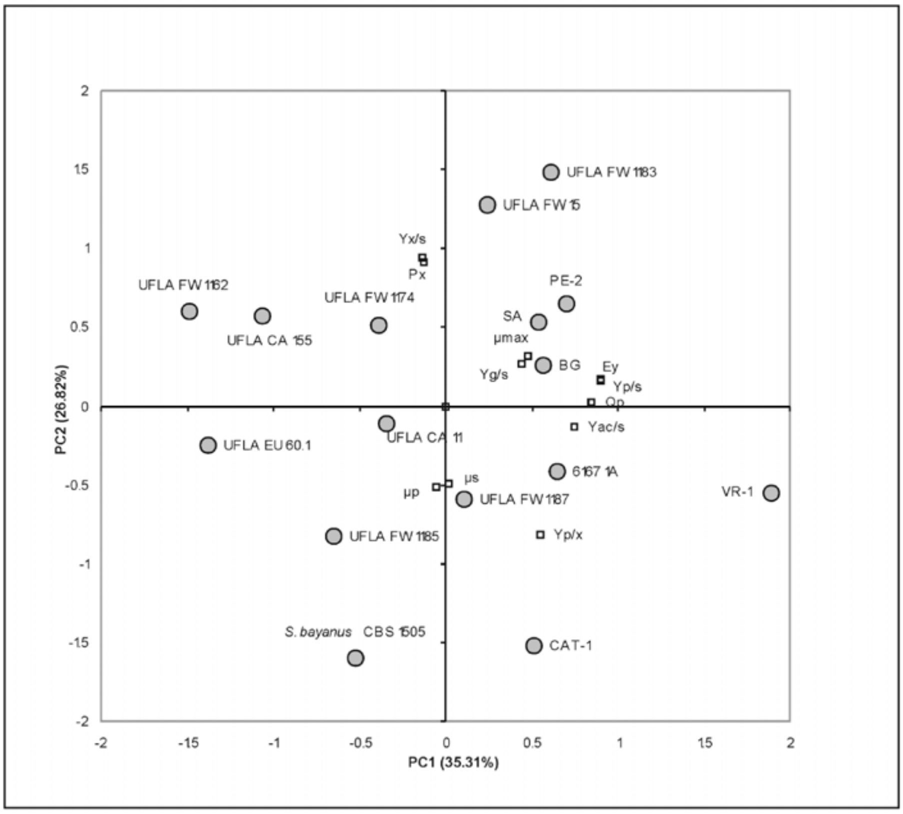

Figure 3. Principal component analysis (PCA) of kinetics parameters in raspberry fermentation.

All the yeast strains from the ethanol industry were grouped on the positive part of PC1. Strains UFLA FW 1183 and UFLA FW 15isolated from fruit fermentations, grouped together with strains SA, PE-2 and BG. These same strains displayed better correlations among the μ_{max}, $Y_{g/s}$, E_f, $Y_{p/s}$ and Q_p parameters (Fig. 3), which indicate that these yeasts are able to grow in raspberry juice (μ_{max}), efficiently convert substrates into ethanol (E_f, $Y_{p/s}$ and Q_p) and efficiently convert substrates into glycerol ($Y_{g/s}$). In the lower right quadrant, strains 6167 1A, UFLA FW 1187, CAT-1 and VR-1 were characterised by their $Y_{ac/s}$, $Y_{p/x}$ and μ_s values (Fig. 3). Yeasts that are typically used for cachaça production characterised by $Y_{ac/s}$ are considered undesirable because the acetic acid may negatively influence the quality of the beverage (Gomes et al., 2007).

Strains UFLA FW 1162, UFLA FW 1174 and UFLA CA 155 (upper left quadrant) formed a group characterised by the $Y_{x/s}$ and P_x (biomass productivity) parameters, which indicates that these strains use higher amounts of substrates for biomass production. This result was confirmed in Fig. 1D, which shows the group of strains with the highest biomass production values found in this work. UFLA EU 60.1, UFLA CA 11, UFLA FW 1185 and *S. bayanus* CBS 1505 were mainly characterised by μ_p (Fig. 3). Table 3 shows the results of ethanol, glycerol, sugars and organic acids identified in raspberry juice and raspberry wines produced by yeasts Residual sucrose was found only in raspberry wine produced by strain UFLA FW 1162; while residual glucose was quantified in raspberry wines obtained with UFLA FW 1174, UFLA CA 11, and UFLA FW 1162 (Table 3). The highest residual sugars concentration measured when UFLA FW 1162 was used to ferment raspberry juice confirms the characteristics of stuck fermentation (Fig. 2A) as proposed by Dubois et al. (1996).

Table 3. Concentration of sugars, organic acids, glycerol and ethanol detected in raspberry must and raspberry wines.

Compounds	Juice	VR-1	PE-2	6167 1A	BG	UFLA FW 1183	UFLA FW 1174	SA	UFLA CA 11
Glycerol	ND	10.11 (0.20)	6.54 (0.12)	5.86 (0..42)	6.80 (0.21)	6.90 (0.35)	4.45 (0.26)	9.02 (0.44)	5.57 (0.51)
Ethanol	ND	71.50 (0.62)	69.34 (0.47)	71.70 (4.32)	71.50 (2.65)	69.77 (5.34)	62.30 (1.89)	68.92 (4.01)	58.92 (2.45)
Succinic acid	3.57 (0.23)	6.20 (0.09)	2.58 (0.19)	7.26 (0.76)	5.79 (0.31)	5.70 (0.23)	6.36 (0.34)	6.00 (0.36)	6.22 (0.32)
Acetic acid	ND	2.27 (0.12)	0.72 (0.21)	0.87 (0.11)	0.71 (0.09)	0.88 (0.05)	0.86 (0.03)	1.03 (0.14)	1.03 (0.04)
Malic acid	2.11 (0.08)	0.61 (0.06)	0.64 (0.08)	1.39 (0.34)	0.67 (0.12)	1.34 (0.07)	0.94 (0.02)	0.38 (0.10)	0.22 (0.00)
Sucrose	72.29 (3.26)	ND	ND	ND	ND	ND	ND	ND	ND
Glucose	36.74 (1.93)	ND	ND	ND	ND	ND	0.40 (0.00)	ND	0.30 (0.06)
Fructose	34,33 (1.36)	1.50 (0.11)	0.34 (0.12)	0.31 (0.12)	0.13 (0.03)	0.41 (0.01)	2.17 (0.19)	0.48 (0.03)	0.92 (0.09)

Table 3. (Continued)

Compounds	**UFLA FW 1185**	**UFLAFW 1187**	**UFLA CA 155**	**UFLA FW 15**	**CAT-1**	**UFLA EU 60.1**	*S. bayanus* **CBS 1505**	**UFLA FW 1162**
Glycerol	7.45 (0.07)	7.74 (0.34)	7.06 (0.17)	7.04 (0.11)	7.04 (0.14)	6.42 (0.10)	6.90 (0.15)	4.68 (0.22)
Ethanol	63.76 (3.12)	70.21 (3.21)	64.26 (1.65)	69.62 (2.71)	66.78 (3.41)	56.94 (0.99)	66.26 (1.57)	55.49 (3.04)
Succinic acid	5.30 (0.18)	6.07 (0.39)	5.16 (0.43)	7.07 (0.81)	5.31 (0.18)	6.13 (0.26)	5.31 (0.33)	5.46 (0.10)
Acetic acid	0.79 (0.04)	1.15 (0.13)	0.52 (0.10)	0.58 (0.09)	0.69 (0.01)	0.43 (0.03)	0.67 (0.09)	0.38 (0.01)
Malic acid	0.77 (0.08)	0.29 (0.04)	0.32 (0.03)	0.62 (0.00)	0.12 (0.02)	0.21 (0.03)	0.11 (0.00)	0.70 (0.11)
Sucrose	ND	ND	ND	ND	ND	ND	ND	10.93 (0.59)
Glucose	ND	ND	ND	ND	ND	ND	ND	6.38 (0.74)
Fructose	0.28 (0.03)	0.34 (0.02)	0.23 (0.03)	0.67 (0.05)	0.27 (0.04)	0.17 (0.01)	1.58 (0.19)	9.78 (0.40)

Numbers between brackets correspond to the standard deviation; ND not detected.

Glycerol concentratation ranged from 4.45 g/L (UFLA FW 1174) to 10.11 g/L (CAT-1). The amounts of glycerol amounts can influence the wine quality. The minimum glycerol concentration in wine is 5 g/L, but it may reach values as high as 15 g/L to 20 g/L. In wine, glycerol affects wine flavour and gives an impression of fullness and softness (Ribéreau-Gayon et al., 2006). Besides the high concentration of glycerol, yeast CAT-1 also showed a high concentration of ethanol (Table 3). Similar amounts of ethanol were measured when yeasts 6167 1A, BG, UFLA FW 1187, UFLA FW 1183, UFLA FW 15, and PE-2 were used in raspberry juice fermentation (Table 3). Although CAT-1 has shown a high ethanol concentration, the highest acetic acid concentration was measured in raspberry wine produced with CAT-1. The high concentration of acetic acid (> 2.0 g/L) was a negative factor for VR-1. Wine containing acetic acid in high concentrations has a pronounced vinegar-like character (Swiegers, et al., 2005).

3.3. Volatile compounds

Twenty-one volatile compounds, which consisted of higher alcohols, ethyl esters, acetates of higher alcohols and volatile fatty acids, were identified and quantified in raspberry wines (Table 4). Alcoholic fermentation leads to the production of ethanol and a series of various by-products, which include carbonyl compounds, alcohols, esters, acids and acetals. All of the by-products potentially influence the quality of the final product. The compositions and concentrations of the by-products may significantly vary in the final product from a few ng/L to hundreds of mg/L (Plutowska and Wardencki, 2008).

Table 4. Concentration of volatile compounds (µg/L) detected in raspberry fruit wines by GC-FID.

Nº	Compounds	VR-1	PE-2	6167 1A	BG	UFLA FW 1183	UFLA FW 1174	SA	UFLA CA 11
	Higher alcohols (7)								
1	1-Propanol	16.87 (3.59)	13.44 (0.22)	231.59 (18.28)	200.48 (18.14)	190.48 (20.73)	459.99 (19.85)	268.78 (0.49)	348.81 (30.64)
2	2-Methyl-1-propanol	30.31 (1.68)	84.25 (3.48)	1245.85 (23.04)	3197.05 (86.27)	2108.38 (580.49)	4846.84 (161.97)	4666.26 (184.63)	2305.89 (115.71)
3	1-Butanol	ND	ND	ND	22.63 (1.7)	12.67 (2.15)	17.45 (1.53)	35.23 (1.92)	38.79 (1.10)
4	2-Methyl-1-butanol + 3-Methyl-1-butanol	39588.04 (588.27)	17821.87 (561.56)	21304.95 (62.17)	32089.42 (416.43)	34736.17 (2326.93)	44231.37 (1019.11)	48296.43 (1259.49)	31914.18 (103.21)
5	3-Methyl-1-pentanol	ND	276.00 (7.43)	288.24 (1.21)	318.45 (7.23)	308.55 (8.70)	246.58 (3.05)	409.40 (0.01)	456.44 (2.18)
6	(*E*)-3-hexen-1-ol	15.30 (3.79)	ND	7.50 (0.17)	11.89 (0.78)	7.99 (1.74)	7.81 (1.38)	ND	10.05 (0.08)
7	2-Phenylethanol	2195.20 (187)	6121.75 (237.95)	8716.8 (22.71)	12379.85 (142.36)	7103.46 (1823.64)	4778.68 (485.35)	8572.72 (314.42)	4371.43 (444.47)
	Ethyl Esters (6)								
8	Ethyl butyrate	30.00 (0.39)	ND	18.56 (3.61)	26.30 (1.63)	55.04 (0.05)	85.51 (11.53)	56.46 (1.47)	65.01 (1.86)
9	Ethyl hexanoate	158.49 (2.83)	112.20 (2.31)	67.22 (2.85)	176.85 (1.15)	119.67 (4.97)	90.74 (0.81)	123.19 (4.94)	114.17 (0.47)
10	Ethyl lactate	8.92 (0.08)	ND	5.81 (0.93)	4.84 (0.47)	9.27 (0.35)	12.02 (1.51)	18.74 (0.60)	10.31 (1.05)
11	Ethyl octanoate	148.74 (1.54)	109.60 (0.30)	50.96 (2.12)	157.47 (4.85)	88.59 (1.70)	67.97 (3.75)	107.34 (1.97)	79.19 (1.69)
12	Ethyl decanoate	21.4 (1.06)	34.91 (3.18)	83.39 (1.87)	14.72 (1.18)	39.52 (8.18)	15.59 (2.57)	11.72 (2.52)	ND

Table 4. (Continued)

Nº	Compounds	UFLA FW 1185	UFLA FW 1187	UFLA CA 155	UFLA FW 15	CAT-1	UFLA EU 60.1	*S. bayanus* CBS 1505	UFLA FW 1162
	Higher alcohols (7)								
1	1-Propanol	335.09 (7.62)	305.56 (50.55)	171.8 (45.40)	509.99 (76.77)	287.26 (35.88)	263.60 (25.40)	299.58 (0.02)	215.08 (13.90)
2	2-Methyl-1-propanol	1771.64 (154.85)	3054.95 (274.99)	1991.1 (358.00)	4703.43 (933.24)	4264.51 (720.66)	2103.40 (188.60)	4110.34 (332.04)	3207.53 (353.00)
3	1-Butanol	25.00 (10.97)	20.70 (8.04)	28.90 (1.70)	38.54 (7.15)	51.33 (6.31)	33.60 (0.40)	23.45 (3.43)	21.41 (2.69)
4	2-Methyl-1-butanol +3-Methyl-1-butanol	43254.8 (155.27)	47391.69 (4619.29)	45669.60 (8119.70)	69853.51 (12577.85)	59682.41 (7922.88)	44644.80 (4141.10)	59065.81 (6206.12)	44586.28 (844.62)
5	3-Methyl-1-pentanol	378.08 (22.81)	312.88 (25.30)	304.70 (40.80)	338.72 (17.17)	314.99 (25.44)	296.20 (15.80)	175.02 (3.02)	274.13 (15.00)
6	(*E*)-3-hexen-1-ol	13.75 (0.38)	16.09 (1.99)	17.40 (2.40)	16.89 (3.17)	ND	9.60 (1.10)	18.58 (0.61)	15.23 (1.07)
7	2-Phenylethanol	5390.64 (238.77)	5366.50 (495.20)	10916.90 (4443.30)	8535.08 (699.17)	13248.44 (178.93)	8455.70 (2579.40)	5306.47 (454.36)	8774.69 (229.86)
	Ethyl Esters (6)								
8	Ethyl butyrate	ND	70.54 (8.26)	61.70 (1.20)	202.57 (128.69)	50.99 (0.96)	59.50 (9.00)	89.99 (3.42)	51.23 (1.20)
9	Ethyl hexanoate	138.54 (1.56)	112.24 (13.42)	107.4 (7.00)	132.28 (4.78)	109.08 (6.52)	99.60 (12.50)	89.13 (4.50)	110.03 (8.24)
10	Ethyl lactate	10.37 (0.33)	6.71 (2.09)	15.60 (4.10)	15.99 (4.04)	7.92 (0.33)	7.90 (1.80)	12.70 (0.64)	10.96 (0.32)
11	Ethyl octanoate	71.85 (0.18)	63.77 (7.32)	81.20 (6.00)	71.05 (3.27)	89.50 (7.12)	58.90 (13.40)	107.44 (1.16)	106.50 (4.18)
12	Ethyl decanoate	ND	11.69 (2.06)	ND	ND	ND	ND	15.92 (4.96)	15.10 (2.88)

Table 4. (Continued)

Nº	Compounds	VR-1	PE-2	6167 1A	BG	UFLA FW 1183	UFLA FW 1174	SA	UFLA CA 11
13	Diethyl succinate	ND	22.61 (4.55)	31.58 (3.51)	57.61 (0.96)	62.13 (20.69)	27.24 (2.53)	45.76 (10.39)	24.16 (0.75)
	Acetates (2)								
14	3-Methylbutyl acetate	591.72 (7.18)	241.92 (3.53)	589.64 (8.34)	653.21 (19.62)	1059.71 (39.73)	1374.81 (42.59)	646.88 (5.64)	472.01 (3.63)
15	2-Phenylethyl acetate	229.92 (6.52)	44.17 (4.54)	87.56 (0.52)	154.36 (0.97)	106.06 (25.74)	26.59 (3.16)	75.19 (2.31)	23.66 (1.46)
	Volatile Acids (6)								
16	Butyric acid	30.10 (4.98)	ND	ND	14.72 (1.18)	ND	17.88 (1.59)	10.17 (2.06)	7.97 (0.00)
17	3-methyl butyric acid	94.57 (0.85)	87.69 (4.11)	ND	85.08 (1.85)	78.30 (19.79)	50.79 (1.74)	59.35 (4.68)	72.42 (7.09)
18	Hexanoic acid	240.35 (17.00)	191.77 (3.35)	115.51 (13.01)	185.68 (1.98)	225.57 (88.19)	137.30 (14.62)	152.42 (7.57)	123 (17.75)
19	Heptanoic acid	24.97 (2.64)	32.99 (2.19)	27.52 (0.13)	23.57 (0.54)	27.81 (12.83)	22.11 (2.20)	13.64 (1.27)	24.45 (2.58)
20	Octanoic acid	1377.95 (73.45)	1060.97 (42.35)	597.72 (5.78)	1051.87 (24.12)	1118.73 (423.13)	593.97 (9.16)	824.89 (1.25)	551.61 (61.63)
21	Decanoic acid	244.51 (5.20)	280.18 (1.20)	122.92 (7.71)	146.49 (11.12)	263.60 (82.32)	203.09 (4.30)	176.04 (0.72)	112.80 (4.29)

Table 4. (Continued)

Nº	Compounds	UFLA FW 1185	UFLA FW 1187	UFLA CA 155	UFLA FW 15	CAT-1	UFLA EU 60.1	*S. bayanus* CBS 1505	UFLA FW 1162
13	Diethyl succinate	59.51 (6.22)	37.67 (2.12)	44.70 (7.70)	32.35 (1.03)	45.36 (2.68)	26.10 (6.40)	25.32 (0.86)	43.20 (7.62)
	Acetates (2)								
14	3-Methylbutyl acetate	557.36 (7.70)	916.89 (69.94)	368.20 (11.00)	1801.77 (35.78)	871.47 (49.90)	623.10 (144.60)	1409.16 (18.50)	903.34 (47.88)
15	2-Phenylethyl acetate	35.98 (2.60)	53.11 (8.76)	104.7 (64.61)	125.76 (2.96	184.46 (10.33)	85.00 (38.50)	20.19 (4.23)	163.01 (5.69)
	Volatile Acids (6)								
16	Butyric acid	25.5 (3.94)	25.75 (0.32)	ND	ND	ND	ND	ND	11.05 (2.74)
17	3-methyl butyric acid	30.14 (5.85)	25.85 (2.39)	29.70 (6.50)	30.61 (0.11)	36.39 (4.10)	37.00 (0.20)	22.92 (0.63)	27.69 (7.08)
18	Hexanoic acid	122.43 (0.43)	122.02 (3.18)	129.90 (27.60)	128.55 (12.67)	110.50 (10.83)	67.80 (38.30)	137.58 (10.52)	97.97 (7.07)
19	Heptanoic acid	18.79 (1.54)	15.95 (0.54)	19.00 (3.40)	15.80 (1.97)	18.62 (3.39)	13.50 (2.90)	11.64 (1.01)	18.26 (0.88)
20	Octanoic acid	689.4 (28.72)	686.78 (25.04)	876.9 (90.20)	766.23 (9.35)	882.77 (87.07)	730.50 (52.00)	675.51 (22.79)	810.05 (26.51)
21	Decanoic acid	199.77 (8.22)	134.75 (7.26)	215.50 (82.60)	115.40 (25.76)	61.77 (2.42)	36.90 (1.10)	43.85 (13.19)	181.99 (10.41)

Numbers between brackets correspond to the standard deviation; ND not detected.

The results attained for the volatile compounds shown in Table 4 were used in principal component analyses (PCA). Three initial principal components (PCs) accounted for 65.88% of the total variance. The first and second PCs explained 36.49% (PC1) and 20.10% (PC2) of the variance, respectively (Fig. 4).

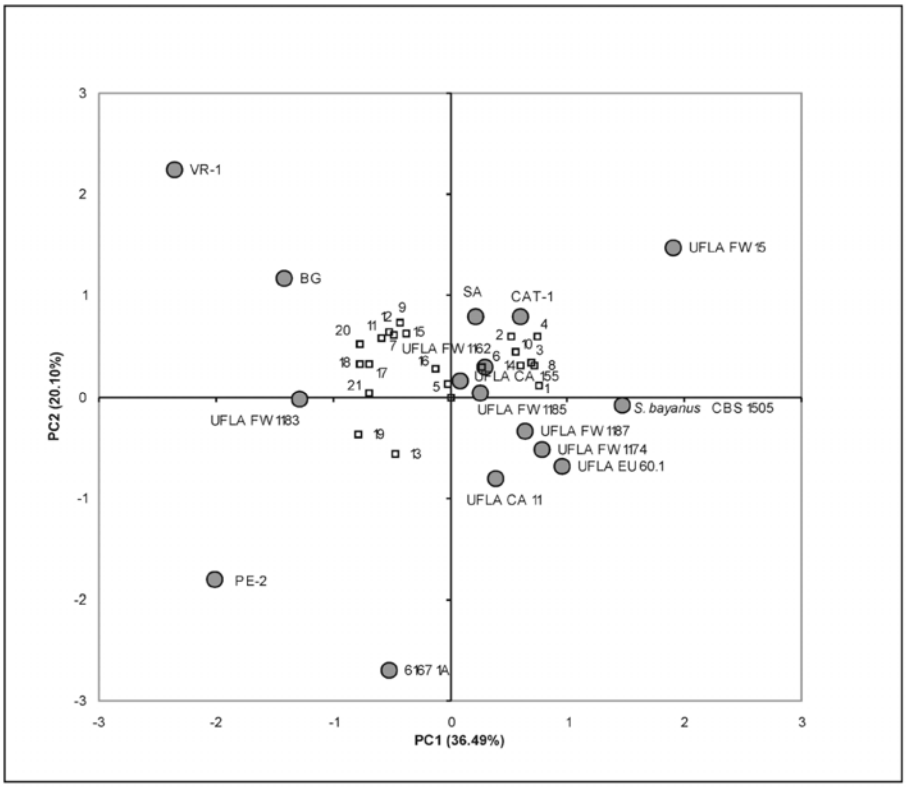

Figure 4. Principal component analysis (PCA) of volatile compounds in raspberry fruit wine. The volatile compounds numbers are referred in Table 3.

As seen in Fig. 4, all the yeasts used in the bioethanol industry, except for strain PE-2, grouped on the positive part of PC2. Yeast strains UFLA FW 1162, UFLA FW 1185, UFLA FW 15, and UFLA CA 155 also grouped on the positive part of PC2. These strains correlated with the production of 2-methyl-1-butanol+3-methyl-1-butanol, 1-butanol, 2-methyl-1-propanol, 1-propanol, (*E*)-3-hexen-1-ol, ethyl butyrate, and 3-methylbutyl acetate (Fig. 4). However, strains BG and VR-1 were primarily characterised by the production of ethyl hexanoate, 2-phenylethanol, ethyl octanoate, 2-phenylethyl acetate, butyric acid, 3-methyl butyric acid, hexanoic acid, octanoic acid, and decanoic acid. In the lower left quadrant, UFLA FW 1183, PE-2 and 6167 1A were only associated with diethyl succinate and heptanoic acid. As seen in Fig. 4, *S. cerevisiae* UFLA FW 1162, UFLA FW 15, UFLA CA 155, SA and CAT-1 were characterised by the production of volatile compounds, including alcohols, acetates and ethyl esters. The BG and VR-1 strains were mainly related to acids, and the raspberry wine produced with these yeasts displayed an overall lower quality than the wine produced with other strains. High levels of volatile acids, such as butyric and isobutyric acid (2-methyl-1-propanoic) may lower the acceptance of the wine because these compounds have a negative effect on the sensory characteristics of the wine (Nikolaou et al., 2006).

To evaluate the global levels of volatile compounds by chemical groups, Table 5 was constructed using the data from Table 4. The raspberry wine produced by UFLA FW 15 strain (454.33 μg/L) contained the highest amounts of ethyl esters (Table 5). However, no significant differences (Scoot-Knott $p<0.05$) were found between the raspberry wines produced with VR-1, BG and UFLA FW 15 yeast strains. Raspberry wines produced by *S. cerevisiae* CAT-1, PE-2, 6167 1A, UFLA CA 1162 , UFLA EU 60.1, UFLA CA 11, UFLA CA 155, UFLA FW 1174, UFLA FW 1187, and *S. bayanus* CBS 1505 had the lowest concentrations of ethyl esters (Table 5). The production of esters by yeasts during the fermentation significantly affects the “fruity” flavours of the wines (Swiergers et al., 2005). Ethyl esters of fatty acids (ethyl butyrate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) are enzymatically produced in wines during the fermentation process and from the ethanolysis of acylCoA formed during the synthesis or degradation of fatty acids (Perestrelo et al., 2006). Among the ethyl esters identified in this work, ethyl butyrate was found in the highest concentration (202.57 μg/L) in the raspberry wine produced with UFLA FW 15 strain (Table 4). The presence of ethyl butyrate may be related to various aroma descriptors, which include “papaya”, “butter”, “sweetish”, “apple”, and “perfumed” (Meilgaard, 1975).

Two acetates of higher alcohols, 3-methylbutyl acetate and 2-phenylethyl acetate, were identified in raspberry wines (Table 4). 3-methylbutyl acetate and 2-phenylethyl acetate give “banana” and “flowery” nuances to the wine (Siebert et al., 2005). The maximum concentration of the acetates, 1927.67 μg/L, was found in the raspberry wine fermented by the UFLA FW 15 strain, while the lowest concentration was found in the raspberry wine fermented by the PE-2 strain. Acetates are formed by the reaction of acetylCoA with higher alcohols in the presence of alcohol acetyltransferase (Yoshioka and Hashimoto, 1981). The activity of alcohol acetyltransferase is widely variable based on the specific strain (Fujii et al., 1996).

The concentration of higher alcohols in the raspberry wines ranged from 25196.33 μg/L (strain PE-2) to 83996.33 μg/L (strain UFLA FW 15) (Table 5) and amyl alcohols (2-methyl-1-butanol+3-methyl-1-butanol) were found in the highest concentrations (Table 4). Higher alcohols, such as amyl alcohols, have aromatic descriptions of “alcoholic”, “sweet” and “choking” and may negatively affect the wine aroma when present in high concentrations. Alcohols like 2-phenylethanol have aromatic descriptions of “rose-like”, “sweet” and “perfume-like” and can positively influence the wine aroma (Falqué et al., 2001).

Table 5. Averages of volatile compounds(µg/L) by chemical groups present in raspberry wines fermented by different yeasts

Total compounds	**VR-1**	**PE-2**	**6167 1A**	**BG**	**UFLA FW 1183**	**UFLA FW 1174**	**SA**	**UFLA CA 11**
Ethyl esters	440.33^{c}	307.67^{a}	257.33^{a}	438.00^{c}	374.00^{b}	299.00^{a}	363.33^{b}	293.00^{a}
Acetates	821.33^{e}	286.00^{a}	677.33^{d}	807.67^{e}	1166.00^{h}	1401.67^{i}	722.33^{d}	495.67^{b}
Volatile acids	2012.33^{c}	1653.67^{b}	863.67^{a}	1492.67^{b}	1714.00^{b}	1025.00^{a}	1236.33^{a}	892.33^{a}
Higher alcohols	64299.00^{c}	25196.33^{a}	31795.00^{a}	48219.67^{b}	44467.67^{b}	54588.67^{c}	62248.67^{c}	39445.33^{b}
Total volatile compounds	67573.99^{d}	27443.67^{a}	33593.33^{a}	50958.01^{b}	47721.67^{b}	57314.34^{c}	64570.66^{d}	41126.33^{b}

Values identified by the same letters are not significantly different at the 0.05 level (Scott-Knott test).

Table 5. (Continued)

Total compounds	**UFLA FW 1185**	**UFLA FW 1187**	**UFLA CA 155**	**UFLA FW 15**	**CAT-1**	**UFLA EU 60.1**	*S. bayanus* **CBS 1505**	**UFLA FW 1162**
Ethyl esters	365.00^{b}	302.67^{a}	310.67^{a}	454.33^{c}	302.67^{a}	252.00^{a}	340.33^{a}	337.00^{a}
Acetates	593.00^{c}	969.67^{f}	472.67^{b}	1927.67^{j}	1055.67^{g}	708.00^{d}	1429.33^{i}	1066.33^{g}
Volatile acids	1086.00^{a}	1011.00^{a}	1271.00^{a}	1056.67^{a}	1110.00^{a}	885.67^{a}	891.33^{a}	1147.00^{a}
Higher alcohols	51169.33^{b}	56468.67^{c}	59100.33^{c}	83996.33^{e}	77848.67^{e}	55807.00^{c}	68999.33^{d}	57094.33^{c}
Total volatile compounds	53213.33^{b}	58752.01^{c}	61154.67^{c}	87435.00^{e}	80317.01^{e}	57653.67^{c}	71660.32^{d}	59644.63^{c}

Values identified by the same letters are not significantly different at the 0.05 level (Scott-Knott test).

The raspberry wine produced by the UFLA FW 15 strain had the highest concentrations of ethyl esters, acetates and alcohols (Table 5). On the contrary, the raspberry wine fermented by the PE-2 strain contained lower concentrations of ethyl esters, acetates and alcohols. According to Torrens et al. (2008), wines produced with yeast strains containing low amounts of esters, higher alcohols and six-carbon alcohols were not well appreciated by consumers. With the exception of the raspberry wine produced by UFLA FW 1183 strain, the other raspberry wines produced with yeasts isolated from fruit fermentations showed low concentrations (Scott-Knott test – letter **a**) of volatile acids (Table 5). Three of the five strains used in the ethanol industry (VR-1, PE-2 and BG) showed the highest concentrations of acids (Scott-Knott test – letters **b** and **c**). The highest concentration of volatile fatty acids found in all raspberry wines was 2012.33 μg/L (strain VR-1), and the most abundant volatile fatty acids were hexanoic, octanoic, and decanoic (Table 4). The presence of high concentrations of acids may negatively influence the qualities of the wines because hexanoic acid has aroma descriptors that include “cheese” and “sweaty”, octanoic acid has aroma descriptors that include “rancid” and “harsh” and decanoic acid has aroma descriptors that include “fatty” (Siebert et al., 2005). Octanoic and decanoic acids can also lead to the inhibition and arrest of fermentation, which ultimately blocks the complete transformation of sugars present in the must (Lanfon-Lafoucarde et al., 1984).

The highest concentrations of total volatile compounds were found in the raspberry wines produced by the CAT-1 and UFLA FW 15 strains (Table 5). The wine made with the VR-1 strain displayed a high concentration of all volatile compounds, but acids were observed in the highest amounts (Scott-Knott test – letter **c**), which is undesirable (Table 5). The concentrations of total volatile compounds present in the wine produced by the *S. bayanus* CBS 1505 werenot different from the concentrations found in the wine produced by VR-1 strain. However, one of the lowest acid concentrations found in this study was obtained from raspberry wine fermented by *S. bayanus* CBS 1505 (Table 5).

3.4. Sensorial analysis

Twelve aromatic descriptors were identified in raspberry wine produced by yeast UFLA FW 15 (Table 6).

Table 6. Frequency, intensity, of descriptors for raspberry produced by strain UFLA FW 15

Descriptors	I (%)	F (%)
Olfactory intensity	60.2	75.0
Olfactory consistency	41.7	75.0
Herbaceous	23.1	50.0
Medicinal	3.7	8.3
Blackberry	26.8	50.0
Floral	8.3	16.6
Tropical	4.6	8.3
Pineapple	2.8	8.3
Tangerine	2.8	8.3
Dried fruit	2.8	8.3
Red fruit	37.9	58.3
Yogurt	4.6	8.3
Resin	3.7	8.3
Balsamic	4.6	8.3
Sulfide	0.0	0.0
Overall	37.9	75.0

I = intensity; F = frequency.

Among the aromatic descriptors identified in raspberry wine fermented by UFLA FW 15, six descriptors were fruity descriptors (*blackberry, tropical fruit, pineapple, mandarin, dried fruit and red fruit*). Some of these descriptors are also found for grape wine, and some of them may be associated to some volatile compounds; among them ethyl butyrate (*blackberry, pineapple, apple, papaya*) may contribute to a good quality of raspberry wine produced using yeast UFLA FW 15.The most frequent aromatic descriptors found were red fruit (37.9%) and blackberry (26.8%) (Table 6).

4. Conclusions

The yeast strains evaluated in this study showed significant differences in the profiles of fermentation kinetics and in the production of volatile compounds during microvinifications of the raspberry juice. In general, yeasts used in the ethanol industry were characterised by high kinetic parameter values, which are related to ethanol production (Q_p, $Y_{p/s}$ and E_f). High concentrations of total volatile compounds were found in raspberry wines produced with UFLA FW 15, *S. bayanus* CBS 1505, CAT-1, SA and VR-1 strains. Despite the high concentrations of total volatile compounds, the raspberry wine produced with VR-1 strain also had the highest concentrations of acids, which can negatively influence the wine quality. Yeast strain UFLA FW 15 showed higher concentrations of desirable compounds, specifically ethyl esters, higher alcohols and acetates. In addition, raspberry wine made with this strain had low concentrations of acids. The raspberry wine obtained with UFLA FW 15 showed good descriptors as *raspberry, cherry, sweet, strawberry*. From the results obtained in this study, we conclude that *S. cerevisiae* strain UFLA FW 15 is the most suitable yeast strain for the production of raspberry wine using the conditions employed in this study. Besides displaying adequate fermentation kinetic parameters, strain UFLA FW 15 also produced a raspberry wine with high concentrations of ethyl esters, acetates and alcohols and low concentrations of acids. These results set a precedence for the large scale production and characterisation (chemical and sensory) of a new fruit wine made from raspberries using a selected *S. cerevisiae* strain and provides a new industrial outlet for raspberry fruits.

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References

Aprea, E., Biasioli, F., Carlin, S., Endrizzi, I., Gasperi, F., 2009. Investigation of volatile compounds in two raspberry cultivars by two headspace techniques: Solid-Phase Microextraction/Gas Chromatography-Mass Spectrometry (SPME/GC-MS) and Proton-Transfer Reaction-Mass Spectrometry (PTR-MS). Journal of Agricultural and Food Chemistry 57, 4011−4018.

Bely, M., Sablayrolles, J.M., Barre, P., 1990. Description of alcoholic fermentation kinetics: its variability and significance. American Journal of Enology and Viticulture 41, 319−324.

Dias, D.R., Schwan, R.F., Lima, L.C., 2003. Metodologia para elaboração de fermentado de cajá (*Spondias mombin* L.). Ciência e Tecnologia de Alimentos 23, 342−350.

Dias, D.R., Schwan, R.F., Freire, E.S., Serôdio, R.D., 2007. Elaboration of a fruit wine from cocoa (*Theobroma cacao* L.) pulp. International Journal of Food Science & Technology 42, 319−329.

Duarte, W.F., Dias, D.R., Oliveira, J.M., Teixeira, J.A., Silva, J.B.A., Schwan, R.F., 2010. Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jabuticaba and umbu. LWT Food Science and Technology doi:10.1016/j.lwt.2010.03.010.

Duarte, W.F., Dias, D.R., Pereira, G.V.M., Gervásio, I.M., Schwan, R.F., 2009. Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production. Journal of Industrial Microbiology & Biotechnology 36, 557−569.

Dubois, C., Manginot, C., Roustan, J.L., Sablayrolles, J.M., Barre, P., 1996. Effect of variety, year, and grape maturity on the kinetics of alcoholic fermentation. American Journal of Enology and Viticulture 47, 363−368.

Falqué, E., Fernández, E., Dubourdieu, D., 2001. Differentiation of white wines by their aromatic index. Talanta 54, 271−281.

Ferreira, D.F., 2008. SISVAR: um programa para análise e ensino de estatística. Revista Symposium 6, 36−41.

Fleet, G.H., 2003. Yeast interaction and wine flavour. International Journal of Food Microbiology 86, 11−22.

Fleet, G.H., Heard, G.M., 1993. Yeast-growth during fermentation. In: Fleet, G.H. (Ed.) Wine Microbiology and Biotechnology. Chur: Harwood Academic Publishers, pp. 27-54.

Fujii, T., Yoshimoto, H., Tamai, Y., 1996. Acetate ester production by *Saccharomyces cerevisiae* lacking the ATF1 gene encoding the alcohol acetyltransferase. Journal of Fermentation and Bioengineering 81, 538−542.

Gomes, F.C.O., Silva, C.L.C., Marini, M.M., Oliveira, E.S., Rosa, C.A., 2007. Use of selected indigenous *Saccharomyces cerevisiae* strains for the production of the traditional cachaça in Brazil. Journal of Applied Microbiology 103, 2438−2447.

Ivorra, C., Pérez-Ortín, J.E., Olmo, M. del., 1999. An inverse correlation between stress resistance and stuck fermentations in wine yeasts. A molecular study. Biotechnology and Bioengineering 64, 698−708.

Kumar, Y.S., Prakasam, R.S., Reddy, O.V.S., 2009. Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology. International Journal of Food Science & Technology 44, 2320–2327.

Lafon-Lafourcade, S., Geneix, C., Ribereau-Gayon, P., 1984. Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast ghosts. Applied and Environmental Microbiology 47, 1246−1249.

Le Duy, A., Zajic, J.E., 1973. A geometrical approach for differentiation of an experimental function at a point applied to growth and product formation. Biotechnology and Bioengineering 15, 805−810.

Liang, L., Zhang, Y., Zhang., L., Zhu, M., Liang, S., Huang, Y., 2008. Study of sugarcane pieces as yeast supports for ethanol production from sugarcane juice and molasses. Journal of Industrial Microbiology and Biotechnology 35, 1605−1613.

Malowicki, S.M.M., Martin, R., Qian, M.C., 2008. Volatile composition in raspberry cultivars grown in the Pacific Northwest determined by stir bar sorptive extraction – gas chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry 56, 4128−4133.

Meilgaard, M.C., 1975. Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. MBAA Technical Quarterly 12, 151–168.

Nikolaou, E., Soufleros E.H., Bouloumpasi, E., Tzanetakis, N., 2006. Selection of indigenous *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results. Food Microbiology 23, 205−211.

Oliveira, E.S., Rosa, C.A., Morgano, M.A., Serra, G.E., 2004. Fermentation characteristics as criteria for selection of cachaça yeast. World Journal of Microbiology and Biotechnology 20, 19−24.

Oliveira, J.M., Faria, M., Sá, F., Barros, F., Araújo, I.M., 2006. C_6-alcohols as varietal markers for assessment of wine origin. Analytica Chimica Acta 563, 300–309.

Perestrelo, R., Fernandes, A., Albuquerque, F., Marques, J., Camara, J., 2006. Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. Analytica Chimica Acta 563, 154–164.

Plutowska, B., Wardencki, W., 2008. Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review. Food Chemistry 107, 449−463.

Querol, A., Férnandez-Espinar, M.T., Olmo, M. del., Barrio, E., 2003. Adaptive evolution of wine yeast. International Journal of Food Microbiology 86, 3−10.

Radler, F., 1993. Yeast: Metabolism of organic acids. In: Fleet, G.H. (Ed.) Wine Microbiology and Biotechnology. Chur: Harwood Academic Publishers, pp. 165-182.

Reddy, L.V.A., Reddy, V.S., 2009. Effect of enzymatic maceration on syntesis of higher alcohols during mango wine fermentation. Journal of Food Quality 32, 34−47.

Ribeiro, C.A.F., Horri, J., 1999. Potencialidades de linhagens de leveduras *Saccharomyces cerevisiae* para fermentação do caldo cana. Scientia Agricola 56, 255−263.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., 2006. Alcohols and other volatile compounds. In: Ribéreau-gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (Eds.) Handbook of enology. The chemistry of wine and stabilization and treatments, vol. 2. England: John Wiley and Sons Ltd, 51-61.

Romano, P., Fiore, C., Paraggio, M., Caruso, M., Capece, A., 2003. Function of yeast species and strains in wine flavour. International Journal of Food Microbiology 86, 169−180.

Selli, S., Canbas, A., Varlet, V., Kelebek, H., Prost, C., Serot, T., 2008. Characterization of the most odor-active volatiles orange wine made from a Turkish cv. Kozan (*Citrus sinensis* L. Osbeck). Journal of Agricultural and Food Chemistry 56, 227−234.

Siebert, T.E., Smyth,. H.E, Capone, D.L., Neuwöhoner, C., Pardon, K.H., Skouroumounis, G. K., Herderich, M. J., Sefton, M. A., Pollnitz, A. P., 2005. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. Analytical and Bioanalytical Chemistry 381, 937–947.

Silva, C.L.C, Vianna, C.R., Cadete, R.M., Santos, R.O., Gomes, F.C.O., Oliveira, E.S., Rosa, C.A., 2009. Selection, growth, and chemo-sensory evaluation of flocculent starter culture strains of *Saccharomyces cerevisiae* in the large-scale production of traditional brazilian cachaça. International Journal of Food Microbiology 131, 203−210.

Sobocan, G., Glavic, P., 2000. Optimization of ethanol fermentation process design. Applied Thermal Engineering 20, 529−543.

Soufleros, E.H., Pissa, P., Petridis, D., Lygerakis, M., Mermelas, K., Boukouvalas, G., Tsimitakes, E., 2001. Instrumental analysis of volatile and other compounds of Greek kiwi wine, sensory evaluation and optimisation of its composition Food Chemistry 75, 487−500.

Swiegers, J.H., Bartowsky, E.J., Henschke, P.A., Pretorius, I.S., 2005. Yeast and bacterial modulation of wine aroma and flavour. Australian Journal of Grape and Wine Research 11 139−173.

Torrens, J., Urpí, P., Riu-Aumatell, M., Vichi, S., López-Tamames, E., Buxaderas, S., 2008. Different commercial yeast strain affecting the volatile and sensory profile of cava base wine. International Journal of Food Microbiology 124, 48−57.

Valero, E., Schuller, D., Cambon, B., Casal, M., Dequin, S., 2005. Dissemination and survival of commercial wine yeast in the vineyard: A large-scale, three-years study. FEMS Yeast Research 5, 959−969.

Weber, C., Liu, R.H., 2002. Antioxidant capacity and anticancer properties of red raspberry. In The 8th International Rubus and Ribes Symposium. Acta Horticulturae 585, 451–455.

Yoshioka, K., Hashimoto, N., 1981. Ester formation by alcohol acetyltransferase from brewers yeast. Agricultural and Biological Chemistry 45, 2183−2190.

Zamora, F., 2008. Biochemistry of alcoholic fermentation. Part I Chemical and Biochemical aspects of winemaking. In: Moreno-Arribas, M.V., Polo, M. C. (Eds.) Wine Chemistry and Biochemistry. New York: Springer, 3-26.

ARTIGO 3 Raspberry (*Rubus idaeus* L.) wine: yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds

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Raspberry (*Rubus idaeus* L.) wine: yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds

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Abstract

To evaluate the potential for fermentation of raspberry pulp, sixteen yeast strains (*S. cerevisiae* and *S. bayanus*) were studied. Volatile compounds were determined by GC−MS, GC−FID, and GC−PFPD. Ethanol, glycerol and organic acids were determined by HPLC. HPLC−DAD was used to analyse phenolic acids. Sensory analysis was performed by trained panellists. After a screening step, CAT-1, UFLA FW 15 and *S. bayanus* CBS 1505 were pre-selected based on the profile of metabolites identified. The beverage produced with CAT-1 showed the highest volatile fatty acid concentration (1542.6 µg/L), whereas the beverage produced with UFLA FW 15 showed the highest concentration of acetates (2211.1 µg/L) and total volatile compounds (5835 µg/L). For volatile sulphur compounds, 566.5 µg/L were found in the beverage produced with *S. bayanus* CBS 1505. The lowest concentration of volatile sulphur compounds (151.9 µg/L) was found for the beverage produced with UFLA FW 15. In the sensory analysis, the beverage produced with UFLA FW 15 was characterised by the descriptors *raspberry, cherry, sweet, strawberry, floral* and *violet*. In conclusion, strain UFLA FW 15 was the yeast that produced a raspberry wine with a good chemical e sensory quality.

Keywords: fruit wine; *Saccharomyces*; fermentation biotechnology; alcoholic beverages; volatile compounds.

1. Introduction

The production of wine from fruits other than grapes has increased in recent years. Apples and oranges have been widely used, but several other fruits have the potential for use in wine production and a number of researchers have found other suitable fruits for wine production. Over the years, fruit wines have been prepared from several different fruits, such as kiwi (Soufleros et al., 2001), banana (Akubor, Obio, Nwadomere & Obiomah, 2003), cajá (Dias, Schwan and Lima, 2003), cocoa (Dias, Schwan, Freire & Sêrodio, 2007), mango (Kumar, Prakasam and Reddy, 2009), gabiroba (Duarte, Dias, Pereira, Gervársio & Schwan, 2009), and cupuassu (Duarte, Dias, Oliveira, Teixeira, Silva & Schwan, 2010). Raspberries, *Rubus idaeus* L., present high polyphenolic phytochemicals, particularly flavonoids such as anthocyanin pigments, which give raspberries their characteristic colour. The phytochemicals in raspberries may have a significant antioxidant activity and may act as a protectant against biological oxidative stress in mammalian cells (Weber and Liu, 2002). Phenolic acids, such as *p*-coumaric, caffeic, ferulic and ellagic acids, are commonly found in raspberries (Häkkinen, Heinonen, Kärenlampi, Mykkänen, Ruuskanen & Törrönen, 1999). The 'Meeker' raspberry variety is popular due to high yields, a long harvest season, resistance to root rot, and machine harvest characteristics. This 'Meeker' fruit has a desirable colour, firm texture, and good sensorial attributes including aroma, sweetness, and acidity (Malowicki, Martin & Qian, 2008). Raspberry fruits that have no standards for "in natura" consumption are used in the production of juices, jam, and sweets; however, in some regions *e.g.*, Campos do Jordão - Brazil, raspberry producers are looking for new alternatives for the use of small and crushed raspberry fruits.

The fermentation process for elaboration of the beverage depends on the performance of yeast to convert sugars into alcohol, esters, and other volatile and non-volatile compounds. Due to the differences in fruit composition, yeast strains used for fermentation have to adapt to different environments *e.g*., sugar composition and concentrations, presence of organic acids, etc. (Duarte, Dias, Pereira, Gervársio & Schwan, 2009).

Actually, the majority of wine elaboration is based on the use of *S. cerevisiae* strains that allow for rapid and reliable fermentation, reducing the risk of sluggish or stuck fermentation, and microbial contamination (Valero, Schuller, Cambon, Casal & Dequin, 2005). It is important to know potential differences in volatile biosynthesis between various strains of yeast to select the best strain that will produce a good quality wine. The use of selected yeast strains can affect the wine composition and sensory profile and can consequently affect the wine quality (Girard, Yuksel, Cliff, Delaquis & Reynolds, 2001).

Alcoholic fermentation leads to a series of byproducts in addition to ethanol. They include carbonyl compounds, alcohols, esters, acids, and acetals, all of which influence the quality of the final beverage. The composition and concentration of the byproducts can vary widely from a few ng/L to hundreds of mg/L (Plutowska & Wardencki, 2008).

This is the first report using raspberries to produce a novel fermented beverage that includes the volatile characterisation of the final product. In this paper, we studied the potential of sixteen different strains of *Saccharomyces* for the fermentation of raspberry pulp and evaluated the influence of different yeasts on the analytical and sensory properties of the final beverage.

2. Materials and methods

2.1. Raspberry must

Raspberry fruits of the Meeker variety were obtained in the city of Vila Verde, North Portugal. Fruits were washed in clean water to remove plant residue. Next, the pulp was extracted manually by mechanical pressure. Seeds and pulp residue were separated from the juice by centrifugation (Relative centrifugal force - *RCF* = 13131*,* 10 min, 25 ºC). The initial Brix value was, on average, 14 and the pH was 3.6. The raspberry must was prepared according to Dias et al. (2003, 2007), with minor modifications. The raspberry pulp was mixed (1:1 v/v) with a sucrose solution to adjust the sugar concentration to 16 ºBrix. Calcium carbonate ($CaCO_3$) was added to increase the pH value to 4.0. Sulphur dioxide, in the form of potassium metabisulphite, was added up to a concentration of 100 mg/L free SO_2 to inhibit bacterial growth.

2.2. Microorganisms

Fifteen *Saccharomyces cerevisiae* strains and one *Saccharomyces bayanus* strain were evaluated. All yeast was obtained from collection of microorganisms in the Microbial Physiology Laboratory/Department of Biology, Federal University of Lavras (UFLA), Brazil.

2.3. Inoculum preparation

Yeast strains were grown in YPD (1% yeast extract; 2% peptone and 2% glucose). Using a platinum loop, yeasts were inoculated into tubes containing 1 mL YPD and then incubated at 28 ºC (24 h). After 24 h, the contents of the tubes were transferred to tubes containing 9 mL YPD and incubated for 24 h at 28 ºC. In the next step, the yeast culture (10 mL) was transferred to an Erlenmeyer flask containing 90 mL YPD, which was incubated for 24 hours at 28 ºC and 200 rpm. After this incubation, the yeast cells were separated from the medium by centrifugation (*RCF* = 4053*,* 5 min, 20 ºC) and washed twice with sterile distilled water.

2.4. Screening of yeast

The yeast previously obtained from 100 mL YPD was re-suspended in 100 mL sterile distilled water to determine the absorbance at 600 nm. The volume of the cell suspension for each of the sixteen yeast strains was adjusted to obtain an inoculum with 1.5 g/L (dry weight) raspberry must (100 mL). After inoculation, Erlenmeyer flasks containing 100 mL raspberry must were incubated at 22 °C without agitation. Over the fermentation period, samples were collected for assessment of biomass (dry weight) and sugar consumption (Brix). At the end of fermentation, biomass was separated from fermented must by centrifugation (*RCF* = 13131*,* 10 min, 20º C). Ethanol, glycerol, organic acids and volatile compounds were identified in fermented beverages. The selection of yeast was based on lower production of organic acids, higher yield of ethanol and higher concentrations of desirable volatile compounds. All experiments were carried out in triplicate.

2.5. Raspberry wine production

The three yeast strains that showed the best results in the screening stage were used for the production of raspberry wine. The inoculum for the three selected yeast strains was obtained as described above in order to obtain a final population, as biomass, corresponding to 1.5 g/L (dry weight). Three litres of raspberry must were utilised for raspberry wine production. All vinifications were carried out in a 5 L bioreactor at 22 °C. Fermentation was monitored by measurement of Brix values and biomass production. The fermentation was considered complete when the Brix level was stable. At the end of fermentation, raspberry fermented musts were transferred to bottles with a capacity of 750 mL and stored at 5 °C for sedimentation of the biomass. After 24 h, the beverages were transferred without aeration to new bottles. After 10 days, beverages were then filtered using cellulose filters and stored at 5 °C in glass bottles filled completely to avoid oxygen entrance.

2.6. Chemical analysis

2.6.1. Chemicals

1-Hexanol, (*Z*)-3-hexen-1-ol, 4-methyl-2-pentanol, 4-methyl-1-pentanol, 2-heptanol, 3-methyl-1-pentanol, 1-heptanol, ethyl propionate, ethyl hexanoate, ethyl pyruvate, ethyl lactate, ethyl octanoate, ethyl 3-hydroxybutanoate, ethyl decanoate, diethyl succinate, diethyl malate, α-ionone, β-ionone, 4-oxo- β-ionol, 3-oxo- α-ionol, 2-nonanone, 4-vinylguaiacol, 4-vinylphenol, N-(2-phenylethyl)acetamide, methanol, 2-phenylethanol, zingerone, methionol, methional, benzothiazole, furfuryl mercaptan, 2-mercaptoethanol, 2-methylthioethanol, 3-mercapto-3-methylbut-1-ol, malic acid, ferulic acid, *p*-coumaric acid were purchased from Aldrich Chemistry (Munich, Germany). 1-Butanol, 2-phenylethyl acetate, 2-methylpropyl acetate, 3-methylbutyl acetate, isobutyric acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, benzoic acid, 3-hydroxy-2-butanone, acetaldehyde, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, α-ionol were purchased from Fluka Analyticals (Seelze, Germany). Ethyl acetate and succinic acid were purchased from Sigma-Aldrich (Saint Luis, EUA). Acetic acid, ethanol and dichloromethane, were purchased from Merck (Darmstadt, Germany). 3-Mercapto-1-hexanol was purchased from Alfa Aesar (Barcelona, Spain). Glycerol and chlorogenic acid were purchased from Sigma (Saint Luis, EUA).

2.6.2. HPLC analysis

Ethanol, glycerol, succinic acid, malic acid, and acetic acid were quantified by high-performance liquid chromatography (HPLC) using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI), UV-visible detector (Jasco 870-UV-visible) and a 67H Chrompack column (6.5 mm x 300 mm) at 37 °C. Five mmol/L sulphuric acid was used as the eluent, at a flow rate of 0.4 mL/min and a sample volume of 20 μL (Duarte et al., 2010). Ethanol, glycerol were identified using RI detector. UV-visible detector was used for idenfitication of succinic acid, malic acid, and acetic acid.

2.6.3. HPLC-DAD analysis

Phenolic acids were analysed after solid-phase extraction according to protocols from Pérez-Magariño, Ortega-Heras and Cano-Mozo (2008), with minor modifications. In a 10 mL culture tube, 5 mL of raspberry wine, 400 μL of 0.05 mmol/L sulphuric acid, and a magnetic stir bar (22.2 mm×4.8 mm) were added and stirred for 3 min. The Isolute SPE C-18 cartridge was first conditioned with 5 mL methanol, followed by 5 mL water; next, 5 mL raspberry wine were percolated through the column. The cartridge was dried with a nitrogen gas stream and phenolic compounds were eluted with 5 mL methanol. After filtration through 0.45 μm cellulose filter, the methanolic extract was evaporated to 1 mL using a rotary evaporator and a 35 ºC water bath. Analysis of phenolic acids (chlorogenic, ferulic and *p*-coumaric) was carried out on a Merck-Hitachi L-7455 liquid chromatograph with a diode array detector (DAD) using a Waters Spheresorb ODS2 column (4.6 mm x 250 mm, 5 μm particle size) and 20 μL of each sample. The chromatographic conditions were modified on the basis of the method proposed by Rodríguez-Delgado, Malovaná, Pérez, Borges & García Montelongo (2001). The solvents were (A) methanol/water/acetic acid (10:2:88; v/v/v) and (B) methanol/water/acetic acid (90:2:8; v/v/v). The gradient was linear at a flow rate of 1.0 mL/min from 0% to 15% solvent B for 15 min, from 15% to 50% solvent B for 10 min, and from 50% to 70% solvent B for 15 min, followed by washing with solvent A and re-equilibration of the column for 20 min. Diode array detection was performed from 240 nm to 400 nm. Peak identity and purity were confirmed. The phenolic compounds analysed were identified by comparing their retention times with their respective standard. The quantification of the different phenolic compounds was carried out at different wavelengths by applying each calibration line constructed using the corresponding standard.

2.6.4. GC-FID analysis

Major volatile compounds in raspberry wines were analysed directly without any previous treatment. A Chrompack CP-9000 gas chromatograph equipped with a Split/Splitless injector and a flame ionisation detector was used. The separation was performed with a CP-Wax 57 CB column (50 m x 0.25 mm i.d., 0.2 μm film thickness; Chrompack). The temperature of the injector and detector were both set to 250 ºC. The oven temperature was held at 50 ºC for 5 min, then programmed to run from 50 ºC to 220 ºC at 3 ºC/min, and then held at 220 ºC for 10 min. Helium 55 (Praxair) was used as the carrier gas at 125 kPa, with a split vent of 15 mL/min. Injections of 1 μL were made in the splitless mode (vent time, 15 s); 4-nonanol (internal standard) was added to the sample to a final concentration of 122.05 mg/L (Duarte et al., 2010). The volatile compounds were identified by comparing the retention times of the samples with those of pure standard compounds. Quantification of volatile compounds was performed with Varian Star Chromatography Workstation software (Version 6.41) after determining the detector response factor for each compound. The quantification of the volatile compounds was expressed as 4-nonanol (internal standard) equivalents.

2.6.5. GC-MS analysis

Minor volatile components in raspberry wines were determined by extraction with dichloromethane according to the method of Oliveira, Faria, Sá, Barros & Araújo (2006) followed by analysis of the extracts by GC–MS using a Varian 4000 gas chromatograph equipped with 1079 split/splitless injector (splitless for 30 s). Samples of 1 µL were injected into a Factor Four VF-Wax$_{MS}$ capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness, Varian). Helium N60 (Air Liquide) was used as the carrier gas at 124 kPa (18 psi). The detector was operated in the electron-impact mode (70 eV), and mass spectra were acquired by scanning over the mass/charge (*m/z*) range of 29 to 260 with an acquisition rate of 610 ms. The temperature of the injector was programmed to run from 20 ºC to 250 ºC at 180 ºC/min and was then maintained at 250 ºC during the analysis. The oven temperature was held at 60 ºC for 5 min, then programmed to run from 60 ºC to 220 ºC at 3 ºC/min and was finally maintained at 250 ºC for 25 min. Volatile compounds were identified using Varian MS workstation software (Version 6.6) by comparing mass spectra and linear retention indices with those of authentic standard compounds injected under the same conditions. 4-Nonanol was chosen as the internal standard and was added to each sample and standard to a final concentration of 305 µg/L. The concentration 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.

2.6.6. GC−PFPD analysis

The analyses of sulphur compounds were made on a Varian CP-3800 gas chromatograph equipped with a GC−PFPD detector operating in sulphur mode. After liquid-liquid extraction with dichloromethane (as described in section 2.6.5), three extracts were mixed and concentrated to 1/3 under a nitrogen stream. Aromatic extracts were injected into a 1079 split/splitless injector (splitless for 30 s). The separation was performed with a CP-Wax 52 CB column

(50 m x 0.25 mm i.d., 0.2 μm film thickness; Chrompack). The oven temperature was programmed to run from 60 °C (5 min) to 200 °C at a rate of 20 °C/min (final hold for 5 min). The carrier gas was helium, with a constant flow rate of 1.2 mL/min. The temperature of the injector and detector was set to 250 °C. The detector voltage was 570 V, the gate delay for sulphur compounds was 6 ms and the gate width was 20 ms. All sulphur compounds were identified by comparing their retention times with those of the pure standards. Ethyl (methylthio) acetate was chosen as the internal standard and was added to each sample and standard to a final concentration of 55 μg/L. The square root of the values for peak area was calculated because the GC−PFPD response is from the emission of two excited sulphur atoms (S_2) corresponding to a second-order, or quadratic, response. The concentration of the volatile compounds was expressed as ethyl (methylthio) acetate equivalents.

2.7. Sensory analysis

Beverages were analysed in triplicate by twelve trained panellists. The evaluation of beverages by sensory analysis was done using quantitative descriptive analysis (QDA) methodology. A constant volume of 30 mL of each raspberry wine was evaluated in wine taster glasses at 12 °C. During the analysis, the wine tasters indicated different perceived descriptors (aroma, colour and flavour) and the intensity of each attribute was rated on a scale from 0 to 9. The data were processed to obtain the Geometric Mean values (GM). The GM was calculated with the following formula:

$$GM = \sqrt{F(\%)I(\%)}$$

where F(%) is the detection frequency of an attribute expressed as a percentage and I(%) is the average intensity expressed as a percentage of the maximum intensity.

2.8. Statistical analysis

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

3. Results and discussion

3.1. Screening of yeast

The yeast strains were selected based on the results from tests of volatile compounds, glycerol, ethanol and, organic acids (Table 1). The higher efficiency of pre-selected yeast for fermentation of raspberry pulp was determined by considering the highest concentrations of volatile compounds (3-methyl-1-butanol, 2-phenylethanol and total higher alcohols), glycerol, and ethanol and the lowest concentrations of organic acids (acetic acid, malic acid, and succinic acid). Of the sixteen yeast strains evaluated, UFLA FW 15, CAT-1, and *S. bayanus* CBS 1505 showed the best performance for fermentation of raspberry pulp (Table 1). Using these yeasts, it was possible to obtain high concentrations of ethanol, glycerol, 2-phenylethanol and 3-methyl-1-butanol. Interesting results (*e.g*., high concentrations of ethanol, glycerol and 2-phenylethanol) were also obtained for VR-1; however, this yeast showed a high concentration of acetic acid (2.3 g/L). The high concentration of acetic acid (> 2.0 g/L) was a negative factor for VR-1. Wine containing acetic acid in high concentrations has a pronounced vinegar-like character (Swiegers, Bartowsky, Henschke & Pretorius, 2005).

Table 1. Concentration of volatile compounds detected in raspberry wines by GC−FID and HPLC during microvinification.

Compounds	VR-1	PE-2	6167 1A	BG	UFLA FW 1183	UFLA FW 1174	SA	UFLACA 11
GC−FID (mg/L)								
Aceltaldehyde	8.0^{a} (0.40)	12.6^{b} (1.77)	1.5^{d} (1.82)	8.7^{a} (0.90)	8.1^{a} (0.62)	15.5^{c} (2.46)	8.4^{a} (0.52)	15.7^{c} (0.28)
Ethyl acetate	7.9^{a} (0.05)	7.9^{a} (0.88)	9.8^{b} (0.42)	9.1^{a} (0.23)	11.1^{c} (0.58)	14.7^{e} (0.11)	8.4^{a} (0.21)	10.1^{b} (0.07)
Methanol	83.0^{b} (2.11)	79.4^{b} (0.27)	68.6^{b} (0.16)	79.2^{b} (4.34)	79.1^{b} (0.91)	61.2^{a} (8.90)	64.4^{a} (11.76)	77.2^{b} (3.35)
1-Propanol	16.5^{a} (0.55)	15.8^{a} (1.06)	23.8^{e} (0.22)	19.2^{c} (0.73)	18.5^{b} (0.50)	21.4^{d} (1.11)	16.6^{a} (0.42)	21.2^{d} (1.09)
2-Methyl-1-propanol	75.0^{i} (2.87)	28.5^{b} (2.39)	32.9^{c} (1.73)	76.1^{i} (1.91)	52.6^{e} (2.45)	63.2^{f} (2.80)	69.9^{h} (1.87)	34.6^{b} (0.96)
2-Methyl-1-butanol	37.8^{g} (1.07)	18.4^{b} (0.63)	19.0^{b} (0.47)	35.6^{f} (0.98)	29.3^{d} (0.72)	29.8^{d} (0.08)	32.1^{e} (0.11)	17.1^{a} (0.10)
3-Methyl-1-butanol	175.5^{h} (7.52)	94.5^{b} (7.28)	95.5^{b} (2.43)	127.3^{d} (1.18)	124.4^{d} (2.82)	130.0^{e} (0.20)	134.3^{e} (0.09)	79.8^{a} (0.14)
2-Phenylethanol	44.3^{h} (3.06)	11.9^{b} (0.94)	18.3^{b} (0.45)	29.4^{g} (0.19)	13.4^{b} (0.12)	12.2^{b} (0.96)	23.7^{f} (1.27)	9.0^{a} (2.27)
Total higher alcohols	349.03^{g}	169.1^{a}	189.5^{b}	287.5^{f}	238.3^{d}	256.6^{e}	276.8^{f}	161.8^{a}
HPLC (g/L)								
Glycerol	10.2^{e} (0.12)	6.7^{c} (0.18)	5.5^{b} (0.46)	7.2^{c} (0.45)	6.5^{c} (0.67)	4.6^{a} (0.21)	8.5^{d} (0.74)	5.3^{b} (0.35)
Ethanol	71.2^{c} (0.38)	69.0^{c} (0.54)	69.5^{c} (3.11)	74.4^{c} (3.80)	72.0^{c} (3.13)	62.8^{b} (0.74)	66.4^{c} (3.62)	59.5^{a} (0.81)
Succinic acid	6.1^{b} (0.12)	2.8^{a} (0.36)	7.1^{b} (0.21)	6.1^{b} (0.40)	6.0^{b} (0.46)	6.5^{b} (0.15)	5.6^{b} (0.59)	6.1^{b} (0.20)
Acetic acid	2.3^{e} (0.03)	0.7^{b} (0.16)	0.9^{c} (0.03)	0.7^{b} (0.07)	0.9^{c} (0.06)	0.9^{c} (0.01)	1.2^{d} (0.26)	0.9^{c} (0.13)
Malic acid	0.5^{d} (0.11)	0.7^{d} (0.03)	1.5^{f} (0.23)	0.6^{c} (0.14)	1.5^{f} (0.17)	1.0^{e} (0.06)	0.5^{b} (0.12)	0.2^{a} (0.01)
Total organic acids	8.9^{e}	4.2^{a}	8.6^{d}	7.3^{c}	8.4^{c}	8.3^{d}	7.3^{b}	7.2^{c}

Table 1. (Continued)

Compounds	**UFLA FW 1185**	**UFLA FW 1187**	**UFLA CA 155**	**UFLA FW 15**	**CAT-1**	**UFLA EU 60.1**	*S. bayanus*	**UFLA FW 1162**
GC−FID (mg/L)								
Aceltaldehyde	14.1^{c} (0.84)	12.1^{b} (0.43)	8.7^{a} (0.76)	11.6^{b} (0.90)	10.8^{b} (1.74)	14.0^{c} (2.29)	11.5^{b} (0.39)	9.9^{a} (0.94)
Ethyl acetate	10.5^{b} (0.23)	9.6^{b} (1.00)	11.4^{c} (0.90)	12.5^{d} (0.53)	10.6^{b} (0.19)	9.7^{b} (0.87)	13.8^{e} (1.16)	11.6^{c} (0.64)
Methanol	77.3^{b} (1.49)	71.9^{b} (1.54)	75.3^{b} (0.20)	77.5^{b} (1.25)	69.4^{b} (4.98)	70.6^{b} (4.21)	71.0^{b} (3.47)	73.0^{b} (2.51)
1-Propanol	16.5^{a} (0.31)	15.3^{a} (1.11)	19.9^{c} (0.58)	19.6^{c} (1.10)	17.2^{b} (0.96)	14.8^{a} (1.32)	22.6^{e} (0.90)	17.7^{b} (1.13)
2-Methyl-1-propanol	26.4^{a} (0.39)	39.4^{d} (1.17)	41.32^{d} (0.59)	52.9^{e} (0.64)	59.3^{f} (0.20)	24.7^{a} (0.10)	63.4^{g} (0.37)	60.5^{f} (0.01)
2-Methyl-1-butanol	15.9^{a} (3.15)	25.0^{c} (0.71)	28.3^{d} (0.43)	33.7^{f} (0.61)	34.2^{f} (0.66)	18.5^{b} (0.06)	28.9^{d} (0.91)	36.8^{g} (1.47)
3-Methyl-1-butanol	117.4^{c} (7.15)	114.7^{c} (4.38)	133.9^{e} (3.76)	149.4^{g} (3.16)	141.0^{f} (2.64)	97.2^{b} (2.46)	126.1^{d} (4.21)	141.0^{f} (1.39)
2-Phenylethanol	13.7^{b} (0.23)	15.9^{b} (0.02)	29.9^{g} (0.49)	22.0^{e} (0.66)	31.3^{g} (2.79)	12.7^{b} (0.60)	19.4^{d} (2.65)	28.5^{g} (1.08)
Total higher alcohols	190.0^{b}	210.3^{c}	253.3^{e}	277.6^{f}	282.9^{f}	167.9^{a}	260.4^{e}	284.5^{f}
HPLC (g/L)								
Glycerol	7.3^{c} (0.13)	7.3^{c} (0.38)	7.0^{c} (0.04)	7.1^{c} (0.08)	6.9^{c} (0.22)	6.5^{c} (0.01)	7.1^{c} (0.26)	4.9^{a} (0.27)
Ethanol	62.1^{b} (2.39)	71.2^{c} (1.38)	62.6^{b} (2.39)	70.3^{c} (0.94)	67.1^{c} (0.49)	55.7^{a} (1.74)	64.2^{b} (2.89)	57.5^{a} (2.87)
Succinic acid	5.2^{b} (0.12)	6.3^{b} (0.26)	5.4^{b} (3.59)	7.2^{b} (0.30)	5.3^{b} (0.07)	6.8^{b} (0.96)	5.4^{b} (0.09)	6.7^{b} (1.73)
Acetic acid	0.7^{b} (0.06)	1.3^{d} (0.18)	0.6^{b} (0.19)	0.6^{b} (0.11)	0.7^{b} (0.05)	0.4^{a} (0.02)	0.7^{b} (0.07)	0.4^{a} (0.03)
Malic acid	0.7^{d} (0.06)	0.2^{a} (0.05)	0.4^{b} (0.07)	0.7^{d} (0.09)	0.6^{c} (0.01)	0.3^{a} (0.09)	0.5^{c} (0.00)	0.8^{d} (0.12)
Total organic acids	6.7^{b}	7.8^{d}	6.4^{b}	8.5^{d}	6.7^{b}	7.5^{d}	6.6^{b}	7.9^{d}

Numbers between brackets correspond to the standard deviation; ND not detected. Values identified by the same letters are not significantly different at the 0.05 level (Scott-Knott test). Data are presented as mean ±SD of triplicate analysis.

Yeast strains PE-2, 6167 1A, UFLA CA 11, UFLA FW 1185, and UFLA EU 60.1 showed low concentrations of higher alcohols, mainly 2-phenylethanol (Table 1). 2-Phenylethanol is an aroma carrier and its presence may contribute to the floral nuance of wines. The aroma characterised by this compound changes with its oxidation from a rose to a hyacinth bouquet (Wondra and Berovic, 2001). Low concentrations of 2-phenylethanol were also found in beverages produced by yeasts UFLA FW 1174 and UFLA FW 1183. However, for all raspberry beverages, 2-phenylethanol was found in concentrations above the odour threshold (Czerny et al., 2008). The lowest amounts of glycerol produced were 4.6 g/L and 4.9 g/L for UFLA FW 1174 and UFLA FW 1162, respectively (Table 1). These concentrations are slightly below those commonly found in grape wines. The minimum glycerol concentration in wine is 5 g/L, but it may reach values as high as 15 g/L to 20 g/L. In wine, glycerol affects wine flavour and gives an impression of fullness and softness (Ribéreau-Gayon, Glories, Maujean & Dubourdieu, 2006).

3.2. Raspberry wine production

Yeast strains CAT-1, *S. bayanus* CBS 1505 and UFLA FW 15 showed similar sugar consumption during raspberry pulp fermentation (Fig. 1). At the end of the fermentation, CAT-1 produced 9.9 g/L of biomass. When UFLA FW 15 and *S. bayanus* CBS 1505 were used for raspberry pulp fermentation, biomass production was 9.4 g/L and 8.3 g/L, respectively (Fig. 1).

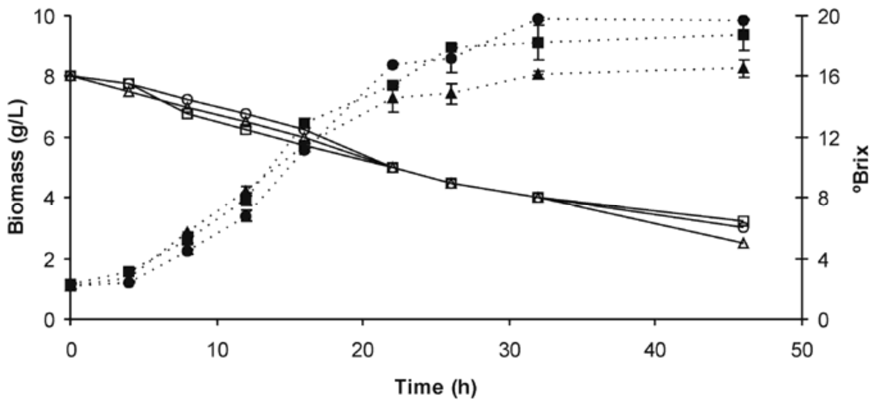

Figure 1 Biomass production and consumption of soluble solids during raspberry pulp fermentation. (*dotted line with filled circle*) biomass CAT-1; (*dotted line with filled square*) biomass UFLA FW 15; (*dotted line with filled diamond*) biomass *S. bayanus* CBS 1505; (*solid line with open circle*) Brix CAT-1; (*solid line with open square*) Brix UFLA FW 15; (*solid line with open diamond*) Brix *S. bayanus* CBS 1505.

3.2.1. HPLC analysis

The must fermented by UFLA FW 15 produced the highest amount of succinic acid (7.9 g/L) (Table 2). This acid at a high concentration can influence negatively the final quality of the wine. The increase in acidity in various wines is correlated with the increase of succinic acid concentration. This is the main acid produced by yeasts. Due to the ability to produce different amounts of succinic acid, yeast strain is the main factor that influences the production of succinic acid during fermentation (Coulter, Godden & Pretorius, 2004). The concentrations of ethanol, glycerol, acetic acid, and malic acid were similar for the three studied yeasts (Table 2). Considering the initial sugars concentration (about 160 g/L) and final ethanol concentration of 75.7 g/L (CAT-1), 70.2 g/L

(*S. bayanus* CBS 1505) and 66.8 g/L (UFLA FW 15), the three yeasts studied showed an efficient fermentation of raspberry.

Table 2 shows the concentrations of chlorogenic acid, ferulic acid and *p*-coumaric acid for raspberry wines. Li, Hydamaka, Lowry & Beta (2009), who were studying various fruits, reported higher concentrations (when compared to our results) of ferulic acid (35 mg/kg) and *p*-coumaric acid (68 mg/kg) in raspberries. Numerous plant species have been analysed for their phenolic content and antioxidant capacity, with berries being among the best sources. Phenolic acids may provide particular health benefits by acting as strong antioxidants or directly affecting specific enzymes. Ferulic acid may be beneficial in the prevention of disorders linked to oxidative stress, diabetes, hypertension, and atherosclerosis (Zhao & Moghadasian, 2008).

Table 2. Concentration of alcohols and acids detected in raspberry wines by HPLC and HPLC−DAD.

Compounds	**Raspberry wines**		
	CAT-1	**UFLA FW 15**	***S. bayanus*** **CBS 1505**
HPLC (g/L)			
Glycerol	6.6±0.06	6.5±0.02	6.1±1.20
Ethanol	75.7±1.37	66.8±1.22	70.2±1.25
Succinic acid	3.9±0.41	7.9±0.60	4.1±0.97
Acetic acid	0.4±0.02	0.2±0.04	0.6±0.02
Malic acid	0.5±0.00	0.5±0.04	0.6±0.11
HPLC−DAD (mg/L)			
Chlorogenic acid	27.5±0.58	17.4±0.87	24.6±1.99
Ferulic acid	10.5±0.15	6.0±0.03	9.9±0.82
p-Coumaric acid	6.2±0.49	4.3±0.52	4.8±0.37

Data are presented as mean ±SD of triplicate analysis.

3.2.2. GC-FID analysis

Table 3 shows the concentrations of compounds identified in raspberry wines by GC-FID. For all yeast strains, the concentrations of the identified compounds were higher than their odour threshold. 3-Methyl-1-butanol was the alcohol present in higher concentrations in raspberry beverages. Similar results regarding the higher concentration of 3-methyl-1-butanol were found by Wondra and Berovic (2001) when they were evaluating different yeast strains. According to these authors, 3-methyl-1-butanol together with its ester contributes to the dry fruit aroma in wine. 2-Methyl-1-propanol and 2-methyl-1-butanol were the other two alcohols present in higher concentrations in raspberry wines (Table 3). The main higher alcohols present after fermentation were 2-methyl-1-propanol and amyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol). Concentrations less than 300 mg/L contribute to a wine's aromatic complexity. At higher levels, their penetrating odours mask the wine's aromatic finesse (Ribéreau-Gayon et al., 2006).

The highest concentration of methanol (92.8 mg/L) was found in the beverage produced with *S. bayanus* CBS 1505. Methanol is a toxic alcohol found in wines; consequently, its concentration must be measured. Methanol is derived from methylated pectic substances (pectins) by the action of pectic esterases.

Table 3. Concentration of volatile compounds (mg/L) detected in raspberry wines by GC−FID; odor threshold and descriptors reported in literature.

No	Compounds	Raspberry wines			Oth (µg/L)	Descriptors
		CAT-1	**UFLA FW 15**	***S. bayanus* CBS 1505**		
1	Acetaldehyde	$9.9^{b}\pm0.26$	$8.5^{a}\pm0.11$	$10.9^{c}\pm0.71$	25[1] *	Fresh, green[1]
2	Ethyl acetate	$27.6^{a}\pm0.34$	$37.4^{b}\pm10.44$	$19.4^{a}\pm3.40$	7500[2] ∞	Solvent, fruity[3]
3	Methanol	$80.6^{a}\pm1.69$	$72.1^{a}\pm5.46$	$92.8^{b}\pm9.06$	-	-
4	1-Propanol	$19.5^{a}\pm0.66$	$22.1^{a}\pm5.10$	$16.0^{a}\pm4.23$	750[3] §	-
5	2-Methyl-1-propanol	$73.6^{a}\pm1.42$	$71.0^{a}\pm14.28$	$89.6^{a}\pm15.15$	550[1] *	Malty[1]
6	2-Methyl-1-butanol	$37.2^{a}\pm3.87$	$34.7^{a}\pm8.09$	$46.4^{b}\pm7.78$	1200[1] *	Malty, solvent-like[1]
7	3-Methyl-1-butanol	$153.5^{a}\pm10.66$	$167.2^{a}\pm37.56$	$151.5^{a}\pm28.01$	220[1] *	Malty[1]
8	2-Phenylethanol	$24.7^{a}\pm1.96$	$21.7^{a}\pm8.91$	$23.4^{a}\pm3.72$	140[1] *	Flowery, honey-like[1]

Oth, odor threshold; ND, not detected; *Olfactory perception threshold in water; ∞ Olfactory perception threshold in hydro-alcoholic solution; § Olfactory difference threshold in beer. Values identified by the same letters are not significantly different at the 0.05 level (Scott-Knott test). Data are presented as mean ±SD of triplicate analysis.

[1] Czerny et al. (2008).
[2] Guth (1997).
[3] Meilgaard (1975).

3.2.3. GC-MS analysis

Thirty-nine volatile compounds were identified by GC−MS in raspberry wines (Table 4) and were grouped as C_6 compounds, alcohols, ethyl esters, acetates, C_{13}-norisoprenoids, volatile phenols, volatile fatty acids, carbonyl compounds, and sulphur compounds.

The ethyl ester group was formed by the largest number (10) of compounds, with significant differences between the seven (ethyl butyrate, ethyl pyruvate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate and diethyl malate) identified compounds. Ethyl esters are one of the most important groups of aromatic compounds in wine, and their concentrations depend on yeast strain, fermentation temperature, aeration, and sugar content. These compounds contribute positively to the overall wine quality, and most of them have a mature flavour and fruity aroma that contribute to the fruity and floral sensory properties of wines (Swiegers et al., 2005). Ethyl hexanoate (fruity, green apple) and ethyl octanoate (apple, fruit and sweet) were the compounds of the ethyl esters group that were found in the highest concentrations in raspberry wines. The highest amount of ethyl butyrate (135.9 µg/L) was measured in the beverage produced by UFLA FW 15 (Table 4). In addition, raspberry wine fermented with *S. baynaus* contained the highest levels of diethyl succinate (13 µg/L), ethyl lactate (31.4 µg/L) and diethyl malate (34.1 µg/L). The results of this work for diethyl succinate and ethyl lactate are in good agreement with those reported by Antonelli, Castellari, Zambonelli & Camacini (1999) and by Hernández-Orte, Cersosimo, Loscos, Cacho, Garcia-Moruno & Ferreira (2008). These authors found the highest concentrations of diethyl succinate in wines produced by *S. bayanus*.

Table 4. Concentration of minor volatile compounds (µg/L) detected in raspberry wines by GC−MS; odor threshold and descriptors reported in literature.

No	Compounds	LRI	Raspberry wines: CAT-1	UFLA FW 15	*S. bayanus*	Oth (µg/L)	Descriptors
	C_6 compounds (2)						
1	1-Hexanol	1348	$12.3^{a}\pm4.96$	$14.7^{a}\pm4.40$	$15.2^{a}\pm3.02$	8000[2 ∞]	Coconut, green leaves, unpleasant[4]
2	(*Z*)-3-hexen-1-ol	1379	$4.5^{a}\pm0.73$	$10.6^{b}\pm1.92$	$5.8^{a}\pm0.99$	3.9[1 *]	Lettuce-like[1]; green leaves[4]
	Total		**16.8^{a}**	**25.4^{a}**	**20.9^{a}**		
	Alcohols (6)						
3	4-Methyl-2-pentanol	1164	$27.1^{a}\pm3.32$	$30.7^{a}\pm9.00$	$30.3^{a}\pm2.13$	-	-
4	1-Butanol	1173	$8.6^{a}\pm2.40$	$18.7^{b}\pm7.36$	$8.7^{a}\pm0.60$	590[1 *]	Malty, solvent-like[1]; fusel, spirituos[3]
5	4-Methyl-1-pentanol	1309	ND	$4.0^{b}\pm1.06$	$5.8^{b}\pm1.00$	-	-
6	2-Heptanol	1315	$316.9^{a}\pm54.32$	$362.6^{a}\pm96.98$	$300.2^{a}\pm39.48$	250[4 §]	Coconut[4]
7	3-Methyl-1-pentanol	1322	$10.6^{a}\pm0.58$	$15.0^{b}\pm1.21$	$14.9^{b}\pm1.66$	-	-
8	1-Heptanol	1449	$12.1^{a}\pm1.43$	$17.0^{a}\pm4.66$	$24.9^{b}\pm0.73$	1000[4 §]	Coconut, ketonic solvent, unpleasant[4]
	Total		**375.2^{a}**	**448.1^{a}**	**384.8^{a}**		
	Ethyl Esters (10)						
9	Ethyl propionate	971	$26.0^{a}\pm7.76$	$28.2^{a}\pm6.15$	$17.1^{a}\pm3.01$	45[2 ∞]	Fruity[3]
10	Ethyl butyrate	1032	$58.0^{a}\pm12.46$	$135.9^{b}\pm36.86$	$54.1^{a}\pm10.70$	20[2 ∞]	Fruity[1, 3]; papaya, apple, perfumed[4]
11	Ethyl hexanoate	1234	$452.2^{a}\pm68.30$	$447.9^{a}\pm36.30$	$394.1^{a}\pm34.95$	14[6 ϕ]	Fruity, green apple[3,4]
12	Ethyl pyruvate	1267	$12.0^{a}\pm3.59$	$24.3^{b}\pm5.22$	$24.2^{b}\pm2.65$	-	Herbaceous, oil painting, forage[4]
13	Ethyl lactate	1338	$4.9^{a}\pm1.29$	$11.4^{a}\pm4.50$	$31.4^{b}\pm7.59$	157 810[7 ∞]	Strawberry, raspberry, perfumed[3,4]
14	Ethyl octanoate	1434	$476.7^{b}\pm20.99$	$449.5^{b}\pm15.23$	$379.7^{a}\pm15.92$	5[6 ϕ]	sweet[3]; apple, fruity[4];
15	Ethyl 3-hydroxybutanoate	1512	$36.9^{a}\pm6.73$	$47.3^{a}\pm10.49$	$51.2^{a}\pm3.40$	-	-
16	Ethyl decanoate	1636	$58.9^{b}\pm6.21$	$71.3^{b}\pm21.60$	$16.0^{a}\pm2.70$	200[6 ϕ]	Fatty acids, fruity, apple, solvent[4]
17	Diethyl succinate	1672	ND	ND	$13.0^{a}\pm2.14$	200 000[7 ∞]	-
18	Diethyl malate	2037	$14.4^{a}\pm1.83$	$23.0^{a}\pm5.08$	$34.1^{b}\pm8.60$	-	-
	Total		**1140.2^{a}**	**1238.6^{a}**	**1014.9^{a}**		
	Acetates (3)						
19	2-Methylpropyl acetate	1009	$71.5^{b}\pm10.59$	$72.8^{b}\pm9.92$	$29.9^{a}\pm4.46$	-	Banana, fruity[3]
20	3-Methylbutyl acetate	1125	$1257.8^{a}\pm113.98$	$1927.0^{b}\pm154.39$	$1293.6^{a}\pm100.09$	30[2 ∞]	Banana[3]
21	2-Phenylethyl acetate	1810	$252.6^{b}\pm20.99$	$211.3^{a}\pm12.64$	$193.4^{a}\pm19.47$	250[2 ∞]	Flowery[3]; apple, honey, roses, sweet[4]
	Total		**1581.9^{a}**	**2211.1^{b}**	**1516.9^{a}**		

Table 4. (Continued)

No	Compounds	LRI	Raspberry wines			Oth (µg/L)	Descriptors
			CAT-1	**UFLA FW 15**	***S. bayanus***		
	C_{13}-Norisoprenoids (5)						
22	α-Ionone	1855	72.0^{a}±6.91	63.0^{a}±3.17	59.2^{a}±11.19	$2.6^{7\,\infty}$	raspberry, cedarwood[4]; floral, perfume[5]
23	α-Ionol	1896	62.7^{a}±7.05	74.7^{a}±17.60	68.0^{a}±6.94	-	Hot tea, lemon-sweet, violet[5]
24	β-Ionone	1943	53.0^{a}±7.17	43.7^{a}±7.29	45.6^{a}±8.00	3.5^{1*}	Flowery, violet-like[1]; raspberry[4]; floral[5]
25	4-Oxo- β-ionol	2640	34.1^{a}±3.98	35.8^{a}±9.96	33.3^{a}±5.05	-	Sweet, fruity, berry[5]
26	3-Oxo- α-ionol	2628	34.4^{a}±7.96	31.0^{a}±7.00	40.4^{a}±3.33	-	-
	Total		**256.1^{a}**	**248.2^{a}**	**246.5^{a}**		
	Volatile phenols (3)						
27	4-Vinylguaiacol	2192	293.7^{c}±24.47	131.9^{b}±37.13	29.3^{a}±3.42	$21^{1\,*}$	Clove-like, smoky[1]; phenolic, bitter[4]
28	4-Vinylphenol	2396	34.3^{a}±8.39	30.5^{a}±4.65	ND	-	-
29	Zingerone	2805	89.7^{a}±9.70	68.0^{a}±19.69	53.6^{a}±13.98	-	Sweet, fruity, cooked pears[5]
	Total		**417.7^{c}**	**230.5^{b}**	**83.0^{a}**		
	Volatile fatty Acids (6)						
30	Isobutyric acid	1579	ND	21.2^{b}±0.95	17.3^{a}±1.55	$200\,000^{2\,\infty}$	Cheese, rancid[3]; sweat, bitter[4];
31	Hexanoic acid	1841	179.9^{b}±17.27	67.2^{a}±0.79	148.1^{b}±27.98	$420^{6\,\phi}$	Cheese, sweaty[3];fatty acids, vegetable oil[4]
32	Octanoic acid	2057	902.4^{b}±102.38	573.6^{a}±116.90	622.4^{a}±72.63	$500^{6\,\phi}$	Rancid, harsh[3]; fatty acids, vegetable oil[4]
33	Decanoic acid	2269	264.3^{b}±27.02	28.6^{a}±6.05	51.8^{a}±8.42	$1000^{6\,\phi}$	Fatty[3]; wax, tallow, rancid, soap[4]
34	Benzoic acid	2451	125.6^{a}±5.89	210.7^{b}±36.17	220.2^{b}±4.15	-	-
35	Dodecanoic acid	2481	70.3^{c}±11.18	34.5^{b}±11.70	14.5^{a}±3.52	-	-
	Total		**1542.6^{b}**	**935.8^{a}**	**1074.3^{a}**		
	Carbonyl compounds (2)						
36	3-Hydroxy-2-butanone	1285	3.8^{a}±0.42	7.3^{a}±3.85	13.1^{b}±3.09	$152\,600^{7\,\infty}$	Fuity, moldy, wood[4]
37	2-Nonanone	1390	29.9^{a}±4.59	31.5^{a}±11.44	23.9^{a}±3.64	-	Sweet, woody, berry, fruity[6]
	Total		**33.7^{a}**	**38.8^{a}**	**37.0^{a}**		
	Sulfur (1)						
38	2-Methyltetrahydrothiofeno-3-one	1533	191.8^{a}±29.25	429.2^{a}±147.36	309.1^{a}±33.52	-	-
	Other (1)						
39	N-(2-phenylethyl)acetamide	2585	17.2^{a}±3.66	29.2^{b}±7.98	31.6^{b}±4.72	-	-
	Total volatile compounds		**5573.3^{b}**	**5835.0^{b}**	**4719.2^{a}**		

LRI, linear retention index; Oth, odor threshold; ND, not detected; *Olfactory perception threshold in water; [∞] Olfactory perception threshold in hydro-alcoholic solution; [§] Olfactory difference threshold in beer; [ϕ] Olfactory threshold in model wine. Values identified by the same letters are not significantly different at the 0.05 level (Scott-Knott test). Data are presented as mean ±SD of triplicate analysis.

[1] Czerny et al. (2008).
[1] Czerny et al. (2008).
[2] Guth (1997).
[3] Siebert et al. (2005).
[4] Meilgaard (1975).
[5] Klesk et al. (2004).
[6] Ferreira et al. (2000).
[7] Etievant (1991).

Raspberry wines fermented with *S. cerevisiae* CAT-1 and UFLA FW 15 showed, respectively, 58.9 µg/L and 71.3 µg/L of ethyl decanoate; raspberry wine produced with *S. bayanus* CBS 1505 contained 16 µg/L of ethyl decanoate. Mateo, Jiménez, Pastor & Huerta (2001) found higher levels of ethyl decanoate in wine fermented by *S. bayanus*. The total content of ethyl esters was higher for the beverage fermented with UFLA FW 15; however, significant differences ($p<0.05$) were not found between the studied yeasts (Table 4).

Raspberry wine fermented with UFLA FW 15 showed the highest concentration of acetates. In this group, 3-methylbutyl acetate was the compound that showed the highest levels in raspberry wines. A maximum value of 1927 µg/L was found in raspberry wine fermented with UFLA FW 15 (Table 4). 3-Methylbutyl acetate is associated with "banana" aromatic notes (Siebert et al., 2005). The synthesis of acetate esters by the wine yeast *Saccharomyces cerevisiae* during fermentation is ascribed to at least three acetyltransferase activities, namely, alcohol acetyltransferase, ethanol acetyltransferase, and isoamyl alcohol acetyltransferase (Lilly, Lambrechts and Pretorius, 2000). 2-Phenylethyl acetate (apple, honey, roses, sweet, and flowery) was found to range from 193.4 µg/L (*S. bayanus*) to 252.6 µg/L (CAT-1). When CAT-1 was used for fermentation of raspberry pulp, 2-phenylethyl was found to be above the odour threshold (Guth, 1997).

In the alcohols group, six compounds were identified (Table 4). 1-Butanol (malty, solvent-like, spirituous) was found in wine fermented with UFLA FW 15 in a concentration approximately two times higher (18.7 µg/L) than the concentration found in raspberry wine fermented with CAT-1 or *S. bayanus* CBS 1505. The beverage obtained by fermentation with *S. bayanus* CBS 1505 produced the highest amount of 1-heptanol (24.9 µg/L). The 3-methyl-1-pentanol content of the raspberry wines varied from 10.6 µg/L (CAT-1) to 14.9 µg/L (UFLA FW 15). According to Liberatore, Pati, Del Nobile & La

Notte (2010), this compound has the odour descriptors of “wine” and “green”. The use of different yeast strains during fermentation contributes considerably to variations in higher alcohol profiles and concentrations in wine (Swiegers et al., 2005). Higher alcohols can have both positive and negative impacts on the aroma and flavour of wine depending on its concentration; they are considered favourable compounds when their total concentration is lower than 300 mg/L (Swiegers et al., 2005; Ribéreau-Gayon et al., 2006).

Six volatile fatty acids were identified in raspberry wines (Table 4). The beverage made with CAT-1 contained 1542.6 µg/L total volatile acids, corresponding to the highest concentration of volatile fatty acids found in this work. With an individual analysis of each identified acid, we can see in Table 4 that for octanoic acid (fatty acid, cheese, harsh, and rancid), decanoic acid (wax, tallow and rancid) and dodecanoic acid, the highest amounts were found when CAT-1 was used in the fermentation of raspberry pulp. Octanoic acid was found at high concentrations in all raspberry wines. With respect to octanoic acid, the obtained results are in good agreement with those reported by other authors (Mateo et al., 2001; Hernández-Orte et al., 2008). When *S. bayanus* was used in fermentation, lower concentrations of octanoic acid were found in wine by Mateo et al. (2001) and Hernández-Orte et al. (2008). For all raspberry beverages, octanoic acid was measured above the odour threshold of 500 µg/L proposed by Ferreira, López and Cacho (2000). Some authors (Soufleros et al., 2001) have found other acids (*e.g.*, isobutyric and butyric) in high concentrations in grape wine. High levels of butyric and isobutyric acid can cause lower acceptance of the wine because these compounds have a negative effect on the sensory character of wines (Nikolaou, Soufleros, Bouloumpasi & Tzanetakis, 2006). In wine, butyric and isobutyric acid are associated with the odour descriptor “rancid” (Liberatore et al., 2010).

Three volatile phenols (4-vinylguaiacol, 4-vinylphenol and zingerone) were identified in raspberry wines. When CAT-1 was used in the fermentation of raspberry must, a total volatile phenol concentration of 417.7 μg/L was found (Table 4), a value approximately five times higher than the concentration present in the beverage produced with *S. bayanus* CBS 1505. In the beverage produced by CAT-1, 4-vinylguaiacol may contribute to aroma descriptors such as "clove-like", "smoky", "phenolic" and "bitter" because this compound was found in high concentrations above the odour threshold of 21 μg/L (Czerny et al., 2008). Vinylphenols (*e.g.*, 4-vinylphenol and 4-vinylguaiacol) can play a role in wine aroma. *Saccharomyces cerevisiae* possesses a type of enzymatic activity, substituted cinnamate carboxy-lyase, which is capable of transforming the phenolic acids in the must (*e.g.*, *p*-coumaric and ferulic acids) into corresponding vinylphenols by non-oxidative decarboxylation. This endocellular activity is constitutive and is only expressed during alcoholic fermentation, with a variable intensity depending on the yeast strain (Chatonnet, Dubourdieu, Boidron & Lavigne, 1993).

There were no significant differences in the concentrations of five compounds identified in the group of C_{13}-norisoprenoids (Table 4). According to Shamaila, Daubeny and Anderson (1993), among the volatile compounds identified in different raspberry cultivars, the most common were terpenes, which included α-pinene, sabinene, γ-terpinene, α- and β-ionone and caryophyllene. α-Ionone, β-ionone, and α-ionol were the three most abundant C_{13}-norisoprenoid compounds in raspberry wines. Compounds such as α-ionone, β-ionone and α-ionol have been identified in raspberry fruit by other authors (Klesk, Qian and Martin, 2004; Aprea, Biasioli, Carlin, Endrizzi & Gasperi, 2009). Aprea et al. (2009) showed that among the C_{13}-isoprenoids found in raspberries (cv. Polka), α-ionone and β-ionone were found in the highest concentrations. In raspberry wines, α-ionone and β-ionone were found in concentrations higher than threshold reported in literature, indicating that these compounds may have contributed to the aroma descriptors “rose”, “floral”, “sweet”, “perfume”, and “artificial raspberry” (α-ionone) and “flowery”, “violet-like”, “floral”, “perfume”, and “raspberry” (β-ionone). These two compounds are indicated as the most relevant for the aroma of raspberries.

Principal component analysis (PCA) was applied to the mean concentration of the volatile compounds from Table 4. The first principal component (PC1) accounted for the 62.62% and the second principal component (PC2) accounted for an additional 37.38% of the total variance (Fig. 2).

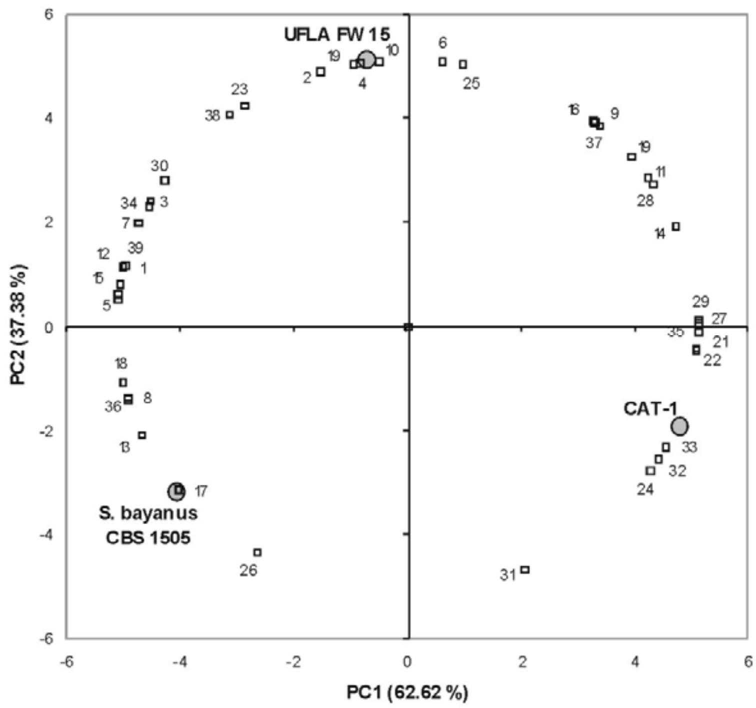

Figure 2 Principal component analysis (PCA) of volatile compounds in fruit raspberry wines by GC−MS. The volatile compounds numbers are referred in Table 4.

Raspberry wine produced by UFLA FW 15, positioned in the upper left quadrant, is more related to (*Z*)-3-hexon-1-ol, 1-butanol, ethyl butyrate, α-ionol, and 2-methyltetrahydrothiofeno-3-one. In the lower right quadrant, the raspberry wine produced with CAT-1 is mainly related to acids (hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid) that have disagreeable smells such as "sweat", "rancid", "bitter", "harsh", and "tallow". The beverage produced with *S. bayanus* CBS 1505 (lower left quadrant) was characterised by 1-heptanol, ethyl lactate, diethyl succinate, diethyl malate, 3-oxo-α-ionol, and 3-hydroxy-2-butanone.

3.2.4. GC−PFPD analysis

Volatile sulphur compounds (VSCs) are generally considered detrimental to wine quality. Some descriptors of these compounds (*e.g*., cabbage, garlic, onion and rubber) are related to their negative effects on wine aroma. However, there are some sulphur compounds (*e.g*. 4-mercapto-2.5-dimethyl(2H)thiophen-3-one, 3-mercaptohexylacetate and 4-mercapto-4-methylpentan-2-one) with more specific descriptors that contribute actively to the wine aroma (Mestres, Busto & Guasch, 2000).

Eight VSCs were identified in raspberry wines (Table 5). The highest amounts of methional (320.5 μg/L), methionol (194.4 μg/L) and total VSCs (566.5 μg/L) were measured in raspberry wine produced by *S. bayanus* CBS 1505. In wine, yeast strains can produce VSCs, and the genetic and physiological nature of the yeast strain determines its ability to release volatile thiols (Swiegers & Pretorius, 2007). When UFLA FW 15 was used in the fermentation of raspberry must, 3-mercapto-1-hexanol was present in the highest concentration (3.9 μg/L). 3-mercapto-1-hexanol is related to "passion fruit" and "grapefruit" aroma descriptors. Yeast strains have variable abilities to release 3-mercapto-1-hexanol from their S-cysteine conjugate (Dubourdieu, Tominaga and Masneuf, 2006). This compound has a very low odour threshold, suggesting that it is among the most potent aroma compounds found in wine, with descriptors as “passion fruit” and “grapefruit”. 2-Furfurylthiol was only identified in the beverages produced with CAT-1 and *S. bayanus* CBS 1505 (Table 5). When *S. bayanus* CBS 1505 was used for raspberry wine production, a 2-Furfurylthiol concentration of 25.2 μg/L was found in the beverage, corresponding to the highest concentration in this work. Due to its characteristic odour and its extremely low perception threshold, this volatile thiol may contribute to the “roast coffee” and “toasty” aroma in certain wines (Tominaga, Guyot-Baltenweck, Peyrot des Gachons & Dubourdieu, 2000).

Table 5. Concentration of volatile sulfur compounds (µg/L) detected in the raspberry wines by GC−PFPD; odor threshold and descriptors reported in literature.

No	Compounds	Raspberry wines			Oth (µg/L)	Descriptors
		CAT-1	**UFLA FW 15**	***S. bayanus* CBS 1505**		
1	Furfuryl mercaptan (2-Furfurylthiol)	$16.9^{a}\pm1.36$	ND	$25.2^{b}\pm0.31$	0.036[1] *	Sulphury, burnt[1]; coffee, toasty[4]
2	Methional	ND	ND	$320.5^{a}\pm10.11$	0.43[1] *	Cooked potato-like[1]; mashed potato, warm, soup-like[5]
3	2-Mercaptoethanol	$4.7^{b}\pm0.48$	ND	$4.1^{a}\pm0.08$	130[3] ∞	Burnt, rubber[3]
4	2-Methylthioethanol	$158.1^{b}\pm14.00$	$60.8^{a}\pm2.48$	ND	250[3] ∞	Cauliflower[3]
5	3-Mercapto-3-methylbut-1-ol	$4.0^{a}\pm0.51$	$5.1^{a}\pm0.02$	$6.6^{b}\pm0.21$	1.5[2] ∞	Cooked leeks [2]
6	Methionol	$128.9^{b}\pm11.51$	$78.5^{a}\pm6.14$	$194.4^{c}\pm27.38$	36[1] *	Cooked potato-like[1]
7	3-Mercapto-1-hexanol	$1.5^{a}\pm0.13$	$3.9^{b}\pm0.10$	$2.4^{a}\pm0.06$	0.06[2] ∞	Passion fruit, grapefruit[2]
8	Benzothiazole	$5.7^{b}\pm0.74$	$3.6^{a}\pm0.12$	$13.2^{c}\pm0.15$	50[3] ∞	Rubber[3]
	Total	**319.7^{b}**	**151.9^{a}**	**566.5^{c}**		

Oth, odor threshold; ND, not detected; *Olfactory perception threshold in water; ∞ Olfactory perception threshold in wine. Values identified by the same letters are not significantly different at the 0.05 level (Scott-Knott test). Data are presented as mean ±SD of triplicate analysis.

[1] Czerny et al. (2008).
[2] Dubourdieu et al. (2006).
[3] Ribéreau-Gayon et al. (2006).
[4] Tominaga et al. (2000).
[5] Meilgaard (1975).

PCA was applied to the VSC data to obtain a more simplified view of the total VSC characters of the raspberry wines. The first and second PCs explained 69.15% (PC1) and 30.85% (PC2) of the variance. The beverage produced with CAT-1 was located in the upper left quadrant (Fig. 3) and was characterised by 2-methylthioethanol (“cauliflower”). The raspberry wine fermented with UFLA FW 15 (lower left quadrant) was associated with 3-mercapto-1-hexanol, suggesting that this raspberry wine may have aroma descriptors such as “passion fruit” and “grapefruit”. In the lower right quadrant of the PCA plot (Fig. 3), raspberry wine fermented with *S. bayanus* CBS 1505 was characterised by the presence of 3-mercapto-3-methylbut-1-ol, methional and benzothiazole. These compounds have aroma descriptors like “cooked leeks”, “cooked potato-like”, “warm”, “soup-like”, and “rubber” (Table 5).

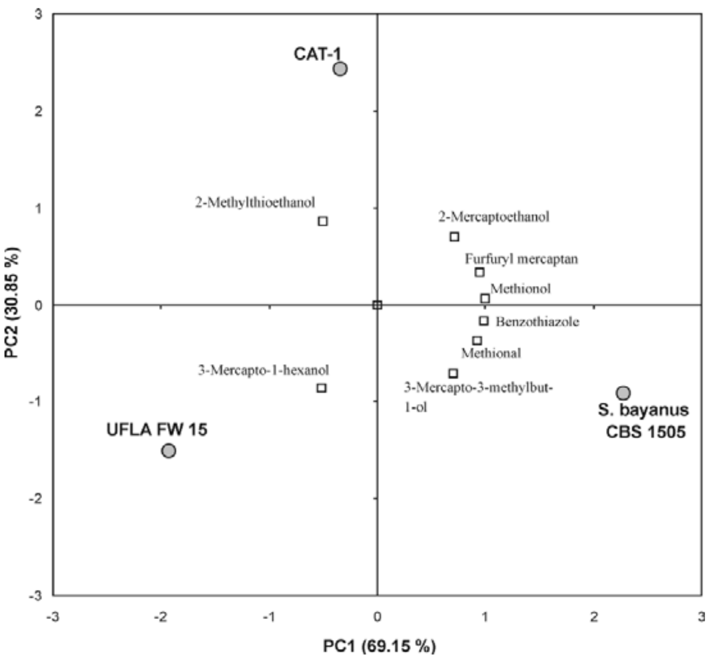

Figure 3 Principal component analysis (PCA) of volatile sulfur compounds in raspberry wines by GC−PFPD.

3.3. Sensory analysis

The three raspberry wines were evaluated by sensory descriptive analysis. Frequency, intensity and the geometric mean of the descriptors analysed are shown in Table 6. Analysis of variance (ANOVA) was used to differentiate the beverages by intensity. In the visual assessment, significant differences were found for the *raspberry* and *strawberry* descriptors. These descriptors showed the highest intensity and frequency in raspberry wine produced with UFLA FW 15. Thirteen aromatic descriptors were identified in raspberry wines. The lowest (4) and highest (12) number of aromatic descriptors were found in beverages produced with CAT-1 and UFLA FW 15, respectively, whereas an intermediate number (8) of aromatic descriptors were found in raspberry wine made with *S. bayanus* CBS 1505 (Table 6). The aromatic descriptor *sulphide* was found only in raspberry wines produced with CAT-1 and *S. bayanus* CBS 1505. Among the aromatic descriptors identified in the raspberry beverage fermented by UFLA FW 15, six descriptors were fruity descriptors (*blackberry, tropical fruit, pineapple, tangerine, dried fruit*, and *red fruit*). The most frequent aromatic descriptors found in raspberry wine produced with UFLA FW 15 were red fruit (37.9%), blackberry (26.8%) and herbaceous (23.1%) (Table 6). Some descriptors may be associated with some volatile compounds; among them, ethyl pyruvate (*herbaceous*) and ethyl butyrate (*blackberry, pineapple, apple, papaya*) may contribute the most in beverages produced from fermentation with UFLA FW 15. However, not all aromatic descriptors could be explained by the studied volatile compounds. Significant differences were found for *consistency*, *body* and *persistence*. In the sensory analysis, the highest overall relative intensity and frequency were found in raspberry wine produced with UFLA FW 15 (Table 6).

Table 6. Frequency, intensity, geometric mean and significant differences of descriptors among raspberry wines.

Descriptors	Raspberry wines CAT-1			UFLA FW 15			*S. bayanus* CBS 1505			Sig.	Groups
Visual assessment	**I (%)**	**F (%)**	**GM**	**I (%)**	**F (%)**	**GM**	**I (%)**	**F (%)**	**GM**		
Cherry	5.6	8.3	6.8	12.0	25.0	17.3	4.6	8.3	6.2	ns	
Raspberry	8.3	25.0	14.4	28.7	50.0	37.9	7.4	16.6	11.1	*	ABB
Violet	3.7	16.6	7.8	1.8	8.3	3.9	0.0	0.0	0.0	ns	
Strawberry	0.0	0.0	0.0	14.8	25.0	19.2	0.0	0.0	0.0	*	ABB
Orange	7.4	16.6	11.1	8.3	16.6	11.8	3.7	8.3	5.5	ns	
Rose	10.2	25.0	15.9	16.7	33.3	23.6	6.5	25.0	12.7	ns	
Olfactory assessment											
Olfactory intensity	23.1	33.3	27.8	60.2	75.0	67.2	17.6	33.3	24.2	**	ABB
Olfactory consistency	11.1	25.0	16.7	41.7	75.0	55.9	8.3	16.6	11.8	**	ABB
Herbaceous	13.9	25.0	18.6	23.1	50.0	34.0	2.8	8.3	4.8	ns	
Medicinal	0.0	0.0	0.0	3.7	8.3	5.5	0.0	0.0	0.0	ns	
Blackberry	15.7	25.0	19.8	26.8	50.0	36.6	10.2	25.0	15.9	ns	
Floral	0.0	0.0	0.0	8.3	16.6	11.8	3.7	8.3	5.5	ns	
Tropical	0.0	0.0	0.0	4.6	8.3	6.2	4.6	8.3	6.2	ns	
Pineapple	0.0	0.0	0.0	2.8	8.3	4.8	0.0	0.0	0.0	ns	
Tangerine	0.0	0.0	0.0	2.8	8.3	4.8	0.0	0.0	0.0	ns	
Dried fruit	0.0	0.0	0.0	2.8	8.3	4.8	3.7	16.6	7.8	ns	
Red fruit	6.5	8.3	7.3	37.9	58.3	47.0	5.6	16.6	9.6	**	ABB
Yogurt	0.0	0.0	0.0	4.6	8.3	6.2	0.0	0.0	0.0	ns	
Resin	0.0	0.0	0.0	3.7	8.3	5.5	0.0	0.0	0.0	ns	
Balsamic	0.0	0.0	0.0	4.6	8.3	6.2	4.6	8.3	6.2	ns	
Sulfide	11.1	25.0	16.7	0.0	0.0	0.0	0.9	8.3	2.8	ns	

Table 6. (Continued)

Descriptors	Raspberry wines									Sig.	Groups
	CAT-1			UFLA FW 15			*S. bayanus* CBS 1505				
Gustative assessment	**I (%)**	**F (%)**	**GM**	**I (%)**	**F (%)**	**GM**	**I (%)**	**F (%)**	**GM**		
Consistency of taste	13.9	50.0	26.3	32.4	91.7	54.5	12.0	41.7	22.4	**	ABB
Sweet	0.9	8.3	2.8	4.6	33.3	12.4	0.9	8.3	2.8	ns	
Salty	3.7	25.0	9.6	3.7	25.0	9.6	0.0	0.0	0.0	ns	
Acid	25.0	41.7	32.3	45.4	91.7	64.5	27.8	41.7	34.0	ns	
Bitter	27.8	50.0	37.3	33.3	75.0	50.0	17.6	33.3	24.2	ns	
Shape	9.3	33.3	17.6	25.9	75.0	44.1	7.41	33.3	15.7	**	ABB
Persistence	10.2	41.7	20.6	25.9	83.3	46.5	10.2	33.3	18.4	*	ABB
Astringency	12.9	41.7	23.2	17.6	50.0	29.7	10.2	41.7	20.6	ns	
Overall	17.6	50.0	29.7	37.9	75.0	53.4	14.8	41.7	24.9	*	ABB

Statistical significance is given by (*) p=0.05, (**) p=0.01 and (ns) not significant. I = intensity; F = frequency; GM = geometric mean.

The results of the sensory analysis are in accordance with those found for the volatile compounds, especially for compounds identified by GC−MS and GC−PFPD (Table 4 and Table 5). The beverage produced with UFLA FW 15 is characterised by the presence of compounds that show pleasant descriptors, *e.g.*, 3-mercapto-1-hexanol (passion fruit and grapefruit), (*Z*)-3-hexon-1-ol (green leaves), ethyl butyrate (papaya, apple, fruity, and perfumed), α-ionol (lemon-sweet and violet) and 3-methylbutyl acetate (banana).

When PCA was applied to the geometric mean (GM), the first principal component (PC1) accounted for the 89.55% of total variance and allowed differentiation between the beverage produced with UFLA FW 15 and beverages fermented with other yeast strains. In the upper left quadrant of the PCA plot (Fig. 4), raspberry wine produced with CAT-1 was characterised by the aromatic descriptor *sulphide*. When UFLA FW 15 was used as the starter culture in raspberry pulp fermentation, the raspberry wine produced was more correlated with the descriptors *raspberry, cherry, sweet, strawberry, floral, violet* and *acid*.

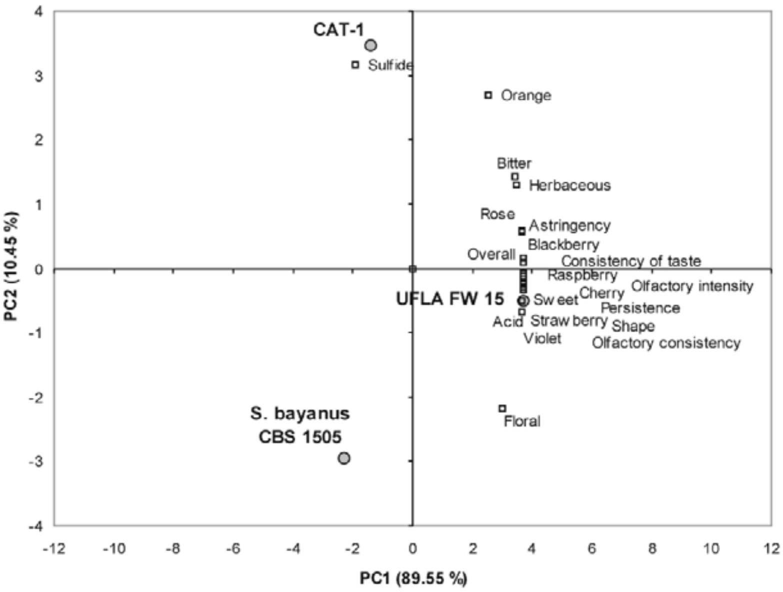

Figure 4 Principal component analysis (PCA) of sensory analysis data.

4. Conclusions

In conclusion, this research has demonstrated that the evaluated yeast strains showed potential to ferment raspberry pulp. However, different profiles for volatile compounds were identified. CAT-1, *S. bayanus* CBS 1505 and UFLA FW 15 were pre-selected for fermentation of raspberry pulp on a larger scale, leading to the production of beverages with peculiar sensory profiles and content of volatile compounds. The characterisation of the raspberry wine obtained, mainly by using GC–MS analysis and GC−PFPD analysis, is in good agreement with sensory analysis showing that UFLA FW 15 had the best results for raspberry wine production. Raspberry wine produced by UFLA FW 15 was characterised by the descriptors *raspberry, cherry, sweet,* and *strawberry;* therefore, this yeast should be used as a starter culture for raspberry wine production. Additionally, based on the characteristics of raspberry wine produced, raspberry fruits showed good potential for use in the production of fermented beverages. It was observed that the technology used here could reveal an alternative use for small-sized raspberry fruit, and thus may provide a new industrial outlet for this fruit.

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References

Akubor, P. I., Obio, S. O., Nwadomere, K. A., & Obiomah, E. (2003). Production and quality evaluation of banana wine, *Plants Foods for Human Nutrition*, *58*, 1–6.

Antonelli, A., Castellari, L., Zambonelli, C., & Camacini, A. (1999). Yeast influence on volatile composition of wines. *Journal of Agricultural and Food Chemistry*, *47*, 1139−1144.

Aprea, E., Biasioli, F., Carlin, S., Endrizzi, I., & Gasperi, F. (2009). Investigation of volatile compounds in two raspberry cultivars by two headspace techniques: Solid-Phase Microextraction/Gas Chromatography-Mass Spectrometry (SPME/GC-MS) and Proton-Transfer Reaction-Mass Spectrometry (PTR-MS). *Journal of Agricultural and Food Chemistry*, *57*, 4011−4018.

Chatonnet, P., Dubourdieu, D., Boidron, J. N., & Lavigne, V. (1993). Synthesis of volatile phenols by *S. cerevisiae* in wines. *Journal of the Science of Food and Agriculture*, *62*, 191−202.

Coulter, A. D., Godden, P. W., & Pretorius, I. S. (2004). Succinic acid-how is it formed, what is its effect on titratable acidity, and what factors influence its concentration in wine? *Austratian & New Zealand Wine Industry Journal*, *19*, 16−20, 22−25.

Czerny, M., Christlbauer, M., Christlbauer, M., Fischer, A., Granvogl, M., Hammer, M., Hartl, C., Hernandez, N. M., & Schieberle, P. (2008). Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *European Food Research and Technology, 228*, 265−273.

Dias, D. R., Schwan, R. F., & Lima, L. C. (2003). Metodologia para elaboração de fermentado de cajá (*Spondias mombin* L.). *Ciência e Tecnologia de Alimentos*, *23*, 342−350.

Dias, D. R., Schwan, R. F., Freire, E. S., & Serôdio, R. D. (2007). Elaboration of a fruit wine from cocoa (*Theobroma cacao* L.) pulp. *International Journal of Food Science & Technology*, *42*, 319−329.

Duarte, W. F., Dias, D. R., Oliveira, J. M., Teixeira, J. A., Silva, J. B. A., & Schwan, R. F. (2010). Characterization of different fruit wines mad from cacao, cupuassu, gabiroba, jabuticaba and umbu. *LWT-Food Science and Technology*, 43:1564−72.

Duarte, W. F., Dias, D. R., Pereira, G. V. M., Gervásio, I. M., & Schwan, R. F. (2009). Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production. *Journal of Industrial Microbiology & Biotechnology*, *36*, 557−569.

Dubourdieu, D., Tominaga, T., & Masneuf, I. (2006). The role of yeasts in grape flavor development during fermentation: The example of Sauvignon Blanc. *American Journal of Enology and Viticulture*, *57*, 81−88.

Etievant, X. P. (1991). Wine. In H. Maarse, Volatile Compounds in Foods and Beverages, (pp. 483–546). New York: Marcel Dekker.

Ferreira, V., López, R., & Cacho, J. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*, *80*, 1659–1667.

Girard, B., Yuksel, D., Cliff, M. A., Delaquis, P., & Reynolds, A. G. (2001). Vinification effects on the sensory, colour and GC profiles of Pinot Noir wines from British Columbia. *Food Research International*, *34*, 483–499.

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry*, *45*, 3027–3032.

Häkkinen, S., Heinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J., & Törrönen, R. (1999). Screening of selected flavonoids and phenolic acids in 19 berries. *Food Research International*, *32*, 345−353.

Hernández-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., Garcia-Moruno, & E., Ferreira, V. (2008). The development of varietal aroma from non-floral grapes by yeasts of different genera. *Food Chemistry*, *107*, 1064−1077.

Klesk, K., Qian, M., & Martin, R. R. (2004). Aroma extraction dilution analysis of cv. Meeker (*Rubus ideaus* L.) red raspberry from Oregon and Washigton. *Journal of Agricultural and Food Chemistry*, *52*, 5155−5161.

Kumar, Y. S., Prakasam, R. S., & Reddy, O. V. S. (2009). Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology. *International Journal of Food Science & Technology*, *44*, 2320–2327.

Li, W., Hydamaka, A. W., Lowry, L., & Beta, T. (2009). Comparison of antioxidant capacity and phenolic compounds of berries, chokecherry and seabuckthorn. *Central European Journal of Biology*, *4*, 499−506.

Liberatore, M. T., Pati, S., Del Nobile, M. A., & La Notte, E. (2010). Aroma quality improvement of Chardonnay white wine by fermentation and ageing in barrique on lees. *Food Research International*, doi:10.1016/j.foodres.2010.01.007

Lilly, M., Lambrechts, M.G., & Pretorius, I. S. (2000). Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. *Applied and Environmental Microbiology*, *66*, 744−753.

Malowicki, S. M. M., Martin, R., & Qian, M. C. (2008). Comparison of sugar, acids, and volatile composition in raspberry Bushy Dwarf Virus-resistant transgenic raspberry and the wild type "Meeker" (*Rubus ideaus* L.). *Journal of Agricultural and Food Chemistry*, *56*, 6648−6655.

Mateo, J. J., Jiménez, M., Pastor, A., & Huerta, T. (2001). Yeast starter cultures affecting wine fermentation and volatiles. *Food Research International*, *34*, 307−314.

Meilgaard, M. C. (1975). Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. *MBAA Technical Quarterly*, *12*, 151–168.

Mestres, M., Busto, O., & Guasch, J. (2000). Analysis of organic sulfur compounds in wine aroma. *Journal of Chromatography A, 881*, 569−581.

Nikolaou, E., Soufleros, E. H., Bouloumpasi, E., & Tzanetakis, N. (2006). Selection of indigenous *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results. *Food Microbiology*, *23*, 205−211.

Oliveira, J. M., Faria, M., Sá, F., Barros, F., & Araújo, I. M. (2006). C_6-alcohols as varietal markers for assessment of wine origin. *Analytica Chimica Acta*, *563*, 300–309.

Pérez-Magariño, S., Ortega-Heras., & Cano-Mozo, E. (2008). Optimization of a solid-phase extraction method using copolymer sorbents for isolation of phenolic compounds in red wine and quantification by HPLC. *Journal of Agricultural and Food Chemistry*, *56*, 11560−11570.

Plutowska, B., & Wardencki, W. (2008). Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review. *Food Chemistry*, *107*, 449−463.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Alcohols and other volatile compounds, In P. Ribéreau-gayon., Y. Glories., A. Maujean., D. Dubourdieu, Handbook of enology. The chemistry of wine and stabilization and treatments, (Vol. 2) (pp, 51-61). England: John Wiley and Sons Ltd.

Rodríguez-Delgado, M. A., Malovaná, S., Pérez, J. P., Borges, T., & García Montelongo, F. J. (2001). Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. *Journal of Chromatography A*, *912*, 240–257.

Shamaila, M., Daubeny, B. S., & Anderson, A. (1993). Sensory, chemical and gas chromatographic evaluation of five raspberry cultivars. *Food Research International*, *26*, 443–449.

Siebert, T. E., Smyth, H. E, Capone, D. L., Neuwöhoner, C., Pardon, K. H., Skouroumounis, G. K., Herderich, M. J., Sefton, M. A., & Pollnitz, A. P. (2005). Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Analytical and Bioanalytical Chemistry*, *381*, 937–947.

Soufleros, E. H., Pissa, P., Petridis, D., Lygerakis, M., Mermelas, K., Boukouvalas, G., & Tsimitakes, E. (2001). Instrumental analysis of volatile and other compounds of Greek kiwi wine, sensory evaluation and optimisation of its composition. *Food Chemistry*, *75*, 487−500.

Swiegers, J. H., & Pretorius, I. S. (2007). Modulation of volatile sulfur compounds by wine yeast. *Applied Microbiology and Biotechnology*, *74*, 954−960.

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Austratian Journal of Grape and Wine Research*, *11*, 139−173.

Tominaga, T., Guyot-Baltenweck, R., Peyrot des Gachons, C., & Dubourdieu, D. (2000). Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *American Journal of Enology and Viticulture*, *51*, 178−181.

Valero, E., Schuller, D., Cambon, B., Casal, M., & Dequin, S. (2005). Dissemination and survival of commercial wine yeast in the vineyard: A large-scale, three-years study. *FEMS Yeast Research*, *5*, 959−969.

Weber, C., & Liu, R. H. (2002). Antioxidant capacity and anticancer properties of red raspberry. In The 8th International Rubus and Ribes Symposium. *Acta Horticulturae*, *585*, 451–455.

Wondra, M., & Berovic, M. (2001). Analyses of aroma components of Chardonnay wine fermented by different yeast strains. *Food Technology and Biotechnology*, *39*, 141−148.

Zhao, Z., & Moghadasian, M. H. (2008). Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chemistry*, *109*, 691−702.

ARTIGO 4 Optimization of fermentation conditions for production of the jabuticaba (*Myrciaria cauliflora*) spirit using the response surface methodology

Journal of Food Science *in press*

Optimization of fermentation conditions for production of the jabuticaba (*Myrciaria cauliflora*) spirit using the response surface methodology

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Abstract

The jabuticaba tree (Brazilian grape tree) is a tree native to Brazil that belongs to the Myrtaceae family. The jabuticaba fruit is used in some regions of Brazil to produce juices, jams, wine and ice cream. In this work, the fermentation conditions (temperature and ºBrix) for producing jabuticaba distillate were optimized using the response surface methodology. The optimal conditions for fermentation were found to be 20 ºC and 22 ºBrix. In repeated experiments to validate the model, experimental data exhibited good agreement with the predicted data. The distillate jabuticaba beverage showed a peculiar chemical composition with twenty volatile compounds that were identified and quantified. Isoamyl alcohols (2-methyl-1-butanol + 3-methyl-1-butanol) were the most abundant volatile compounds identified in jabuticaba spirit. Sensory analysis by tasters showed overall approval of jabuticaba distillate. In principal component analysis, when the beverage was evaluated by panelists under 24 years old tended to give favorable ratings of aroma and taste, as well as high overall scores. The group of panelists between the ages of 25 and 53 years old generally gave high marks for appearance in the principal component analysis.

Keywords: *Saccharomyces cerevisiae* UFLA CA 11; fermentation biotechnology; alcoholic beverages; volatile compounds.

Practical Application: This study describes the study of fermentation conditions of jabuticaba pulp for production of jabuticaba spirit. Based on the results of this work the proposed method can be an alternative for the use of the jabuticaba fruit, and may provide a new industrial outlet for this fruit.

Introduction

In Brazil, although most of the fruit production is destined for fresh consumption, beverage production is a potential use for fruits such as the jabuticaba (*Myrciaria jaboticaba* Berg). The jabuticaba tree, also known as the "Brazilian grape tree," is a tree native to Brazil that belongs to the Myrtaceae family. Its fruits are purplish black and their skin and pulp have a sweet taste and low acidity. Jabuticaba fruits are consumed fresh and in processed forms such as jams, juices and liqueurs (Barros and others 1996; Magalhães and others 1996).

Over the years, new and diverse methods for processing fruits have been studied in an effort to minimize production losses, generate more profits and to introduce new products to the market (Duarte and others 2009). Several works have reported the use of fruit in producing fruit wine (Soufleros and others 2001; Akubor and others 2003; Dias and others 2003, 2007; Selli and others 2008; Kumar and others 2009; Duarte and others 2010a,b,c). However, there have been few studies on the use of fruit or fruit pulp in the production of distilled beverages. Among the studies about the use of fruit for production of distillates some authors have tested fruits such as orange (Da Porto and others 2002), marula (Fundira and others 2002) and Koumaro (Soufleros and others 2005).

Although there have been some studies (Silva and others 2008) of the use of jabuticaba in the production of fermented beverages, there is no information in the literature on the optimization of fermentation conditions and the use of jabuticaba pulp for the production of spirits. The fermentation conditions (e.g. temperature and ºBrix) can exert both positive and negative influence on the quality of beverage. The interaction between temperature and ºBrix can determine the final quality of the beverage (Llauradó and others 2002).

According to Nwabueze (2010), some of the techniques for process optimization, which have not undergone due consideration for relevant experimental design, are scientifically unreliable and irreproducible. For optimization, mathematical modeling, such as response surface methodology (RSM), provides a precise map leading to successful optimization. Currently, several statistical experimental design methods have been used for bioprocess optimization. RSM is one of the most commonly used and most suitable methods for identifying the effect of individual variables and for seeking the optimum conditions for a multivariable system efficiently (Kumar and others 2009). Some authors (De León-Rodríguez and others 2008; Kumar and others 2009) have used RSM to optimize fermentation conditions for producing fermented beverages and distillates. The aims of this work were to optimize the fermentation process for a novel spirit produced from jabuticaba pulp using RSM and central composite design (CCD) and to evaluate the chemical and sensory quality of jabuticaba spirit.

Materials and methods

Fruits

The Ponhema variety of jabuticaba (*Myrciaria cauliflora*) fruit was harvested between October and December of 2009 in the city of Itapira, São Paulo, Brazil. The jabuticaba fruits were washed in 1% v/v sodium hypochlorite and then in clean water. The jabuticaba pulp was extracted using a mechanical depulper and stored in 2-L polystyrene bags at -20 °C.

Jabuticaba must

The initial Brix and pH of jabuticaba pulp were 12±0.3 ºBrix and 3.4±0.0, respectively. According to the methodology proposed by Dias and others (2007), jabuticaba pulp was defrosted at room temperature and was adjusted to the given ºBrix degree (14, 18 and 22 ºBrix) using sucrose syrup.

Sucrose was used because it is easily purchased in the market and its price is lower than that of fructose and glucose. To inhibit bacterial growth, sulfur dioxide, in the form of potassium metabisulfite, was added at a concentration of 100 mg/L. The pH of the pulp was adjusted to 4.0 by the addition of $CaCO_3$.

Inoculum preparation

Active dry yeast, *Saccharomyces cerevisiae* of the strain UFLA CA 11, was grown in YPD (1% yeast extract; 2% peptone and 2% glucose) at a concentration of 2%. In the first step of the experiment (optimization), after 24 h of incubation at 28 ºC at 200 rpm, the yeast cells were separated from the medium by centrifugation (*RCF* = 4053*,* 5 min, 20 ºC) and washed twice with sterile distilled water. The yeast biomass was transferred to flasks containing 250 mL of jabuticaba must. After optimization, the second step of the experiment was carried out in optimized conditions. The strain UFLA CA 11 was pre-grown in YPD and after 24 h of incubation the cells were separated from the medium by centrifugation. The biomass was inoculated into flasks containing 4 L of jabuticaba must.

Distillation

After fermentation, the distillation process was performed in distiller with a working capacity of 6 L. The temperature of the fermented jabuticaba must was kept between 91 and 97ºC. The distillate was separated into three fractions. The first fraction (head fraction) was collected separately and standardized to a volume corresponding to about 10% of the total volume of cachaça. The intermediate fraction (heart fraction) was then collected until an ethanol concentration of about 42% v/v was reached. The last fraction (tail fraction), corresponding to 10% of the volume of spirit produced, was also collected. The final beverage was stored in glass bottles and maintained at 20 ºC for physico-chemical and sensory analysis.

Chemical analysis

Standard physico-chemical analysis

Analyses of pH, density, ethanol content and the concentrations of volatile acids, higher alcohols, aldehydes, esters, methanol and secondary metabolites were performed according to the methodologies proposed by Fernandes and others (2007) and Brazil (1988).

HPLC analysis

Ethanol, glycerol, organic acids (acetic acid, malic acid) and carbohydrates (glucose, sucrose and fructose) were quantified by high-performance liquid chromatography (HPLC). Analyses were carried out using a Shimadzu chromatograph, model LC-10A*i* (Shimadzu Corp., Japan), equipped with a dual detection system consisting of a UV detector and a refractive index detector (RID – 10A SPD-10A*i*). A Shimadzu ion exclusion column (Shim-pack SCR-101H, 7.9 mm X 30 cm) was operated at a temperature of 30 °C using 100 mM perchloric acid as the eluent at a flow rate of 0.6 mL^{-1}. The acids were detected via UV absorbance (210 nm), while the sugars and ethanol were detected via RID. Individual sugars, acids, glycerol and ethanol were identified by comparison of their retention times with the retention times of certified standards. The quantification of alcohols, sugars and acids were performed using calibration curves obtained from standard compounds. All samples were examined in duplicate (Duarte and others 2009).

GC-FID analysis

Major volatile compounds in the fermented jabuticaba must and in the jabuticaba distilled beverage were analyzed directly without any prior treatment. The minor volatile compounds were determined after extraction with dichloromethane according to Oliveira and others (2006). Analysis was performed using a gas chromatography (GC) Shimadzu model 17A, equipped with an FID (flame ionization detector) and using a capillary column of silica DB Wax (30 m x 0.25 mm i.d. x 0.25 μm) (J&W Scientific). Operating conditions were as follows: the oven temperature was maintained at 50 ºC for 5 min, raised to 190 ºC by increments of 3 ºC min^{-1} and then kept at 190 ºC for 10 min. Injector and detector temperatures were kept at 240 ºC, and the carrier gas (N_2) was kept at a flow rate of 1.2 mL min^{-1}. Injections of 1 μL were made in the split mode (1:10). The identification of volatile compounds was done by comparing the retention times of the samples with those of standard compounds injected at the same conditions. The quantification of the volatile compounds was expressed as 4-nonanol (internal standard) equivalents. For injection without any treatment, the internal standard was used at a concentration of 126 mg L^{-1}, whereas for extraction with dichloromethane, 4-nonanol was used in a final concentration of 312 μg L^{-1} (Duarte and others 2010a).

Experimental design and optimization by response surface methodology (RSM)

Response surface methodology (RSM) was used to study the effects of temperature (X_1) and ºBrix (X_2) (independent variables) on the quality attributes of the jabuticaba beverage. For each independent variable, different levels were considered (Table 1). The amounts of ethanol (Y_1), glycerol (Y_2), acetic acid (Y_3), malic acid (Y_4), volumetric productivity of ethanol (Qp) (Y_5) and volatile compounds (Y_6) were chosen as dependent variables. For Qp determination, the equations presented below were used.

$$[Q_p = (P_f - P_i) / t_f]$$

where P_i is the initial concentration of ethanol, P_f is the ethanol concentration at the end of fermentation and t_f is the total time of fermentation.

Table 1. Coded and actual values of factors of the central composite design

Factor	Name	Low actual	High actual	Low coded	High coded
X_1	Temperature	20	30	-1	+1
X_2	Brix	14	22	-1	+1
Response	**Name**	**Obs.**	**Min.**	**Max.**	**Mean**
Y_1	Ethanol (g L^{-1})	14	39.35	82.83	61.48
Y_2	Glycerol (g L^{-1})	14	5.95	11.22	8.24
Y_3	Acetic acid (g L^{-1})	14	0.72	3.02	2.06
Y_4	Malic acid (g L^{-1})	14	1.51	3.57	3.10
Y_5	Q_p (g L h^{-1})	14	0.57	1.86	1.29
Y_6	Volatile compounds (mg L^{-1})	14	421.59	1033.55	741.63

The experiment was established based on a face-center central composite design. The complete design considered 14 experiments, which included 6 replications at the center point (0).

The behavior of the response surface was investigated for response function (Yi) using the polynomial regression equation. The generalized response surface model is
given below.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (1)$$

where Y is the response variable, X_1 and X_2 are independent variables for temperature and initial sugar concentration (ºBrix), respectively. β_0 is the intercept term, β_1 and β_2 are the linear coefficients, β_{12} is the interactive coefficient and β_{11} and β_{22} are quadratic coefficients.

Sensory evaluation

The sensory analysis was carried out with non-trained tasters. The panelists were selected for participation on the basis of their preference for dry (less than 5 g L^{-1} of sugar) wines, interest and availability. The jabuticaba spirit was evaluated by 50 panelists of both sexes from 19 to 53 years of age. Ten-milliliter samples were served in clear glasses with a capacity of 25 mL. The evaluation sessions took place between 9:00 and 10:00 a.m. at room temperature (20–22 ºC) under white light. The samples were evaluated for taste, aroma, appearance and overall impression, according to the hedonic scale of nine categories: Extremely Dislike = 1, Very Much Dislike = 2; Moderately Dislike = 3; Slightly Dislike = 4, Neither Like nor Dislike = 5, Slightly Like = 6; Moderately Like = 7; Very Much Like = 8 and Extremely Like = 9. The sensory analysis was performed in two sensory sessions, each lasting 1 hour.

Statistical analysis

The Principal Component Analyses were performed using the software XLSTAT 7.5.2 (Addinsoft, New York, NY, USA). The experimental design matrix, data analysis and optimization procedure were performed using Design-Expert, Version 8.0 (STAT-EASE Inc., Minneapolis, USA).

Results and discussion

Optimization of fermentation conditions

RSM is a procedure that allows us to quickly and efficiently obtain a general idea of the optimum conditions (Ratnam and others 2005). A total of 14 experiments with different combinations of temperature and ºBrix were conducted. A central composite design with 3 levels for the 2 factors (X_1: temperature; X_2: ºBrix) was used in this work. The experimental design and the results are displayed in Tables 1 and 2. The concentrations of ethanol (Y_1), glycerol (Y_2), acetic acid (Y_3), malic acid (Y_4), Q_p (Y_5) and volatile compounds (Y_6) ranged from 39.35 g L^{-1} to 82.83 g L^{-1}, 5.94 g L^{-1} to 11.91 g L^{-1}, 0.72 g L^{-1} to 3.01 g L^{-1}, 1.50 g L^{-1} to 3.57 g L^{-1}, 0.56 g L h^{-1} to 1.86 g L h^{-1} and 421.59 mg L^{-1} to 1033.55 mg L^{-1}, respectively (Table 2).

Table 2. Central composite design matrix

Run nº	Temperature (º C)	Brix	Ethanol (g L^{-1})	Glycerol (g L^{-1})	Acetic acid (g L^{-1})	Malic acid (g L^{-1})	Q_p (g L h^{-1})	Volatile compounds (mg L^{-1})*
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
1	0	0	63.92	8.08	2.31	3.32	1.33	738.60
2	0	0	62.79	7.44	2.19	3.07	1.31	717.26
3	0	0	59.75	7.43	2.21	3.46	1.24	727.85
4	0	0	65.04	7.93	2.34	3.43	1.35	774.54
5	0	0	63.82	7.67	2.31	3.57	1.33	741.19
6	0	0	62.14	7.64	2.32	3.07	1.20	751.88
7	-1	0	65.57	8.98	1.70	3.32	1.10	780.34
8	+1	0	67.14	8.08	2.31	3.54	1.86	777.50
9	-1	-1	40.83	6.04	0.72	3.28	0.57	579.43
10	0	-1	39.35	5.95	0.80	3.40	1.09	421.59
11	+1	-1	42.11	6.76	0.99	1.90	1.75	454.68
12	-1	+1	82.83	10.99	2.84	1.51	1.17	1005.63
13	0	+1	71.77	11.22	2.32	3.08	1.20	878.77
14	+1	+1	73.62	11.10	3.01	3.44	1.53	1033.55

* Majoritary compounds were ethyl acetate, 1-propanol, 2-methyl-1-propanol, isoamyl alcohols, propionic acid, 2,3-butanediol, isobutyric acid, 1,2-propanediol, butyric acid, 2-phenylethanol, octanoic acid, and decanoic acid.

The experimental results of the CCD (central composite design) were fitted with a second-order polynomial equation. From the results of multiple regression analysis and based on the analysis of variance (ANOVA) data, the mathematical models for Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6, as functions of temperature (X_1) and ºBrix (X_2), can be expressed by the equations (2), (3), (4), (5), (6) and (7).

$$Y_1 = 62.81 - 1.06X_1 + 17.65X_2 - 2.62\, X_1X_2 + 3.84X_1^2 - 6.96\, X_2^2 \qquad (2)$$

$$Y_2 = 7.78 - 0.01X_1 + 2.43X_2 - 0.15\,X_1X_2 + 0.51X_1^2 + 0.56\,X_2^2 \quad (3)$$

$$Y_3 = 2.28 + 0.17X_1 + 0.95X_2 - 0.02\,X_1X_2 - 0.03X_1^2 - 0.47\,X_2^2 \quad (4)$$

$$Y_4 = 3.42 + 0.13X_1 - 0.09X_2 + 0.83\,X_1X_2 - 0.28X_1^2 - 0.46\,X_2^2 \quad (5)$$

$$Y_5 = 1.32 + 0.39X_1 + 0.08X_2 - 0.21\,X_1X_2 + 0.14X_1^2 - 0.19\,X_2^2 \quad (6)$$

$$Y_6 = 732.35 - 16.61X_1 + 243.71X_2 + 38.17\,X_1X_2 + 75.20X_1^2 - 53.53\,X_2^2 \quad (7)$$

The statistical significance of equations 2 to 7 listed above was checked by the F-test analysis of variance, which indicated that the regressions are statistically significant ($P < 0.005$) (Table 3). The determination coefficient (R^2) values for all response variables were higher than 0.85; this value was considered sufficiently good. The lowest value for the signal/noise (9.84) ratio was found for response Y_4 (malic acid), indicating that the models could be used to investigate the design space (Sansonetti and others 2010). Lack of fit was not significant for all six dependent variables, indicating fitness of the model for all six responses.

Table 3. Analysis of variance for the experimental results of the central composite design

Source	df	***F*-value**						***P*-value**					
		$\boldsymbol{Y_1}$	$\boldsymbol{Y_2}$	$\boldsymbol{Y_3}$	$\boldsymbol{Y_4}$	$\boldsymbol{Y_5}$	$\boldsymbol{Y_6}$	$\boldsymbol{Y_1}$	$\boldsymbol{Y_2}$	$\boldsymbol{Y_3}$	$\boldsymbol{Y_4}$	$\boldsymbol{Y_5}$	$\boldsymbol{Y_6}$
Model	5	96.24	44.08	13.98	9.63	160.59	76.28	< 0.0001	< 0.0001	0.0009	0.0031	< 0.0001	< 0.0001
X_1 Temperature	1	1.58	0.000493	2.01	1.14	588.98	1.66	0.2436	0.9457	0.1936	0.3168	< 0.0001	0.2342
X_2 ºBrix	1	440.14	203.97	58.98	0.59	25.64	356.23	< 0.0001	< 0.0001	< 0.0001	0.4650	0.0010	< 0.0001
X_1X_2	1	6.48	0.51	0.027	31.06	110.97	5.83	0.0344	0.4937	0.8745	0.0005	< 0.0001	0.0423
X_1^2	1	9.85	4.20	0.030	2.44	37.22	16.02	0.0138	0.0745	0.8675	0.1567	0.0003	0.0039
X_2^2	1	32.27	5.10	6.92	6.85	68.87	8.12	0.0005	0.0538	0.0302	0.0308	< 0.0001	0.0215
Residual	8												
Lack of Fit	3	1.67	4.95	3.33	3.63	1.09	5.07	0.2880	0.0587	0.1144	0.0995	0.4324	0.0563
Pure Error	5												
Total	13												

The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable. The response surface curves are shown in Fig. 1. In Fig. 1A, it can be observed that both the linear and quadratic coefficients of the ºBrix (X_2) affected the ethanol concentration (Y_1), while for temperature (X_1), only the linear effect was statistically significant. These results differ from those in a previous report (Kumar et al., 2009), in which both the linear and quadratic coefficients of the temperature affected the ethanol production in mango pulp fermentation. For the response Y_2, in Fig. 1B, the glycerol concentration was positively affected by a linear effect of ºBrix. The maximum concentration of glycerol observed was 11.22 g L^{-1} (Table 1). In grape wine, this compound can affect the wine flavor and gives an impression of fullness and softness (Ribéreau-Gayon and others 2006). The predicted effect of temperature and ºBrix on the response Y_3 is showed in Fig. 1C. It is clear from Fig. 1C that the response of acetic acid concentration was influenced by both the linear and quadratic effects of ºBrix. The concentration of acetic acid (Y_3) in jabuticaba fermented must is similar to the amounts of acetic acid found in previous studies using raspberry pulp (Duarte and others 2010a,b) showing that strain UFLA CA 11 produces acetic acid in considerable quantities. The malic acid concentration (Y_4) decreased considerably as the ºBrix increased, indicating that the ºBrix has a significant effect on the malic acid concentration (Fig. 1D). According to Duarte and others (2009), high levels of malic acid (2.7 g/L) negatively influence the sensory quality of the beverage. Fig. 1E shows the effects of temperature and ºBrix on volumetric productivity of ethanol (Q_p). For Q_p, all of the model terms were statistically significant ($p < 0.001$). Similar results for Q_p were found by León-Rodríguez and others (2008), in whose report both linear and quadratic coefficients of the temperature and the initial sugar concentration affected the Q_p. The effects of temperature and ºBrix on the concentration of volatile compounds are shown in Fig 1F. Similar to our observations for other variables, an increase in ºBrix results in an increase in the volatile compound concentration.

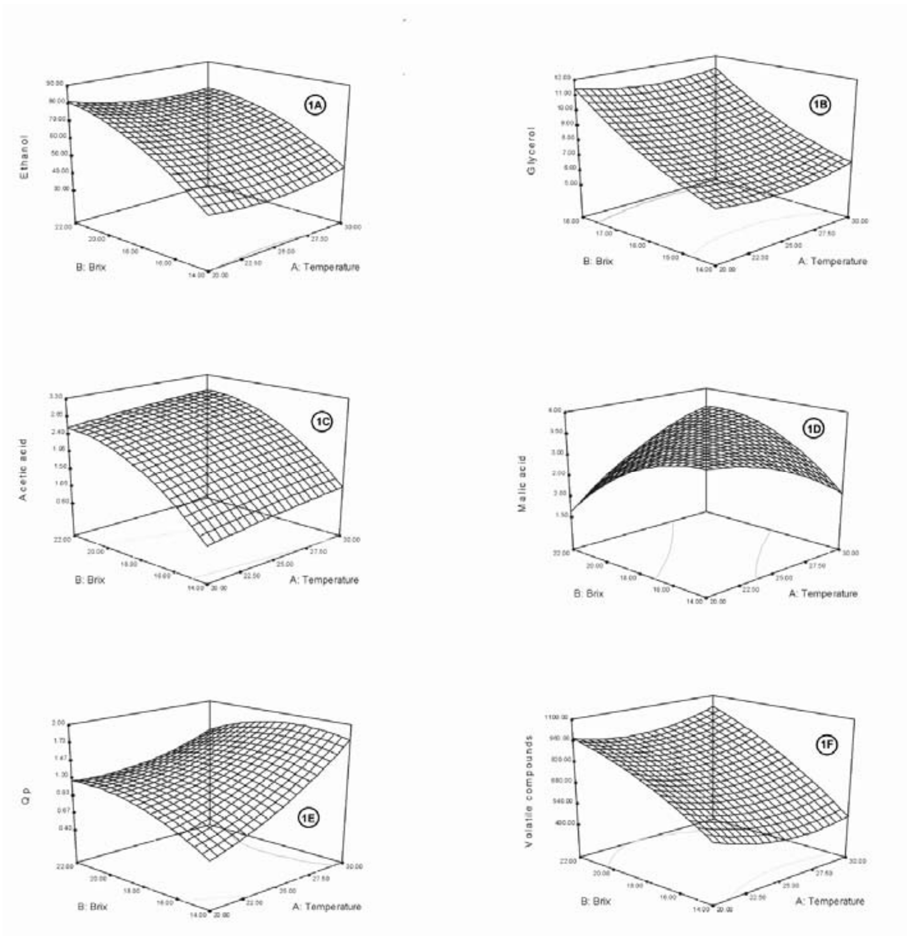

Figure 1. Response surface for dependent variables. 1A: response surface for ethanol (Y_1); 1B: response surface for glycerol (Y_2); 1C: response surface for acetic acid (Y_3); 1D: response surface for malic acid (Y_4); 1E: response surface for Q_p (Y_5); 1F: response surface for volatile compounds (Y_6).

The response surfaces showed the effects of temperature and ºBrix on the concentrations of ethanol, glycerol, acetic acid, malic acid, volatile compounds, and volumetric productivity of ethanol. Based on the models, the independent variables were evaluated in order to maximize ethanol, glycerol, volatile compounds and Q_p and to minimize acetic acid and malic acid. The optimum concentrations of the variables were obtained by graphical and numerical analysis using the Design-Expert® 8.0, based on the criteria of desirability.

Validation of the optimized conditions

In order to confirm the optimized fermentation conditions, the predicted experiments were performed. The model predicted the optimal values (coded) of the two studied variables, which were $X_1 = -1$ and $X_2 = +1$, corresponding to the values of temperature and initial ºBrix of 20 ºC and 22 ºBrix, respectively. Table 4 shows predicted and experimental data for Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6. All predicted and experimental values corresponded well. After the validation process, a new experiment was carried out in optimized conditions to produce the jabuticaba distillate.

Table 4. Experimental and predicted values for dependent variables

Variable	Optimum levels	Y_1 (g L^{-1})		Y_2 (g L^{-1})		Y_3 (g L^{-1})		Y_4 (g L^{-1})		Y_5 (g L h^{-1})		Y_6 (mg L^{-1})	
		Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.
X_1 Temperature (ºC)	20	81.04	87.40	11.43	11.91	2.57	2.18	1.63	1.74	1.16	1.22	976.16	963.98
X_2 ºBrix	22												

Chemical analysis of distilled beverage

HPLC analysis

Table 5 shows the results from HPLC analysis of the jabuticaba spirit. The glycerol concentration in the distilled beverage was 0.20 g L^{-1}. This concentration is approximately 55 times lower than the concentration measured in fermented must of jabuticaba. For acetic acid, the concentration measured in the distilled beverage (0.37 g L^{-1}) was approximately 5 times lower than the concentration found in fermented jabuticaba. Although the concentration of acetic acid in the spirit may be decreased by the distillation process**,** in this work we tried to minimize the concentration of acetic acid in the fermented must in order to further reduce the negative effects of acetic acid in the final beverage**.** The high concentrations of acetic acid cause a pronounced vinegar-like character in the beverage (Swiegers, Bartowsky, Henschke & Pretorius, 2005).The change in the concentration of acetic acid may have been caused by the distillation process (temperature 90-94 ºC), in which some compounds cannot be fully volatilized due to their boiling points (e.g., the boiling point of glycerol is 290 ºC) or result from the separation of the three fractions (head, heart and tail) of the distillate. The head, heart and tail fractions were collected as proposed by (Campos and others 2010). The main objective of the separation into fractions is to ensure that the heart fraction has a low concentration of toxic and negative sensory compounds (e.g., acetic acid), acceptable concentrations of ethanol and compounds that are favorable to the aroma and flavor of the beverage (Reche and others 2007). The reduction in the concentration of acetic acid is an interesting result because this compound may negatively influence the quality of the beverage (Gomes and others 2007). The methanol content of jabuticaba distillate was 0.85 g L^{-1} (Table 5). Methanol is formed from the enzymatic hydrolysis of the methoxy groups of pectin during fermentation, and its content depends on the degree of maceration, particularly that of the skins, which are high in pectin content (Peinado and others 2004). In the present work, jabuticaba pulp was extracted by a mechanical depulper. Therefore, the skin residues in the pulp are probably responsible the concentration of methanol found in the jabuticaba distillate.

Table 5. Concentration of compounds identified in jabuticaba distillate by HPLC

Compounds	Concentrations (g L^{-1})
Glycerol	0.20 ± 0.07
Methanol	0.85 ± 0.03
Ethanol	340.53 ± 17.27
Acetic Acid	0.37 ± 0.01

GC-FID analysis

A total of twenty volatile compounds were identified and quantified in jabuticaba distillate (Table 6). For major compounds, isoamyl alcohols were the most abundant compounds measured in distilled jabuticaba. The concentration of 476.57 mg L^{-1} found in this work was higher than the values reported by García-Llobodanin and others (2008) in pear distillates. The isoamyl alcohols, 2-methyl-1-butanol and 3-methyl-1-butanol, could be synthesized by yeast during the fermentation through deamination and decarboxylation reactions from the corresponding amino acids, *iso*-leucine and leucine, respectively (Boulton and others 1996). 2-phenylethanol, another important higher alcohol, was found at a concentration of 14.96 mg L^{-1} in jabuticaba distillate (Table 6). This compound is an aroma carrier, and its presence may contribute to the floral nuances of the beverage (Wondra and Berovic 2001). Some authors (Schehl and others 2005; García-Llobodanin and others 2008; Hernández-Goméz and others 2005) have found 2-phenylethanol in variable concentrations depending on the yeast and fruit used in the production of the distilled beverages.

Table 6. Concentration of volatile compounds identified in jabuticaba distillate by GC-FID

Major volatile Compounds	**Concentration (mg/L)**	**Descriptors**
Acetaldehyde	11.20 ± 2.97	Fresh, green[a]
Ethyl acetate	43.60 ± 10.09	Solvent, fruity[e]; nail polish[d]
1-Propanol	112.53 ± 23.75	-
2-Methyl-1-propanol	166.55 ± 34.50	Malty[a]
Isoamyl alcohol (2-Methyl-1-butanol + 3-Methyl-1-butanol)	476.57 ± 101.38	Malty, solvent-like[a] /Malty[a]
2,3-butanediol	7.36 ± 5.07	-
2-Phenylethanol	14.96 ± 3.26	Flowery, honey-like[a]
Minor volatile compounds	**Concentration (µg/L)**	
2,3-Butanedione	190.80 ± 55.60	-
Ethyl butyrate	185.90 ± 11.60	Fruity[a c]; papaya, butter, sweet, apple, perfumed[e]
Isoamyl acetate	482.9 ± 47.50	Banana[c]
1-Butanol	142.90 ± 20.50	Malty, solvent-like[a]; fusel, spirituous[c]
Furfural	88.70 ± 13.80	Almonds[d]
Propionic acid	70.50 ± 13.90	Vinegar[c]
Isobutyric acid	1125.60 ± 5.60	Sweat, bitter[e]; cheese, rancid[c]
Butyric acid	194.50 ± 20.00	Sweaty[a]; cheese, rancid[c]
Diethylsuccinate	804.50 ± 7.70	-
Phenylethyl acetate	368.30 ± 29.10	Apple, honey, roses, sweet[e]; flowery[c]
Hexanoic Acid	943.70 ± 13.70	Fatty acids, vegetable oil[e]; cheese, sweaty[c]
Octanoic Acid	3740.00 ± 138.40	Fatty acids, vegetable oil[e]; rancid, harsh[c]
Decanoic Acid	1656.30 ± 208.90	Wax, tallow, rancid, soap[e]; fatty[c]

[a] Czerny and others (2008). [b] Guth (1997). [c] Siebert and others (2005). [d] Boidron and others (1988). [e] Meilgaard (1975). [f] Ferreira and others (2000). [g] Swiegers and Pretorius, (2005).

Acetaldehyde (with methanol and furfural) is the most negative compound in distillate (García-Llobodanin and others 2008). Acetaldehyde was measured at a concentration of 22.56 mg L^{-1} in jabuticaba fermented must, whereas in jabuticaba distillate, the acetaldehyde concentration was 11.20 mg L^{-1}, indicating that the distillation process and separation of the head fraction have an influence on the final distilled beverage. Ethyl acetate is another compound that may adversely affect the quality of wine due to its unpleasant flavor in high concentrations. In our spirit, ethyl acetate was found at a concentration of 43.60 mg L^{-1} (Table 6). According to Tešević and others (2009), at low concentrations (50–80 mg L^{-1}), ethyl acetate has a positive impact on the flavor.

Thirteen minor volatile compounds were identified in the final spirit (Table 6). Although these compounds were found in lower concentrations, their presence is important for the final sensory quality of the beverage. The aroma of the beverage results from the combination of several hundred compounds in concentrations ranging from 10^{-10} to 10^{-1} g L^{-1} (Rapp and Mandery 1986). Six volatile fatty acids, propionic acid, isobutyric acid, butyric acid, hexanoic acid, octanoic acid and decanoic acid, were identified and quantified (Table 6). Short-chain fatty acids, such as isobutyric, butyric and isovaleric acids, are minor compounds and their odor may be as strong as that of acetic acid; therefore, these acids can contribute significantly to the aromas of wines and spirits. Long chain fatty acids, such as hexanoic, octanoic, decanoic and dodecanoic acid, have smaller flavor effects on the distillates (Soufleros and others 2001). Octanoic acid and decanoic acid were the most abundant minor compounds, found at concentrations of 3740.00 µg L^{-1} and 1656.30 µg L^{-1}, respectively. According to Siebert and others (2005), octanoic acid and hexanoic acid are associated with the descriptors "rancid/harsh" and "fatty," respectively. Isoamyl acetate and phenylethyl acetate were the acetates identified here (Table 6). These two compounds were also identified by Dragone and others (2009) in a distilled beverage produced from cheese whey. Both the concentrations of isoamyl acetate and of phenylethyl acetate reported by these authors were approximately twice as high as the concentrations found in jabuticaba beverage. Isoamyl acetate and phenylethyl acetate are important to the quality of the beverage because they are the main compounds responsible for descriptors as fruity and flowery (Ferreira and others 1999). As seen in Table 6, furfural was found at a concentration of 88.70 $\mu g\ L^{-1}$. Furfural concentrations in some cider spirits as reported by Madrera and others (2010) were two times lower than the concentration found in jabuticaba beverage. According to Hernández-Goméz and others (2005), furfural is produced by acid hydrolysis or during the heating of polysaccharides containing hexose or pentose fragments. In the European Union, this compound is allowed (1000 g h L^{-1} of anhydrous alcohol) because it is naturally present in fruits and other foodstuffs, whereas in Brazil it is allowed at a maximum concentration of 50 mg L^{-1} in any spirits (Brazil 2005). Two ethyl esters were found in jabuticaba, ethyl butyrate and diethylsuccinate, the concentrations of which were 185.90 $\mu g\ L^{-1}$ and 804.50 $\mu g\ L^{-1}$, respectively.

According to some authors, ethyl butyrate is characterized as having a fruity aroma, similar to papayas and apples (Meilgaard 1975; Siebert and others 2005; Czery and others 2008). Duarte and others (2010a) found ethyl butyrate at a concentration of 12.80 $\mu g\ L^{-1}$ in fermented jabuticaba beverage. The high amounts of ethyl butyrate measured in jabuticaba distillate may be the result of concentration by the distillation process.

Physico-chemical analyses

According to Brazilian law, spirits need to be of a standard quality measured by parameters set by the Ministry of Agriculture (MAPA). Table 7 shows the results for parameters evaluated in a routine analysis of spirits and the limits set by Brazil (2005) for each parameter. The relative density value of 0.96 is considered a normal value for a distillate beverage. The volatile acidity (as in acetic acid) was found in jabuticaba distillate at a concentration of 139.33 mg 100 mL^{-1} of anhydrous alcohol (Table 7). This concentration of volatile acidity is higher than the value found by Asquieri and others (2009) in a distillate produced using jabuticaba skins and the sediment from fermentation for jabuticaba wine production. According to Silva and others (2006), acidity has a negative influence on the sensory quality of beverage. Some differences were found between the results obtained by GC-FID and physico-chemical analysis. Although according to physico-chemical analysis (Table 7), furfural and esters (as in ethyl acetate) were not detected, in results obtained by GC-FID, furfural and ethyl acetate were detected. This difference in results may be related to the higher sensitivity of GC-FID compared to the methods proposed by Brazil (2005) for use in physical-chemical analysis.

Table 7. Results of physico-chemical analyses of jabuticaba distillate and limit of each parameter in accordance with Brazil (2005).

Parameters	Limit		Jabuticaba beverage
	Min.	**Max.**	
Organoleptic characteristics	-	-	Normal
Relative density (g/cm^3)	-	-	0.96
Copper (mg/L)	-	5	ND
Dry extract (g/L)	-	-	0.048
Alcoholic degree (GL)	38	54	38.64
Volatile acidity as acetic acid (mg /100 mL anhydrous alcohol)	-	150	139.33
Higher alcohols (mg /100 mL anhydrous alcohol)	-	360	187.16
Furfural (mg /100 mL anhydrous alcohol)	-	5	ND
Aldehydes (as acetic aldehyde) (mg /100 mL anhydrous alcohol)	-	30	11.18
Esters (as ethyl acetate) (mg /100 mL anhydrous alcohol)	-	200	ND
Total secondary compounds (mg /100 mL anhydrous alcohol)	200	650	337.67
Total sugars (g/L in sucrose)	> 6	≤ 30	ND

ND – not detected

Sensory evaluation

The jabuticaba distillate was subjected to sensory analysis to assess its acceptance among consumers. In the sensory analysis, the attributes of appearance, aroma, taste and overall impression were evaluated using the hedonic scale. Fig. 2 shows the distribution of individual notes for each point on the hedonic scale for the different evaluated attributes. As seen in Fig. 2, a greater number of panelists chose values above seven on the hedonic scale, demonstrating that the jabuticaba distillate showed great acceptance by the tasters.

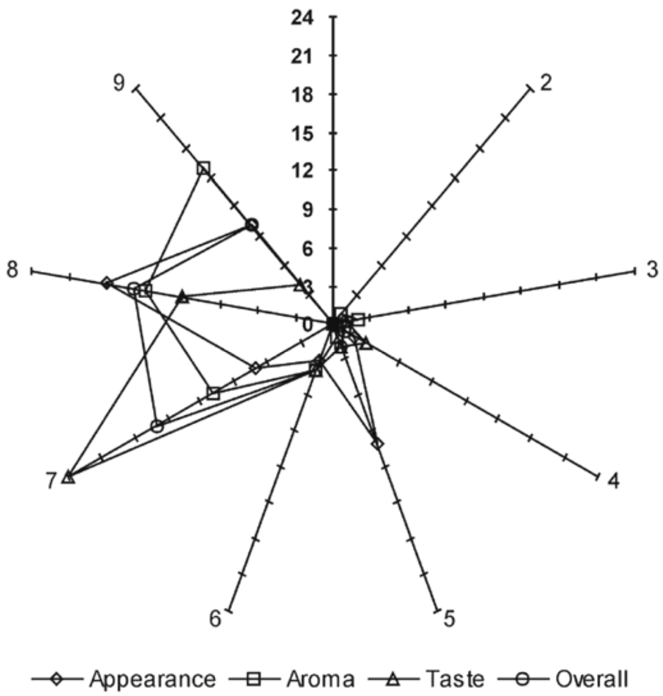

Figure 2. Distribution of number of panelists in sensory analysis. Numbers 1 to 9 range from extremely disliked (1) to extremely liked (9).

To analyze the correlation between the age of tasters and attributes evaluated in the sensory analysis, the data (age and grades for each attribute) were submitted to principal component analysis. From the 50 total panelists, two groups were formed according to the age of the panelists. The first group was formed of panelists aged 19 to 24 (represented by numbers from 1 to 24) and a second group consisted of panelists aged 25 to 53 years old (represented by numbers from 25 to 50). Fig. 3 shows the result of PCA. First and second principal components (PC1 and PC2) accounted for 79.89% of the total variance and allowed for differentiation between panelists up to 24 years old and panelists between 25 and 53 years old. On the negative side of PC2, the group of panelists younger than 24 was correlated with the attributes aroma, taste and overall impression; on the positive side of PC2, the other group (panelists aged 25 to 53 years old) was characterized by the appearance attribute (Fig. 3).

These results showed that the jabuticaba beverage was more accepted by panelists under 24 years old because there was a greater correlation between positive values for the attributes aroma and taste with the younger group of panelists.

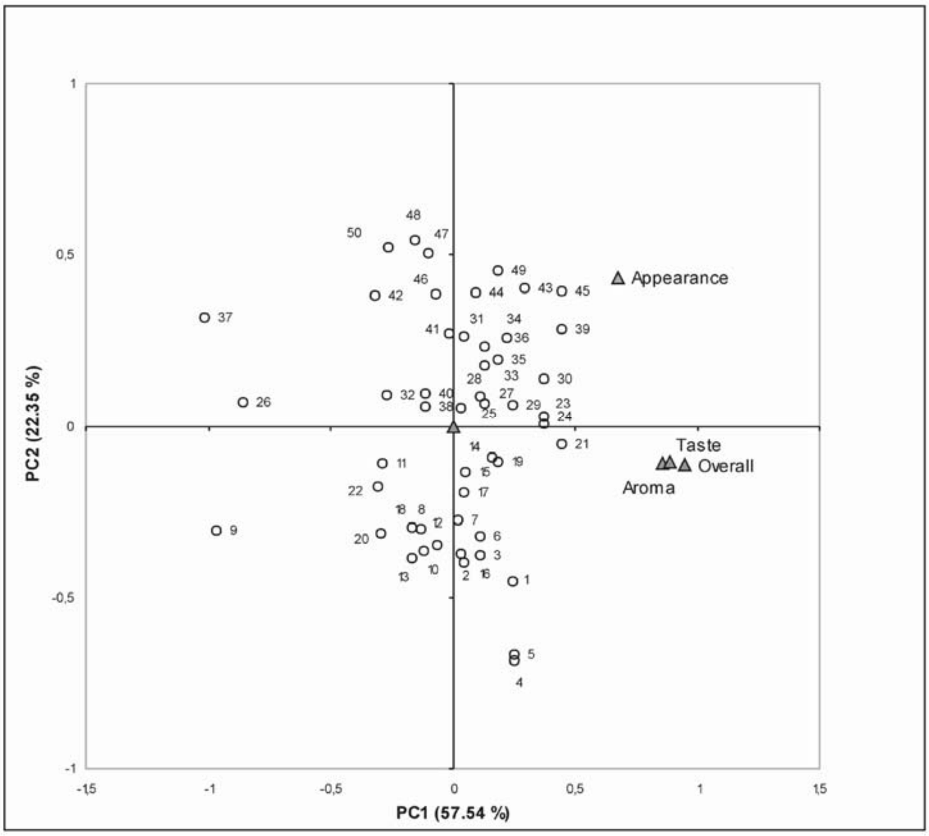

Figure 3. Principal component analysis of sensory attributes.

Conclusions

From the results of this study, it is possible to conclude that the use of jabuticaba for the production of spirits is a viable alternative usage of this fruit. The jabuticaba beverage presented some differences (e.g., concentration of some volatile compounds) compared to other fruit distillates. It also showed great acceptance in the sensory evaluation, especially for younger panelists, showing the potential of jabuticaba spirit as a new product that may be appropriate for a particular niche market. Considering the chemical characteristics of the jabuticaba beverage and the good overall results obtained in the sensory analysis, it was also possible to conclude that the optimization of fermentation conditions using response surface methodology is a good tool for improving the quality of fermented and distilled beverages produced from fruits.

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References

Akubor PI, Obio SO, Nwadomere KA, Obiomah E. 2005 Production and quality evaluation of banana wine. *Plant Foods Hum Nutr* 58:1-6.

Asquieri ER, Silva AGM, Cândido MA. 2009. Jabuticaba fruit aguardiente made from skin and sediments resulting from the production of fermented jabuticaba. Ciênc Tecnol Alimen 29:896−04.

Barros RS, Finger FL, Magalhães MM. 1996. Changes in non-structural carbohydrates in developing fruit of *Myciaria jaboticaba*. Sci Hort 66:209−15.

Boidron JN, Chatonnet P, Pons M. 1988. Influence du bois sur certaines substances odorantes des vins. Connaissance de la Vigne et du vin. 22:275–94.

Boulton RB, Singleton VL, Bisson LF, Kunkee RE. 1995. Principles and practices of winemaking. New York: Chapman and Hall.

Brasil. 2005. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº. 13, de 29 de junho de 2005. Aprova o regulamento técnico para fixação dos padrões de identidade e qualidade para aguardente de cana e para cachaça. Brasília: Diário Oficial da União, seção 1, pp. 3–4, de 30 de junho de 2005.

Brasil. 1988. Lei n. 7678, de 08 de out. de 1988. Brasília: Ministério da Agricultura e do Abastecimento, [Internet document] http://oc4j.agricultura.gov.br/agrolegis/do/consultaLei. accessed 21/12/2009.

Campos CR, Silva CF, Dias DR, Basso LC, Amorin HV, Schwan RF. 2010. Features of Saccharomyces cerevisiae as a culture starter for the production of the distilled sugar cane beverage cachaça in Brazil. J Appl Microbiol 108:1871–79.

Czerny M, Christlbauer M, Christlbauer M, Fischer A, Granvogl M, Hammer M, Hartl C, Hernandez NM, Schieberle P. 2008. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. Eur Food Res Technol 228:265−73.

Da Porto C, Pizzale L, Bravin M, Conte LS. 2003. Analyses of orange spirit flavour by direct-injection gas chromatography-mass spectrometry and headspace solid-phase microextraction/GC-MC. Flavour Frag J 18:66−72.

De León-Rodríguez A, Escalante-Minakata P, de la Rosa APB, Blaschek HP. 2008. Optimization of fermentation conditions for the production of the mescal from Agave salmiana using response surface methodology. Chem Eng Process 47:76−82.

Dias DR, Schwan RF, Lima LC. 2003. Metodologia para elaboração de fermentado de cajá (*Spondias mombin* L.). Ciênc Tecnol Alimen 23:342−50.

Dias DR, Schwan RF, Freire ES, Serôdio RD. 2007. Elaboration of a fruit wine from cocoa (Theobroma cacao L.) pulp. Int J Food SciTechnol 42:319−29.

Dragone G, Mussatto SI, Oliveira JM, Teixeira JA. 2009. Characterisation of volatile compounds in an alcoholic beverage produced by whey fermentation. Food Chem 112:929−35.

Duarte WF, Dias DR, Oliveira JM, Teixeira JA, Silva JBA, Schwan RF. 2010a. Characterization of different fruit wines mad from cacao, cupuassu, gabiroba, jabuticaba and umbu. LWT-Food Sci Technol 43:1564−72.

Duarte WF, Dragone G, Dias DR, Oliveira JM, Silva JBA, Teixeira JA, Schwan RF. 2010b. Fermentative behavior of Saccharomyces strains during microvinification of raspberry (Rubus idaeus L.). Int J Food Microbiol 143:173−82.

Duarte WF, Dias DR, Oliveira JM, Vilanova M, Silva JBA, Teixeira JA, Schwan RF. 2010c. Raspberry (*Rubus idaeus* L.) wine: yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds. Food Res Int 43:2303−2314.

Duarte WF, Dias DR, Pereira, GVM, Gervásio IM, Schwan RF. 2009. Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production. J Ind Microbiol Biotechnol 36:557−69.

Fernandes WJ, Cardoso M. G, Vilela FJ, Morais AR, Silva VF, Nelson DL. 2007. Physicochemical quality of a blend of domestic cachaças from the south of Minas Gerais. J Food Compos Anal 20:257–61.

Ferreira V, Hernández-Orte P, Escudero A, López R, Cacho J. 1999. Semipreparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. J Chromatogr A 864:77–88.

Ferreira V, López R, Cacho, J. 2000. Quantitative determination of the odorants of young red wines from different grape varieties. J Sci Food Agric 80:1659–67.

Fundira M, Blom M, Pretorius IS, Van Rensburg P. (2002). Comparison of commercial enzymes for the processing of Marula pulp, wine and spirits. J Food Sci 67:2346–51.

García-Llobodanin L, Ferrando LM, Güell C, López F. 2008. Pear distillates: influence of the raw material used on final quality. Eur Food Res Technol 228:75−82.

Gomes FCO, Silva CLC, Marini MM, Oliveira ES, Rosa CA. 2007. Use of selected indigenous Saccharomyces cerevisiae strains for the production of the traditional cachaça in Brazil. J Appl Microbiol 103:2438−47.

Guth H. 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. J Agric Food Chem 45:3027–32.

Hernández-Gómez LF, Úbeda-Iranzo J, Briones-Pérez A. 2005. Role of maceration in improving melon spirit. Eur Food Res Technol 220:55−62.

Hernández-Gómez LF, Úbeda-Iranzo J, García-Romero E, Briones-Pérez A. 2005. Comparative production of different melon distillates: Chemical and sensory analyses. Food Chem 90:115−25.

Kumar YS, Prakasam RS, Reddy OVS. 2009. Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology. Int J Food Sci Technol 44:2320–27.

Llauradó J, Rozès N, Robert R, Mas A, Contantí M. (2002). Low temperature alcoholic fermentations in high sugar concentration grape musts. J Food Sci 67:268–73.

Madrera RR, Lobo AP, Alonso JJM. 2010. Effect of cider maturation on the chemical and sensory characteristics of fresh cider spirits. Food Res Int 43:70–78.

Magalhães MM, Barros RS, Finger FL. 1996. Changes in structural carbohydrates in developing fruit of *Myciaria jaboticaba*. Sci Hort 66:17−22.

Meilgaard MC. 1975. Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. MBAA Techn Quart 12;151–68.

Nwabueze TU. 2010. Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. Int J Food Sci Technol 45:1768−76.

Oliveira JM, Faria M, Sá F, Barros F, Araújo IM. 2006. C_6-alcohols as varietal markers for assessment of wine origin. Anal Chim Acta 563:300–09.

Peinado RA, Moreno JA, Muñoz D, Medina M, Moreno J. 2004. Gas chromatographic quantification of major volatile compounds and polyols in wine by direct injection. J Agric Food Chem 52:6389–93.

Rapp A, Mandery G. 1986. Wine aroma. Experientia. 42:873–84.

Ratnam BVV, Rao SS, Rao MD, Rao MN, Ayyanna C. 2005. Optimization of medium constituents and fermentation conditions for the production of ethanol from palmyra jaggery using response surface methodology. World J Microbiol Biotechnol 21:399–04.

Reche RV, Neto AFL, Silva AA, Galinaro CA, Osti RZ, Franco DW. 2007. Influence of type of distillation apparatus on chemical profiles of Brazilian cachaças. J Agric Food Chem 55:6603–6608.

Ribéreau-Gayon P, Glories Y, Maujea, A, Dubourdieu D. 2006. Alcohols and other volatile compounds, In P. Ribéreau-Gayon, Y, Glories, A. Maujean, D Dubourdieu, Handbook of enology. The chemistry of wine and stabilization and treatments, (Vol. 2) (pp, 51-61). England: John Wiley and Sons Ltd.

Sansonetti S, Curcio S, Calabrò V, Iorio G. 2010. Optimization of ricotta cheese whey (RCW) fermentation by response surface methodology. Bioresour Technol, 101:9156–62.

Schehl B, Lachenmeier D, Senn T, Heinisch JJ. 2005. Effect of the stone content on the quality of plum and cherry spirits produced from mash fermentations with commercial and laboratory yeast strains. J Agric Food Chem 53:8230−38.

Selli S, Canbas A, Varlet V, Kelebek H, Prost C, Serot T. 2008. Characterization of the most odor-active volatiles of orange wine made from a Turkish cv. Kozan (*Citrus sinensis* L. Osbeck). J Agric Food Chem 56:227−34.

Siebert TE, Smyth HE, Capone DL, Neuwöhoner C, Pardon KH, Skouroumounis GK, Herderich MJ, Sefton MA, Pollnitz AP. 2005. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. Anal Bioanal Chem 381:937–47.

Silva CLC, Rosa CA, Oliveira ES. 2006. Studies on the kinetic parameters for alcoholic fermentation by flocculent Saccharomyces cerevisiae strains nonhydrogen sulfide-producing strains. World J Microbiol Biotechnol 22:857–63.

Silva PHA, Faria, FC, Tonon B, Mota SJD, Pinto VT. 2008. Avaliação da composição química de fermentados alcoólicos de jabuticaba (Myrciaria jabuticaba). Quím Nova 31:595−00.

Swiergers JH, Pretorius IS. 2005. Yeast modulation of wine flavour. Adv Appl Microbiol 57:131–75.

Soufleros EH, Mygdalia SA, Natskoulis P. 2005. Production process and characterization of the traditional Greek fruit distillate "Koumaro" by aromatic and mineral composition. J Food Compos Anal 18:699–16.

Soufleros EH, Pissa P, Petridis D, Lygerakis M, Mermelas K, Boukouvalas G, Tsimitakes E. 2001. Instrumental analysis of volatile and other compounds of Greek kiwi wine, sensory evaluation and optimisation of its composition. Food Chem 75:487−00.

Swiegers JH, Bartowsky EJ, Henschke PA, Pretorius IS. 2005. Yeast and bacterial modulation of wine aroma and flavour. Aust J Grape Wine Res 11:139−73.

Tešević V, Nikićević N, Milosavljević S, Bajić D,Vajs V, Vučković I. 2009. Characterization of volatile compounds of "Drenja", an alcoholic beverage obtained from the fruits of cornelian cherry. J Serbian Chem Soc 74:117−28.

Wondra M, Berovic M. 2001. Analyses of aroma components of Chardonnay wine fermented by different yeast strains. Food Technol Biotechnol 39:141−48.